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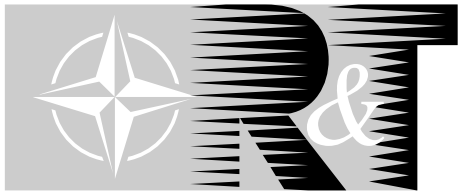
NORTH ATLANTIC TREATY ORGANIZATION



RESEARCH AND TECHNOLOGY ORGANIZATION

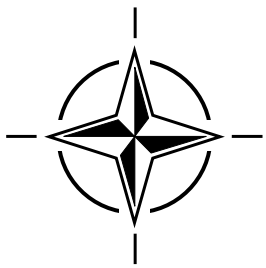
BP 25, 7 RUE ANCELLE, F-92201 NEUILLY-SUR-SEINE CEDEX, FRANCE

RTO MEETING PROCEEDINGS 42

The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation

(les Effets d'activités militaires prolongées sur l'homme. Changements physiologiques et biochimiques. Moyens possibles de récupération rapide.)

Papers presented at a workshop organised by the former DRG of NATO in Oslo, Norway, 3-5 April 1995.



Published March 2001

Distribution and Availability on Back Cover

Form SF298 Citation Data

Report Date *("DD MON YYYY")* 01032001	**Report Type** N/A	**Dates Covered (from... to)** *("DD MON YYYY")*

Title and Subtitle The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation	**Contract or Grant Number**
	Program Element Number
Authors	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Research and Technology Organization North Atlantic Treaty Organization BP 25, 7 rue Ancelle, F-92201 Neuilly-sur-Seine Cedex, France	**Performing Organization Number(s)**
Sponsoring/Monitoring Agency Name(s) and Address(es)	**Monitoring Agency Acronym**
	Monitoring Agency Report Number(s)
Distribution/Availability Statement Approved for public release, distribution unlimited	
Supplementary Notes	
Abstract	
Subject Terms	
Document Classification unclassified	**Classification of SF298** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** unlimited
Number of Pages 134	

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RTO-MP-042
AC/323(HFM)TP/23

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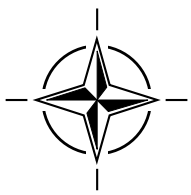

The Research and Technology Organization (RTO) of NATO

RTO is the single focus in NATO for Defence Research and Technology activities. Its mission is to conduct and promote cooperative research and information exchange. The objective is to support the development and effective use of national defence research and technology and to meet the military needs of the Alliance, to maintain a technological lead, and to provide advice to NATO and national decision makers. The RTO performs its mission with the support of an extensive network of national experts. It also ensures effective coordination with other NATO bodies involved in R&T activities.

RTO reports both to the Military Committee of NATO and to the Conference of National Armament Directors. It comprises a Research and Technology Board (RTB) as the highest level of national representation and the Research and Technology Agency (RTA), a dedicated staff with its headquarters in Neuilly, near Paris, France. In order to facilitate contacts with the military users and other NATO activities, a small part of the RTA staff is located in NATO Headquarters in Brussels. The Brussels staff also coordinates RTO's cooperation with nations in Middle and Eastern Europe, to which RTO attaches particular importance especially as working together in the field of research is one of the more promising areas of initial cooperation.

The total spectrum of R&T activities is covered by the following 7 bodies:

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS Studies, Analysis and Simulation Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These bodies are made up of national representatives as well as generally recognised 'world class' scientists. They also provide a communication link to military users and other NATO bodies. RTO's scientific and technological work is carried out by Technical Teams, created for specific activities and with a specific duration. Such Technical Teams can organise workshops, symposia, field trials, lecture series and training courses. An important function of these Technical Teams is to ensure the continuity of the expert networks.

RTO builds upon earlier cooperation in defence research and technology as set-up under the Advisory Group for Aerospace Research and Development (AGARD) and the Defence Research Group (DRG). AGARD and the DRG share common roots in that they were both established at the initiative of Dr Theodore von Kármán, a leading aerospace scientist, who early on recognised the importance of scientific support for the Allied Armed Forces. RTO is capitalising on these common roots in order to provide the Alliance and the NATO nations with a strong scientific and technological basis that will guarantee a solid base for the future.

Published March 2001

ISBN 92-837-1054-1

Printed by St. Joseph Ottawa/Hull
(A St. Joseph Corporation Company)
45 Sacré-Cœur Blvd., Hull (Québec), Canada J8X 1C6

The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation

(RTO MP-042)

Executive Summary

Panel 8 of the Defence Research Group (DRG) held a Workshop on the medical consequences of prolonged continuous military operations at Soria Moria, Oslo, 3-5 April 1995.

The participants agreed that the key questions raised by this workshop were:

– How to select and train personnel for continuous operations

– How to intervene to enhance physical and mental performance during continuous operations

The participants concluded that although the studies presented were of considerable interest, too scarce data exist to answer these key questions. Most data available are from studies of one stress factor at a time, and more effort should be done on studies of combined stress under field conditions. The workshop also agreed that further studies of physiological and psychological parameters during continuous operations during field conditions are highly needed. Whether special recommendations should be given for the treatment of wounded personnel participating in continuous military operations, and possible consequences for the long-term health of personnel participating in continuous military operations were also discussed by the workshop. This question is of great importance and can only be answered through further research.

The other key question about how to intervene during continuous operations in order to maintain the physical and mental performance, by such means as work-rest schedules, nutrition and drugs, has mainly been carried out in laboratory experiments and only scarcely addressed in a systematic manner during continuous field conditions. The workshop participants agreed that the question of possible interventions was the most significant issue to be addressed in further studies. The methodological problems with field models and how to measure the effect of interventions during field conditions were discussed. One suggestion was that a multifactorial intervention study could be performed e.g. during a NATO exercise. Effect of the interventions should be measured in a standard manner on mental and physical performance by the performance measures used e.g. during a “Best ranger competition” in US in addition to studies of physiological, biochemical and immunological functions, as well as sleep quality and effects of nocturnal sleep recovery. Canada, Germany, France, Norway, US and UK are likely to participate in these collaborative studies, and the workshop participants suggest that the Human Factors and Medicine Panel should establish a Task Group to address this important question.

les Effets d'activités militaires prolongées sur l'homme. Changements physiologiques et biochimiques. Moyens possibles de récupération rapide.

(RTO MP-042)

Synthèse

Le Panel 8 du Groupe sur la recherche pour la défense (GRD) a organisé un atelier sur les conséquences médicales d'opérations militaires continues prolongées à Santa Moria, Oslo, du 3 au 5 avril 1995.

Les participants ont reconnu la nature clé des questions suivantes soulevées par l'atelier :

– la sélection et l'entraînement des personnels en vue de la réalisation d'opérations continues
– les possibilités d'intervention en vue d'améliorer les performances physiques et mentales lors d'opérations continues

Les participants ont conclu que bien que les études présentées fussent d'un grand intérêt, trop peu de données existaient pour pouvoir formuler des réponses à ces questions clés. La plupart des données disponibles sont issues d'études de facteurs de stress isolés, et il y a lieu d'entreprendre des études du stress complexe dans des conditions opérationnelles. Les participants se sont également accordés à penser que la nécessité se faisait sentir d'études complémentaires des paramètres physiologiques et psychologiques présents lors d'opérations continues en conditions opérationnelles. Les participants à l'atelier ont aussi discuté de l'opportunité de l'élaboration de recommandations spéciales concernant les soins à dispenser aux personnels blessés lors d'opérations militaires continues, et des conséquences possibles pour la santé à long terme des personnels engagés dans ces mêmes opérations. La question est d'une grande importance et ne trouvera de réponse que par le biais de recherches complémentaires.

L'autre question clé, qui concerne les possibilités d'intervention pendant les opérations continues afin d'assurer le maintien des performances physiques et mentales, par le biais de cycles de travail-repos, de la nutrition et des médicaments, a été principalement abordée sous la forme d'expériences de laboratoire, et rarement de façon systématique en conditions opérationnelles. Les participants à l'atelier se sont accordés à dire que le sujet des interventions possibles était la question la plus intéressante à étudier à l'avenir. Les problèmes méthodologiques associés aux modèles des conditions opérationnelles et au calcul des effets des interventions en conditions opérationnelles ont été discutés. Il a été proposé d'entreprendre une étude d'intervention multifactorielle, par exemple lors d'un exercice de l'OTAN. Les effets des interventions sur les performances physiques et mentales devraient être calculés de façon normalisée en faisant appel par exemple, aux méthodes employées lors de la "Best ranger competition" aux USA, en plus des études réalisées sur les fonctions physiologiques, biochimiques et immunologiques, sur la qualité du sommeil et sur les effets de la récupération du sommeil nocturne. Il est vraisemblable que le Canada, l'Allemagne, la France, la Norvège, les Etats-Unis et le Royaume-Uni participent à ces études menées en coopération et les participants à l'atelier ont proposé à la commission sur les facteurs humains et la médecine de créer un groupe de travail pour examiner cette question importante.

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Endocrine and Metabolic Changes during Exhaustive Multifactorial Military Stress. Results from Studies during the Ranger Training Course of the Norwegian Military Academy

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INTRODUCTION

The endocrine and metabolic system is strongly affected by stress and its regulation is vital for both physical and mental performance particularily during physical stress, energy deficiency and sleep deprivation. In the present paper we have studied the endocrine and metabolic changes in male cadets from the Norwegian Military Academy during a strenuous military training course.

THE EXPERIMENTAL MODEL

All experiments were performed in male cadets from the Norwegian Military Academy during their ranger training courses at the end of the first year (June) or beginning of the second year (August-September) at the Academy. The course is part of the cadets' obligatory training program at the Academy. In spite of this we were allowed to do some standardization of the training program and to introduce differences between groups in order to study the impact of different stress factors. The cadets were between 21 and 28 years of age and physically well trained and healthy.

The courses normally lasted from 5 to 7 days. The cadets had continuous physical infantry activities around the clock corresponding to 35 % of their maximal oxygen uptake (Waldum and Huser 1974, Aakvaag et al 1978a) as measured by continuous heart rate recordings.

The work load corresponded to a daily energy consumption of 34.000 to 46.000 KJ, which is in accordance with the estimated energy loss during the course. The total weight loss depended on the food supply during the course. In the middle of the course each cadet got a half hen contains 2500 to 3400 KJ mainly in the form of proteins and was often the only food they received. For these cadets the weight loss reached 8-12 kg during the course. However, in some courses, the cadets consumed as much as 4200-6300 KJ daily in the form of bread, biscuits and other carbohydrate sources, which prevented some of the weight loss during the course, in particular loss of muscle proteins and glycogen reserves. From calliper measurements and needle biopsies the estimated fat loss is 3-4 kg during the course (Rognum et al 1982).

The cadets were normally not allowed any organized sleep during the course except for the cadets participating in a 7 day course who got 3-4 hours of sleep on day 5. From heart rate recordings, from wrist actigraphy (Vitalog) and from our own and officers' observation, the cadets' sleep during the course has been estimated to 1-3 hours totally.

BLOOD SAMPLING

Blood samples were taken by venipuncture in the antecubital vein, whereas repeated sampling was performed trough an indwelling plastic canulla also in the antecubital vein. The blood was drawn into pre-chilled vacuum tubes containing the necessary anticoagulants, such as EDTA or heparin, or also substances necessary to conserve the hormones investigated, such as the peptidase inhibitor aprotinin. The blood for preparation of plasma was centrifuged immediately in a refrigerated centrifuge for approximately 20 minutes, and the plasma

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

was frozen at -80 °C on dry ice. Blood for preparation of serum was allowed to clot at room temperature for 30 minutes before centrifugation. The samples were kept at - 80 °C in an ultrafreezer until analyzed.

BIOCHEMICAL ANALYSIS

Catecholamines in plasma were analyzed using a radioenzymatic method (s-adenosyl methionin as a tritiated methyl donor transferred to the amines by catechol-o-methyl transferase), which is a modification of the method of daPrada and Zürcher (1976). Total concentration of catecholamines was analyzed with the same method after desulphatation of the sulphate group by a sulphatase (Sigma S-1629). The plasma concentration of the conjugated catecholamines was calculated by subtracting the concentration of the free amines from the total concentration of catecholamines in blood. All the other hormone analyses were performed with radioimmunoassays with commercial kits, the methods and antibodies or radiolabeled tracers are described in the different papers. Glucose was analyzed with three different methods, first the hexokinase method (Boehringer), then by an oxygen sensitive electrode (Beckmann Analyser 2), and lastly by the Kodak Ektakem DT2 system.

STATISTICAL ANALYSIS

Since our experiments often include serial samples from the same persons, such as every morning, or responses to different types of stimulation, we had to use an analysis of variance for repeated measures. The data were analyzed by means of commercial statistical programs such as SPSS (Manova) or BMDP (4V, 5V, 8V). If the requirements for normal distribution or equal variance were not satisfied, the results were transformed by logarithmic transformation or analyzed with non-parametric methods. However, since the non-parametric methods are rather few and without possibilities to treat many groups in a repeated analysis the parametric methods were preferred. Student’s t-test has been used to identify significant differences.

RESULTS AND DISUSSION

The catecholamines and adrenergic receptors

Catecholamines are important regulators of homeostasis and are indispensable for adequate physiological responses to different environmental conditions and demands such as exercise, fasting, cold stress, surgery, and diseases (von Euler 1974, Unger et al 1980, Kuchel et al 1986, Landsberg and Young 1992). The adrenal medulla is the only source for adrenaline, whereas noradrenaline has a dual origin. Two third of circulating noradrenaline derives from the sympathetic nervous system and the rest from the adrenals. In addition to a small adrenal secretion of dopamine, plasma dopamine derives mainly from the sympathetic ganglion interneurones where dopamine is assumed to be a transmitter. Physical exercise stimulates the release of noradrenaline more than adrenaline, whereas the opposite is the case for mental stress and hypogycemia (von Euler 1974, Åkerstedt et al 1983).

The role of the conjugated plasma catecholamines has been a matter of dispute. In humans the conjugated amines are mainly sulphated, and there are high concentrations of the phenol-sulpho-transferase in the liver, in the gastro-intestinal tract and in blood platelets, and the plasma levels of conjugated amines increase after ingestion of amine-rich food. Sulphatation might therefore be the body’s protection against food amines which could, if they were absorbed unchanged, induce dramatic changes in blood pressure and pulse frequency and cause anxiety and catabolic stress (Mielke and Strobel 1994). Fig. 2, 3, and 4 shows that conjugation does not serve to inactivate catecholamines after short term exercise, since there is no increase in the conjugated amine levels in the recovery period after the bicycle exercise test. Since the cadets did not ingest any significant amounts of nutrients during the course, the only source for the increased plasma levels of conjugated amines during the course had to be sulphoconjugation of free circulating amines. Sulphoconjugation therefore serves to inactivate catecholamines during prolonged exercise. Conjugated amines do not serve as a source for the free catecholamines during exercise, since there was no decrease in the conjugated catecholamines simultaneously with the increase in the free amines. Large interindividual variations have been found for the plasma conjugated catecholamines, particularly for dopamine. During the ranger course this large interindividual variation disappeared, which indicates that the variations are due to the ingestion of food that contains different amounts of amines.

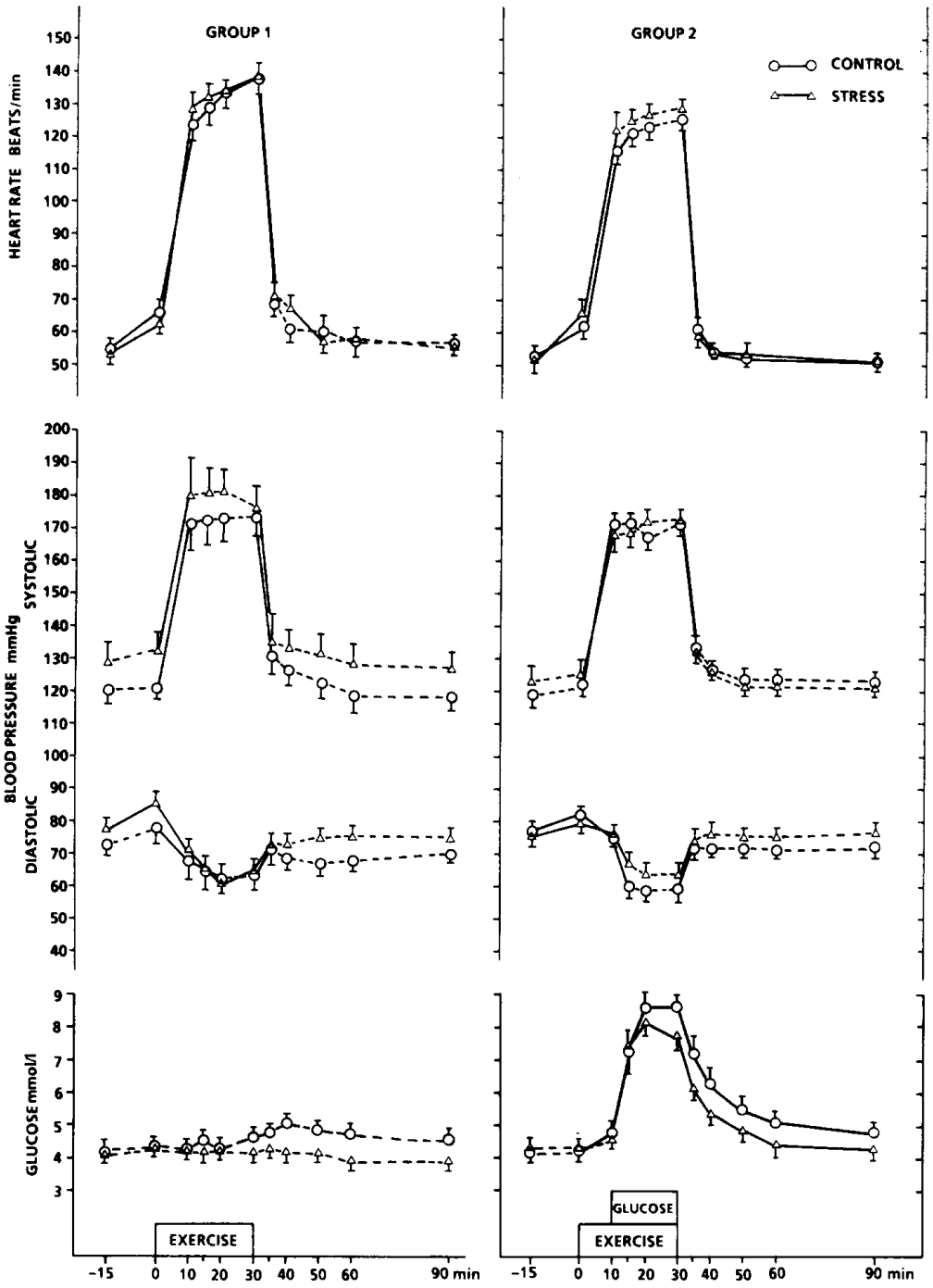

Fig. 1. The pulse rate, blood pressure and plasma glucose responses to 30 min ergometer exercise with approximately 60% of VO_2max in a control experiment and after a 5-day ranger training course with heavy physical exercise combined with energy and sleep deprivation. In contrast to Group 1 the subjects of Group 2 received 25 g of glucose intravenously during the last 20 min of exercises. The levels are presented as means and the vertical bars represent the standard error of the mean. Point to point variations significant at *P<0.01* are indicated by thick lines.

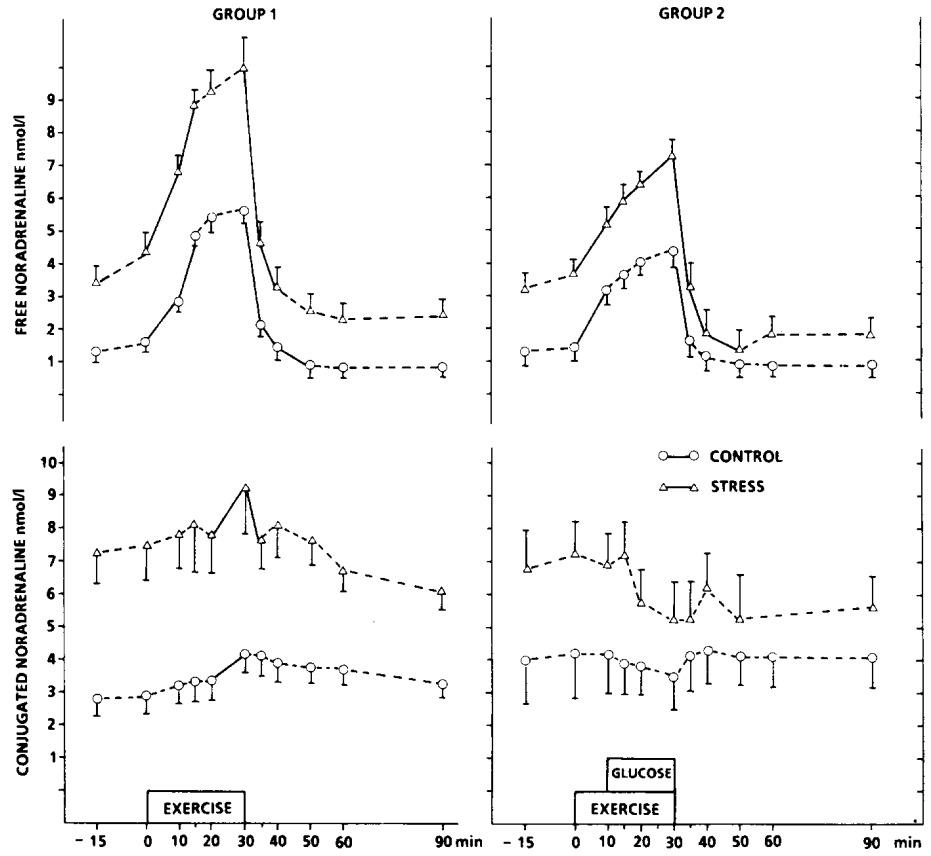

Fig. 2. Plasma levels of free and conjugated noradrenaline. For details, see Fig. 1.

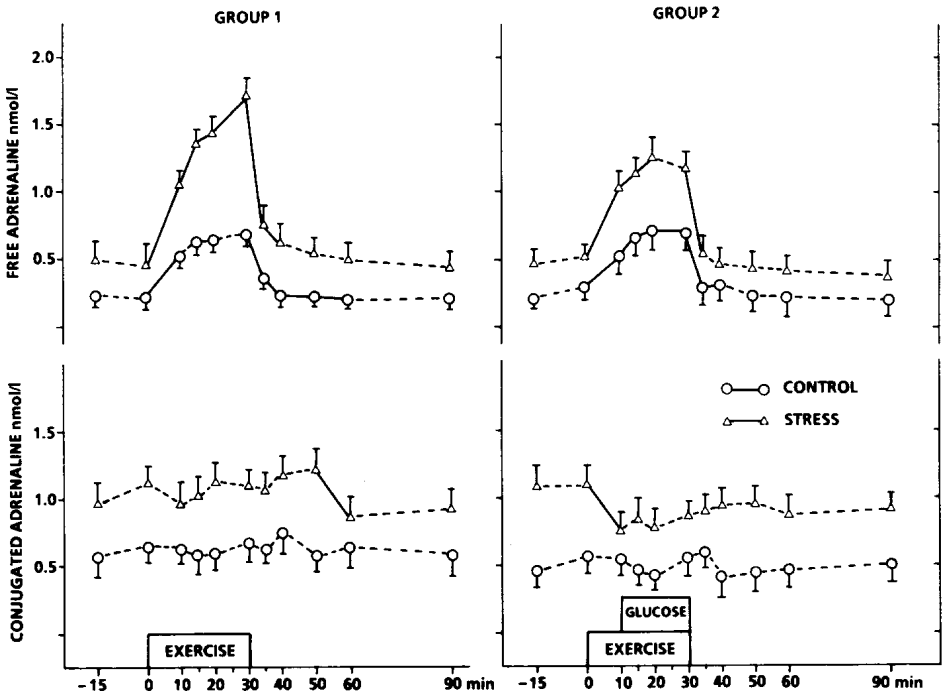

Fig. 3. The plasma free and conjugated adrenaline. For details, see Fig. 1.

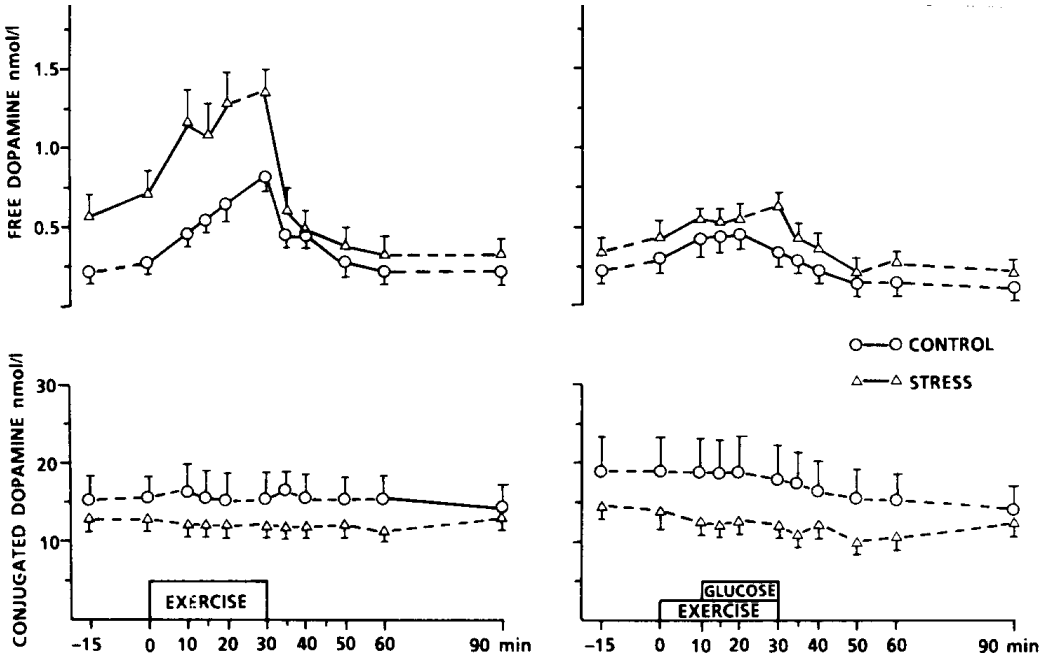

Fig. 4. Plasma free and conjugated dopamine. For details, see Fig. 1.

Ahlquist (1948) classified the adrenergic receptors as α and ß, based on the rank order of potency of the different catecholamines in the vascular beds. The receptors have further been classified into α_1, α_2, $ß_1$, $ß_2$, D_1 and D_2 adrenoceptors, based on ligand-binding studies and responses to synthetic agonists and antagonists (Hoffman and Lefkowitz 1992a, b). The α_1 receptor is postsynaptic and located on the effector tissues such as vascular smooth muscle. Stimulation of this receptor may cause vasoconstriction, increased peripheral vascular resistance and increased blood pressure, pupillary dilation and intestinal and urine bladder relaxation. In contrast many α_2 receptors are located presynaptically and their stimulation inhibits the noradrenaline secretion. α_2-receptor stimulation decreases sympathetic nerve activity and causes aggregation of platelets (Keiser 1995). $ß_1$-adrenoceptor stimulation causes positive inotropic and chronotropic effects in the heart, lipolysis and increased renin secretion by the kidney. Stimulation of the $ß_2$ receptor causes bronchodilation, vasodilatation particularly in the skeletal muscle, glycogenolysis, smooth muscle relaxation and increased release of noradrenaline from sympathetic nerves. Adrenaline is a much more powerful stimulant of the $ß_2$ receptor than noradrenaline, whereas they have approximately equal potency at the $ß_1$ and α_2 receptor. For the α_1 receptor, noradrenaline is the most powerful stimulator. Dopamine is a weak stimulator of the α and ß receptors, and in addition it has its own receptor, the D_1 receptor, which induces vasodilatation in the coronary, renal, mesenteric and cerebral vascular beds. Stimulation of the D_1 receptor in the kidney causes natriuresis and diuresis. This may be a mechanism for the natriuresis and diuresis during the first days of starvation. D_2-receptors are located presynaptically in the sympathetic nerve endings, and their stimulation inhibits release of noradrenaline and sympathetic ganglion transmission. Stimulation of the dopamine receptors in the brain causes emesis and inhibition of prolactin release. The decreased prolactin levels during the course from rather high precourse levels may be due to dopamine inhibition of prolactin secretion. Both dopamine receptors mediate their effects through the adenylate cyclase. With high catecholamine levels for prolonged periods of time, such as in patients with pheochromocytoma, a desensitization may occur by several mechanisms; internalization of receptors, decreased binding affinity of the receptor on the cell surface, uncoupling of the receptor and decreased sensitivity of the adenylate cyclase activity (Keiser 1995). The present investigation demonstrates an adrenergic desensitization during prolonged stress in that both pulse rate and blood pressure and their responses to short term physical exercise are almost unchanged in spite of considerably increased plasma catecholamine responses (Opstad et al 1980, Fig 1, 2, 3 and 4). This is well in accordance with the decrease in the leukocyte adrenergic receptors during the course (Fig 5). The high correlation between increased plasma catecholamines and reduced number of adrenergic receptors indicates a homologous down-regulation during the course. In contrast, the increased number of adrenergic receptors at the end of the course might be explained by a heterologous upregulation, because corticosteroids, which are

increased during the course, are known to stimulate the synthesis of adrenergic receptors. Thyroid hormones are also known to stimulate the synthesis of adrenergic receptors which should have given the opposite results, since all thyroid hormones decrease during the course (Opstad et al 1984). Another mechanism which may explain the increased cateholamine levels, is decreased reuptake into sympathetic nerve terminals, which is the main mechanism for noradrenaline inactivation. Decreased reuptake in sympathetic nerve terminals is probably the reason for increased noradrenaline responses to stress in aged people (Esler et al 1995).

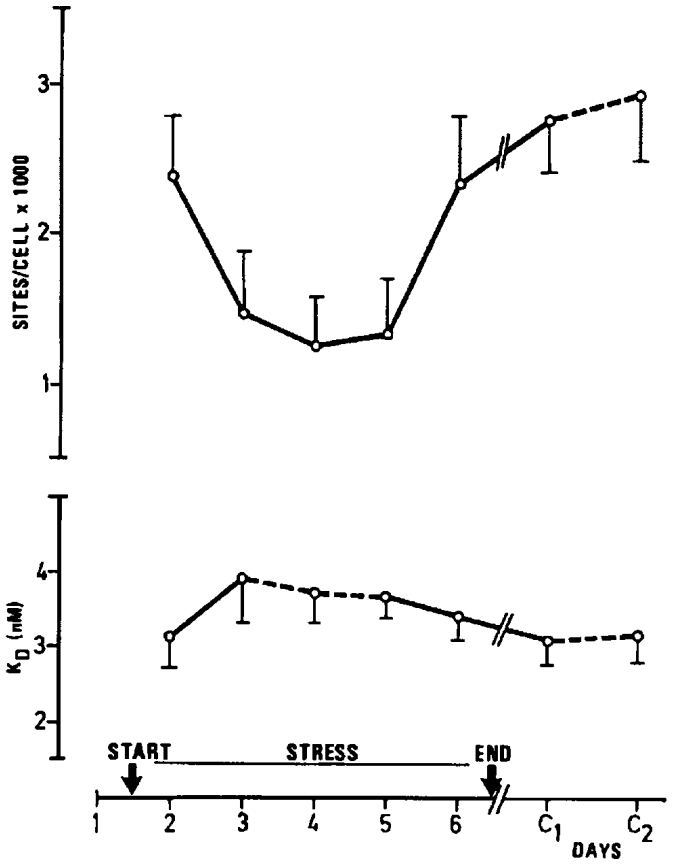

Fig. 5. The upper curve shows the total number of HBI-sites on mononuclear cells each day during the ranger training course with heavy physical exercise, energy and sleep deficiency. C_1 and C_2 are the control values obtained several weeks after the course. Values are given as means and SEM. Day to day variations statistically significant at P<0.01 are indicated by thick lines and not significant changes by dotted lines. The dissociation constant Kd is shown in the lower curve.

Fig 7 shows that the adrenergic desensitization during the course is not only due to reduced number of adrenergic receptors, but is also due to reduced adenylate cyclase activity, in that the cAMP response to adrenaline stimulation was reduced during the course, both in its sensitivity and its maximal response (Opstad 1990, 1991). The adrenergic desensitization seems to be due to the prolonged physical exercise, since there was minimal effects of sleep or food deprivation on the catecholamine responses to bicycle exercise during the course. One explanation for this is that the effect of exercise dominated over a small effect of food or sleep deficiency. However, there was a surprisingly small effect of glucose infusion on the plasma catecholamines during a bicycle exercise test (Fig 2 and 3), since it is known that particularly adrenaline is very sensitive to decreased plasma glucose levels (von Euler 1974). High stress levels and associated hormones have traditionally been associated with unfavourable survival rate. More recently it has been shown in a population study that low resting plasma adrenaline levels were associated with an unfavourable survival rate (Christensen and Jensen 1994).

The increased catecholamine responses to bicycle exercise shows that there was no sign of exhaustion in the sympathoadrenal nervous system during the course, and that the mechanism for decreased performance is to find in a desensitization of the peripheral tissue to nervous and hormone stimulation. During prolonged stress with high turnover in the sympathoadrenal system it has been proposed to give supplements of tyrosine, the precursor in the synthesis of catecholamines, to prevent exhaustion (Ahlers et al 1994, Liebermann 1994). There was no indication of such a need during the ranger course of 5-7 days since there was no deficiency in the cadets' catecholamine response to exercise.

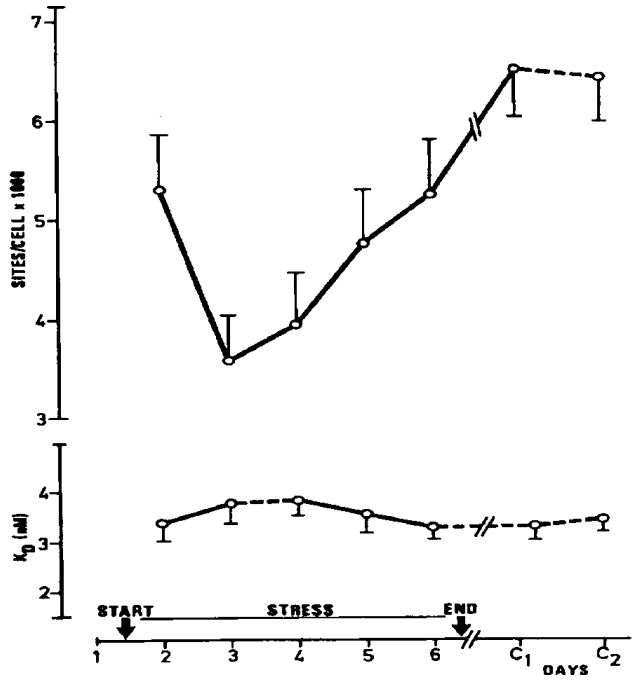

Fig. 6. Total number of HBI-sites, and values for the dissociation constant Kd for granulocytes during the ranger training course. For detail see Fig. 3.

Catecholamines normally do not pass the blood-brain barrier. The possible action of plasma catecholamines in the CNS therefore has to be through locations where the blood brain barrier is fenestrated. Most often this is in the basic parts of the brain, particularly in the hypothalamus. However, there is no information on possible effects of circulating catecholamines on the CNS. During an adrenaline or noradrenaline infusion test lasting for 20 minutes during the ranger training course a striking clinical difference was observed between the two hormones, in that the subjects given adrenaline became very alert, and stayed awake in spite of prolonged sleep deprivation. In contrast the subjects given noradrenaline got drowsy and tended to fall asleep. If this effect was mediated via increased plasma glucose levels, the same effects should have been observed in subjects given glucose intravenously. Since adrenaline does not pass the blood-brain barrier, it is tempting to suggest that this effect is due to secondary mechanisms. In the brain, noradrenaline serves as neurotransmitter for the neurones in the locus coeruleus, which have a widespread distribution of their fibres through most of the brain.

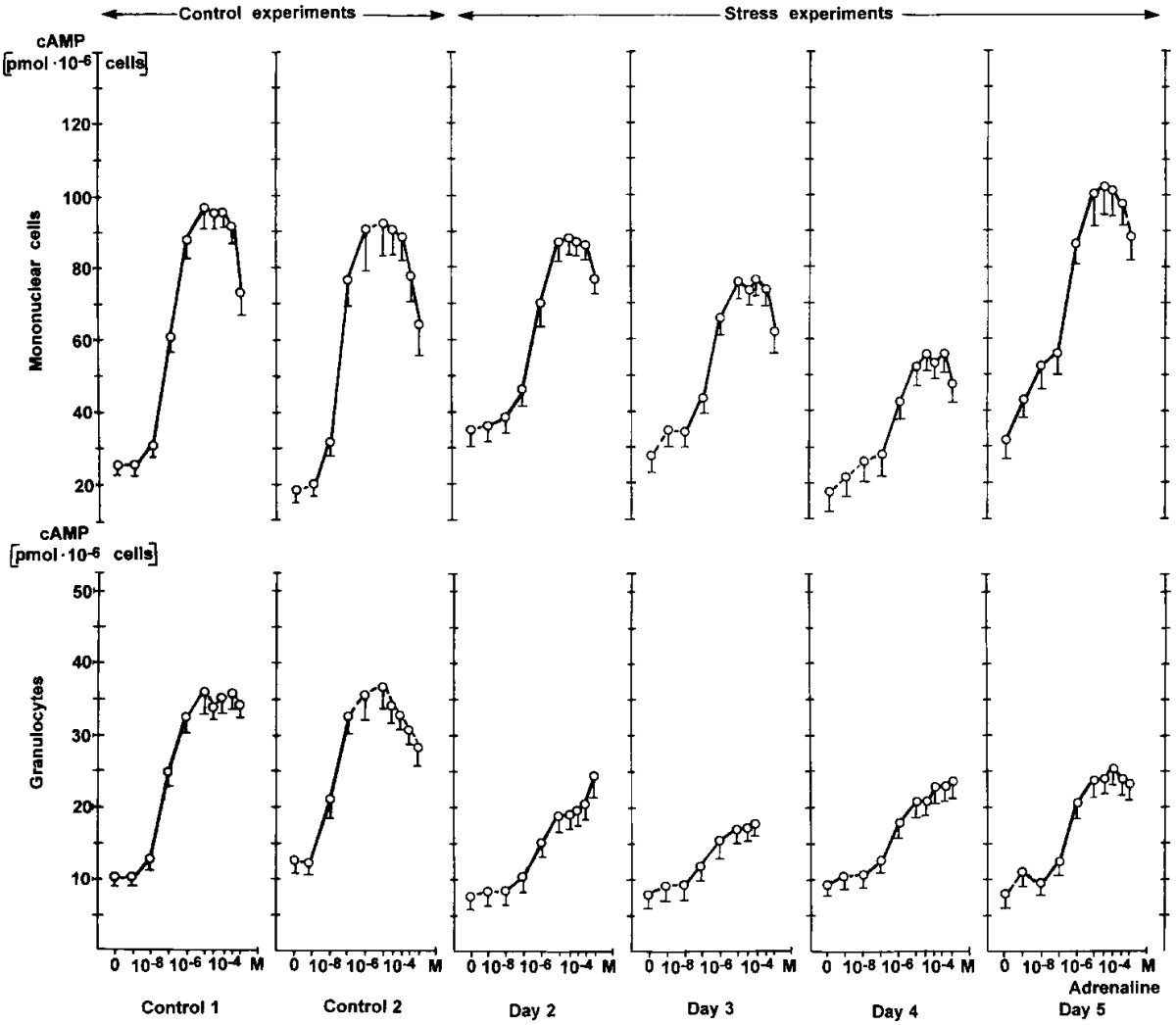

Fig. 7. Cyclic adenosine monophosphate (cAMP) response to adrenaline stimulation in human mononuclear cells and granulocytes during a 5-day military training course with heavy physical activities, sleep and energy deficiency. The experiments were performed on days 2-5 and in two control experiments performed while the cadets had normal activities at the training Academy. The results are shown as means and SEM. Time to time variations statistically significant at P<0.01 are shown with thick lines.

ADRENAL STEROIDS

The hypothalamo-pituitary-adrenocortical activation was considered by Selye as a main physiological reaction to stress with the following shrinkage of the thymus, spleen, lymphatic structure and deep bleeding ulcers in the stomach and upper gut. The anti-inflammatory effects of the glucocorticoids were in opposition to the disease of adaptation of Selye and were therefore not recognized for decades (Selye 1946, Munck and Náray-Fejes-Tóth 1995).

The adrenal cortex can be divided in 3 separate zones, the zona glomerulosa which produces the mineralocorticoids, zona reticulosa which produces the glucocorticosteroids and zona fasciculata which produces the adrenal androgens (Parker 1989, 1995). There are no sharp distinctions between the different zones, and in addition there is considerable crossreactivity in their physiological effects, and there is to some extent an interconversion of the different steroids in the peripheral tissues, particularly in the liver and in fat tissue but lately also demonstrated for muscle tissue. The mineralocorticoids are mainly regulated by the renin-angiotensin axis, but are also stimulated by ACTH. Glucocorticoids are almost exclusively regulated by ACTH, whereas adrenal androgens beside ACTH may also be stimulated by a polypeptide isolated from the pituitary that is different from ACTH, however, this is still under debate (Parker 1995).

The 4-5 fold increase in the plasma levels of aldosterone during the course is mainly due to reduced intake of food and by that the intake of NaCl. However, NaCl/K excretion is strongly regulated, and by a 90 % decrease in the urine excretion of NaCl, plasma levels of NaCl were maintained constant. When extra food was given containing approximately 20 g/24h of NaCl for each cadet, both plasma renin activity and aldosterone levels were reduced by more than 50 %, but still the salt excretion from the kidneys was reduced by 50 % compared to normal (Opstad et al 1985b, Opstad et al 1994). Clinically only a few cadets showed symptoms of salt deficiency, and only in connection with exercise and high environmental temperature. Therefore, in spite of the very strong regulation of salt balance by the kidney, occasionally small extra challenge was sufficient to cause clinical symptoms of hyponatremia.

The increase in glucocorticoids during the course is due to a combination of physical exercise and energy deficiency. During the bicycle exercise test the plasma cortisol levels were lower both during the exercise test and during recovery in the well fed subjects. To rise the plasma levels of cortisol in rested subjects the exercise has to last for at least 60 minutes and at more than 60 % of maximal oxygen uptake (Sundsfjord et al 1975). However, during the course this rise starts earlier and at lower exercise intensity (Opstad et al 1980). It is well established that cortisol stimulates energy mobilization at many levels. First of all cortisol stimulates gluconeogenesis through the stimulation of relevant hepatic enzyme systems. In addition cortisol mediates its action through the stimulation of the synthesis of adrenergic β-receptors, making adrenaline more efficient. Thereby cortisol counterbalances the stress-induced homologous downregulation of the β-receptors and is an important mechanism for preventing adrenaline from loosing its efficiency during prolonged exhausting stress. This will contribute to maintain physical as well as mental performance capacity. Cortisol may also contribute during prolonged physical strain to minimize all inflammatory processes which might be painful and which might prevent soldiers from performing their tasks. Cortisol passes the blood brain barrier and influences a number of brain functions such as mental performance and memorization (Funder 1991). The antiinflammatory response may sometimes even be lifesaving, since it prevents the inflammatory process from being harmful for the body.

In contrast to glucocorticoids and mineralocorticoids, which increase during the training courses, there is a decrease in the adrenal androgens such as dihydroepiandrosterone, androstendione and 17α-OH progesterone (Fig 8). ACTH is known to stimulate the adrenal secretion of all steroids. The decrease found for ACTH levels could then well explain the decreased secretion of andrenal androgens. However, ACTH levels measured do not necessarily reflect the mean level of ACTH stimulation of the adrenals. To avoid testing the acute effects of exercise, but rather a steady physiological state, the cadets were not allowed any significant exercise just before testing or blood sampling. The problem withe this experimental procedure is that the subjects during the course are rarely in a steady state situation but are rather in a state of activation or in a state of recovery. In the present case ACTH, which has a short half life of only some minutes, therefore recovers faster then cortisol, which has a half life of approximately 90 minutes. During this period cortisol will, in addition, exercise a negative feedback on ACTH production which is stronger than normal and by consequence lead to lower plasma ACTH levels than normal. So in spite of the measured ACTH levels, ACTH may well be responsible for the increased cortisol levels. ACTH will also contribute to the increased aldosterone levels but in combination with an even stronger and more important regulator, the renin-angiotensin system (Parker 1995). In contrast to the free adrenal androgens, the sulphated form, dihydroepiandrosterone-sulphate (DHEA-S) which circulates in the plasma in micromolar concentration and with a half life of several days, increases during the course (Fig 8). This is probably due to increased secretion from the adrenals and therefore shows that the adrenal gland may differentiate its secretion of the different androgens or steroids. The increased levels of DHEA-S might also originate from peripheral sulphatation of the free androgens, or there may even be a combination of the increased secretion and peripheral sulphatation of free androgens. Our original hypothesis that increased adrenal androgens could compensate for effects of decreased testicular androgens is not verified. This is also supported by the hypogonadic clinical symptoms during the course such as almost no beard growth, reduced muscle strength and less aggressive behaviour. It is presumed that a main reason for the decreased adrenal androgens during the course is the physical strain particularly during night time. However, the decrease of unconjugated adrenal androgens found during sleep deprivation by Åkerstedt et al (1980) may indicate that also sleep is important to preserve the increase in adrenal androgens during night time.

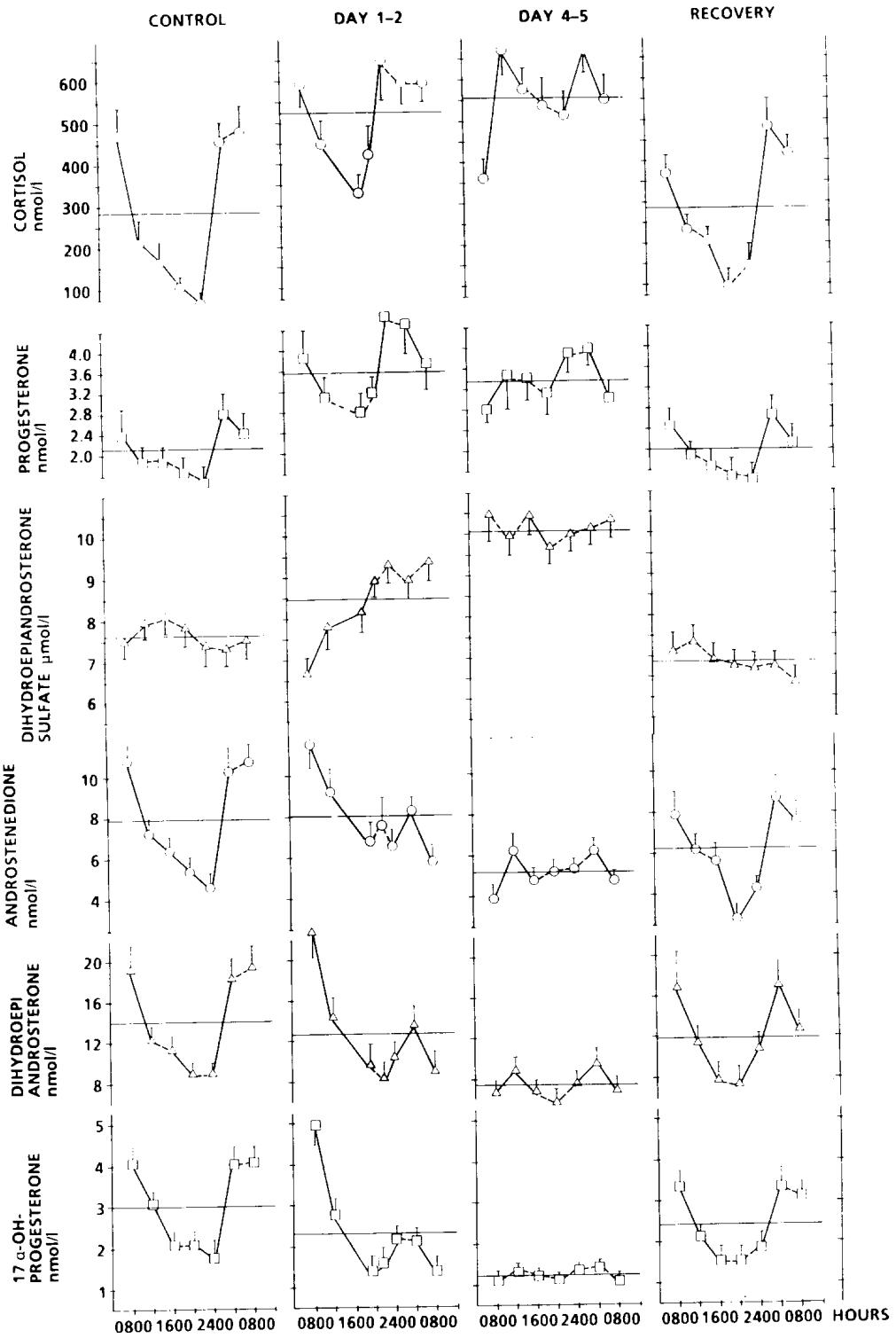

Fig. 8. The circadian rhythm for Cortisol, progesterone, dihydroepiandrosterone sulfate, androstenedione, dihydroepiandrosterone and 17α-hydroxyprogesterone during a control experiment with normal school activities (left column), during the first 24 h of continuous activities (mid-left) and from 72 to 97h of activities (mid-right) during a military training course with continuous physical activities almost without sleep and with limited amounts og food. The recovery experiment (right column) was performed 4-5 days after the course, whilw thw cadets had normal shool activities. The blood samples was collected at 4 hrs intervals. The results are expressed as means ±sem. The time to time variations that were statistically significant at p<0.01 are shown with thick lines and those that were not significant by dotted lines. Horizontal lines indicate 24-h means.

Like all other free steroids, the glucocorticoids pass the blood-brain barrier and are known to influence behaviour, mood, neuronal excitability and electrical activity. Behavioural changes are observed both in excess states such as Cushing's disease and in deficient states such as Addison's disease. Sleep disorders are often associated with glucocorticoid therapy (McEwen 1979). Adrenalectomy leads to the loss of neurones in the hippocampal formation, particularly in the dentate gyrus (Sapolsky et al 1991), whereas very high levels of glucocorticoids have been shown to cause the death of hippocampal CA3 pyramidal cells and to potentiate neuronal death evoked by toxic substances (Packan and Sapolsky 1990, Stein-Behrens et al 1992, Munch and Náray-Fejes-Tóth 1995).

Glucocorticoid receptors are widely distributed in neurones and glial cells throughout the brain (Funder 1991, Power et al 1991), whereas the mineralocorticoid receptor is mainly localized in the hippocampus and septum. In spite of the fact that mineralocorticoid receptors have a lower affinity for the glucocorticoids than for aldosterone, this is compensated by far higher concentrations of glucocorticoids. There are small areas where the mineralocorticoid receptor is protected against glucocorticoid effects by 11β-hydroxysteroid dehydrogenase and is by that aldosterone selective. In the limbic structure mineralocorticoid receptors mediate glucocorticoid effects. Studies in hippocampal slices have shown that low concentrations of glucocorticoids, when only the mineralocorticoid receptors are activated, give enhanced neuronal excitability. In contrast, high concentrations which activate the glucocorticoid receptors, suppress hippocampal excitability (Jöels and De Kloet 1989, Kerr et al 1989). In addition to the electrophysiological effects, glucocorticoids inhibit glucose transport in hippocampal neurones and glial cells, they affect glycerol-phosphate dehydrogenase (McCarthy and deVillis 1980) and glutamine synthetase in astrocytes (Hellermayer et al 1981) and induction of K^+ channel mRNA synthesis and channel expression in pituitary cells (Levitan et al 1991).

Also the adrenal androgens pass the blood brain barrier, but in contrast to the glucocorticoids, mineralocorticoids and testosterone, the cerebral concentration of pregnenolone, DHEA and their sulphate and fatty esters is considerably higher than in plasma. In addition the sulphate and fatty acid esters do not cross the blood brain barrier, and it has been shown that their variations are independent of the plasma variations. It is also shown that the oligodendrocytes have the enzymes (Cytochrom P-450) necessary to convert cholesterol to Δ5-3β-OH androgens and their conjugated and lipoid derivatives. Moreover, DHEA and its sulphate persisted for several weeks after pharmacological or surgical glandular suppression. This contrasts with testosterone, glucocorticoids and mineralocorticoids which disappear in the brain after the removal of their respective glands, and which normally have lower concentrations in the brain than in plasma (Corpéchot et al 1983, Denner et al 1990, Akwa et al 1991, 1992, Robel et al 1991, Vourch et al 1992). As for plasma steroids, brain steroids show a rather strong circadian rhythm with the highest levels during the dark period. The acrophase of corticosterone in plasma preceded the acrophase of brain DHEA and pregnenolone, indicating an independence between plasma and brain steroids. DHEAS has been shown to interact with rat forebrain membrane γ-amino butyric acid (GABA) receptor complex as a non-competitive negative neuromodulator. The GABA receptor is an oligomeric protein complex that, when activated by an agonist, produces an increase in neuronal membrane conductance to Cl^- ions, resulting in membrane hyperpolarization and reduced neuronal excitability (Chavatal and Kettenmann 1991, Demirgoron et al 1991, Robel et al 1991). Thus, adrenal androgens cause neuronal excitation and regulate neuronal and glial growth *in vitro* (Carette and Poulain 1984, Bologna et al 1987, Muntwyler and Bologna 1989), and also affect memory and aggressive behaviour in mice (Young et al 1991, Flood et al 1992). Pregnenolone, DHEA and DHEAS have also been found in the peripheral nerve tissue and might be trophic factors for these nerves (Akwa et al 1991, Chvatal and Kettenmann 1991, Demirgoron et al 1991, Morfin et al 1992). Nasman et al (1991) have shown that plasma DHEAS was decreased in patients with Alzheimer's disease. Morris et al (1987) have shown that concentration of gonadal and adrenal androgens is related to female libido. Plasma adrenal androgens show a peak concentration in the third decade of life, and then decrease gradually to very low levels in senescence. A decrease is also found for gonadal androgens with ageing with considerable interindividual alterations, however, the decrease is far less pronounced than for the adrenal androgens (Zumoff et al 1982, Davidson et al 1983, Tenover et al 1987a, b, 1988, Swerdloff and Wang 1993a, b, Winters 1995). In contrast to the androgens, the classical stress hormones, the glucocorticoids, the catecholamines, and the other counterregulatory hormones increase with age (Landsberg and Young 1992, Munck and Náray-Fejes-Tóth 1995). It has been speculated wheather the hormonal alterations may be one of the mechanisms behind the

process of ageing. In this case the demonstrated alterations found during stress will promote the process of ageing. However, since “adrenal androgens” probably do not have the same source in brain and plasma, and since we and others have not investigated the effect of stress on brain androgens, we do not know exactly the possible consequences for the central nervous system of alterations in these hormones.

TESTICULAR ANDROGENS

The testicular androgens are steroids that are responsible for the development of the male phenotype. They have three main effects: stimulation of masculine sexual characteristics, anabolic function by stimulating the increase of muscle mass, and influence on behaviour, particularly by stimulating initiative and aggressiveness. Plasma testosterone derives for 95 % from the testis, and is its most important and potent androgenic hormone. The rest (5%) derives from conversion of androgen precursors to testosterone in peripheral tissue and also for a very small part from direct adrenal secretion (Catlin 1995, Handelsman 1995, Hiipakka and Shutsung 1995, Kretser et al 1995).

Androstenedione and dihydroepiandrosterone are also secreted from the testis but at rather low rate. Their biological effects are small, but they may serve as precursors for the peripheral synthesis of testosterone or oestrogens. The androgens affect the development, growth and function of a wide variety of tissues and cell types by their interaction with the intracellular androgen receptor. Androgen-receptor complexes bind to specific sequences of DNA and modulate the rate of specific gene transcription (Kretser et al 1995). The biological effects of androgens in different tissues are determined by the tissue concentration of androgen receptors and also by the tissue concentration of the enzyme 5α-reductase which converts testosterone to dihydrotestosterone which has a 5 times higher affinity for the androgen receptor than testosterone. Tissues containing androgen receptors include the reproductive organs, brain, kidney, liver, skin, skeletal muscle, cardiac muscle, bone, larynx, thymus, hematopoietic and lipid tissue. Although a small portion of 5α-dihydrotestosterone (DHT) is secreted from the testis, most of the circulating DHT derives from peripheral metabolism of testosterone in various tissues. The tissue sensitivity to androgenic hormones is also dependent on the tissue content of 5 α-reductase, which is necessary for the conversion of testosterone to dihydrotestosterone, since this enzyme may convert androgens to the most potent androgen DHT (Hiipakka and Shutsung 1995).

During the ranger training course there is a dramatic decrease in the plasma levels of both free and total testosterone and dihydrotestosterone. The nocturnal increase in the plasma levels of testosterone was completely abolished during the course, showing that night activity is even more deleterious for anabolism and recovery than day activity (Fig 9).

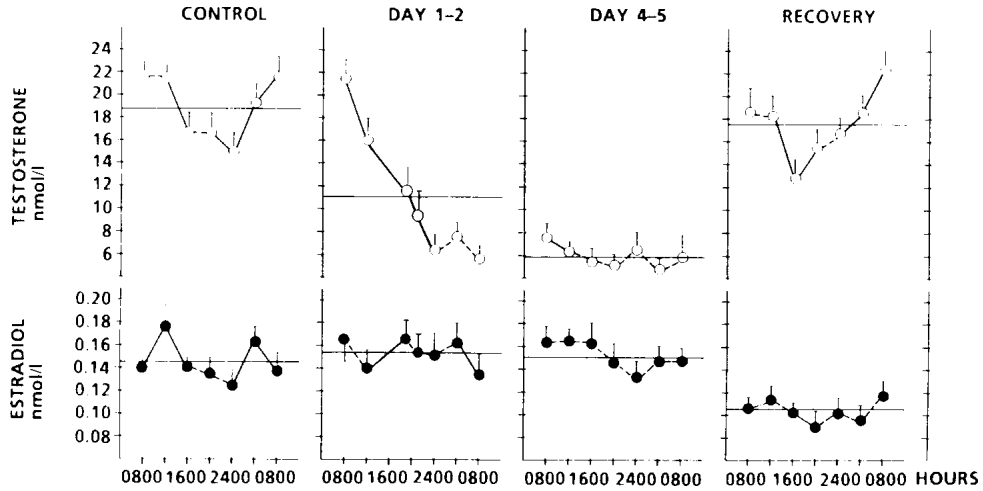

Fig. 9. Alterations in the circadian rhythm for testosterone and estradiol in a control experiment, during short and prolonged continuous stress and during recovery. For details, see Fig. 8.

The decrease in dihydrotestosterone probably reflects the decrease in androgen precursors since the percent decrease in testosterone and dihydrotestosterone are similar. From the present results we do not have any indication of alterations in the 5α-reductase activity in androgen target tissues during the course.

The decrease in testicular androgens was mainly due to the physical strain since no significant effect was found when the cadets were given extra food. A slower decrease was seen in subjects given 3 hours of extra sleep each night, however, all cadets reached the same level on the last day of the course (Opstad and Aakvaag 1982, 1983, Elias and Wilson 1993). Others have shown that extra food might reduce the decrement shown to take place for testosterone during a military training course (Guezennec et al 1994). The present papers also show that the decrease in testosterone is due to reduced secretion of LH/FSH. Further the increased LH/FSH response to GnRH stimulation indicates that there is a reduced hypothalamic GnRH secretion during the course leading to an increased sensitivity of the LH/FSH producing cells to GnRH stimulation. This shows that androgen secretion during the course is regulated from the hypothalamus and its inputs from other brain areas.

The clinical signs of reduced androgen activity are present since the beard growth during the whole course corresponds to a normal growth of one day, and this beard growth takes mainly place during the first day of the course. This corresponds well to the alterations in the androgen hormones. During the course the cadets become less aggressive, show less initiative, become more defensive and depressive, which is also in accordance with the alterations in the plasma levels of androgens (Opstad et al 1978, Myhrer 1987).

Like other steroids, testosterone and dihydrotestosterone may cross the blood brain barrier and bind to cerebral androgen receptors. Androgen receptors are mainly localized in the medial preoptic area, bed nucleus of the stria terminalis, amygdala, hippocampus, thalamus and several hypothalamic nuclei including the periventricular nuclei, supraoptic, and ventromedial nuclei and median eminence. In addition there are androgen receptors in other areas of the brain such as the frontal cortex etc., but these areas have lower receptor densities than the classical sites (Wortsman et al 1987, Sar et al 1990, Jones and Pfaff 1991, Takeda et al 1991, Genazzani et al 1992, Burgess and Handa 1993, Menard and Harlan 1993, Clancy et al 1994). In addition androgens may act through the oestrogen receptor since aromatase irreversibly transforms testosterone to oestradiol and androstendione to oestrone. This enzyme was originally found in ovary and placenta, but has also been localized to the mammalian brain particularly in the medial preoptic area, septal region, the bed nucleus of the stria terminalis and the tuberal hypothalamus (Balthazart and Foidart 1993, Hutchison 1993). The “limbic-telencephalic” aromatase-immunoreactivity is shown to be independent of gonadectomy, whereas the hypothalamic aromatase-immunoreactivity disappears after gonadectomy (Jakab et al 1993). Occupation of oestrogen receptors in the male brain is dependent on brain aromatase activity, whereas the occupation of oestrogen receptors in the female brain is more dependent on circulating oestrogen particularly during the preovulatory oestrogen surge. In addition both oestrogen and androgen receptor concentrations decrease after gonadectomy and reappear after substitution and are further increased by anabolic steroid abuse (Sar et al 1990, Takeda et al 1991, Menard and Harlan 1993, Catlin 1995). Dihydrotestosterone has a 4-5 times higher affinity for the androgen receptor than testosterone, and tissue sensitivity for testosterone may therefore be quite dependent on the tissue concentration of 5 α-reductase which converts testosterone to dihydrostosterone. Tissues such as the external genitalia and accessory sex glands have high concentrations of 5 α-reductase, and congenital deficiency in this enzyme causes female external genitalia. However, the significance of this enzyme in the brain sensitivity for androgens is less investigated. In contrast, aromatase has been shown to be necessary for the sexual differentiation of the fetal brain in rats and also for the adult (Kalra and Kalra 1991, Swerdloff et al 1992, Hutchison 1993, Jacab et al 1993). Abuse of anabolic steroids promote aggressiveness and motivation which might be an important contribution to increased performance (Moritani and DeVries 1979, Catlin 1995). A positive correlation has also been found between aggressiveness and blood testosterone levels during puberty, and adulthood in prison population, adolescent boys and military veterans (Hines and Green 1991). Testosterone is also shown to decrease with ageing, and some look upon aged men as androgen deficient and believe that reduced testosterone levels are responsible for asthenia, decreasing muscle mass, osteoporosis, and decreased sexual activity (Swerdloff and Wang 1993a, b, Winters 1995).

The protein wasting during physical stress is probably also enhanced by the decrease in the plasma androgens. However, in contrast to the decreased beard growth, which is a rather specific androgenic effect, the protein wasting and alteration in behaviour are not specific and therefore have additional explanations. Although sleep deprivation affects testosterone secretion, there are a multitude of other mechanisms that are responsible for the behavioural consequences of sleep deprivation, and the decrease in testosterone is only one of them. The most important reason for protein wasting is probably the state of fasting during the course with lack of carbohydrates and proteins and an extremely high and continuous need for energy. Although there was no significant effect of fasting on plasma androgens during the course, it has been shown by others that fasting may affect plasma androgen levels. It has been speculated whether the cadets' combat performance, such as aggressiveness, initiative and muscle strength could be improved by giving the cadets androgens. However, the "wisdom of the body" might indicate that high androgen activity is incompatible with a high energy production. Adrogens in such extreme conditions could disturb this mechanism and force the body to take the energy from other more critical sources for survival than the tissues containing androgen receptors. This might be a very hazardous experiment. In contrast, androgens could probably ameliorate or shorten the cadets recovery period if the androgen substitution is combined with an adequate diet.

THYROID HORMONES

Thyroid hormones have a myriad of physiological functions and induce alterations in almost all metabolic pathways and organs (Dumont and Vassart 1995, Jameson and deGroot 1995, Nicoloff and LoPresti 1995, Refetoff and Nicoloff 1995, Sarne and Refoteff 1995). Thyroid secretion is mainly regulated by thyroid stimulating hormone (TSH) through the hypothalamo-pituitary axis (Wilber 1995). Thyroid hormones increase oxygen consumption, affect protein, carbohydrate, lipid and vitamin metabolism. These hormones also interact with a number of other hormones, peptides and growth factors so that many of their effects occur trough interaction with other endocrine systems. The main effects of thyroid hormones on metabolism and cellular differentiation, development and growth are closely interrelated and represent a complex integration of pathways both at the cellular level and in terms of whole body physiology. Many of the developmental effects are not reversed by later treatment with hormones, suggesting that thyroid hormones act in combination with other differentiation factors that may not be available later in life. Clinically alterations in thyroid hormones were long the basis for the measurement of basal metabolic rate or oxygen consumption which are increased in hyperthyroidism and reduced in hypothyroidism. Measurement of oxygen consumption in individual tissues has shown that the metabolic effects of thyroid hormones on oxygen consumption are highly variable in different organs and tissues with marked effects in the heart, skeletal muscle, liver, kidney and gastrointestinal organs, whereas the brain, spleen and gonad tissues are metabolically less responsive. The pituitary gland shows paradoxical response since there is increased metabolic activity in hypothyroidism and reduced activity in hyperthyroidism. These variable tissue responses are partly due to tissue presence of receptors for thyroid hormones. Oxygen consumption is, however, not a marker of thyroid hormone effects in all tissues. This is for instance the case for thyroid hormone effects in the brain which shows one of the most pronounced clinical effects of hypo- and hyper-thyroidism. Most of the effects of thyroid hormones are now considered to occur through the actions of nuclear receptors that cause alterations in gene expression. The thyroid stimulation of energy production also leads to increased heat production which will ameliorate the cadets' cold tolerance (Jameson and deGroot 1995). During the ranger course there is an increase in oxygen consumption both at rest and during work in spite of the decreased levels of thyroid hormones (Bahr et al 1991).

All plasma thyroxin (T4) derives from thyroid secretion, whereas only 5-10 % of T3 and 1-3 % of rT3 derive from thyroid secretion. The rest originates from peripheral conversion of T4, mainly in the liver. Only 0.02 % of T4 and 0.3 % of T3 circulate in the free form, the rest is bound to plasma proteins such as thyroxin binding globulin (TBG), thyroxin binding prealbumin (TBPA) and albumin. Nuclear receptor saturation of 75 % in the brain and pituitary and 50 % in liver and kidney in spite of a plasma concentration of $2x10^{-11}$ M for T4 and $6x10^{-12}$ M for T3 versus a dissociation constant for the thyroid receptor of $2x10^{-9}$ M for T4 and $2x10^{-10}$ M for T3 indicates active transport mechanisms across plasma membranes. This is supported by the fact that for instance the concentration of T3 is 50 times greater in the erythrocytes than in plasma (Osty et al 1990, Nagashima et al 1993, Jameson and deGroot 1995).

Clinical observation of the cadets during the ranger training course showed that all had symptoms of hypothyroidism, since they shivered, were easily freezing, had slow motions and were also mentally slower than normal. The thyroid studies performed showed a decrease in thyroid hormones corresponding to the half life of T4 (Aakvaag et al 1978a, b, Opstad and Aakvaag 1981, 1983). An initial increase during the first day (12 hours) of activities was due to exercise, whereas the following decrease corresponding to the half-life of T4 was due to energy deficiency. The plasma concentration of rT3 also increased during the first day of activities due to exercise, but continued to increase during the course due to energy deficiency. This finding is well in accordance with the decreased plasma levels of thyroid hormones during fasting or starvation (Palmblad et al 1977, Jung et al 1980). There were no corresponding alterations in the plasma levels of thyroid stimulating hormone (TSH) during the course (Fig 11). Surprisingly the cadets that were allowed 3 hours of sleep each night showed the strongest decrease in TSH, whereas energy deficiency, which caused the difference in thyroid hormones, caused only moderately higher TSH levels. This is well in accordance with later published data on the inhibitory effect of sleep on TSH (Opstad et al 1984, Parker et al 1987).

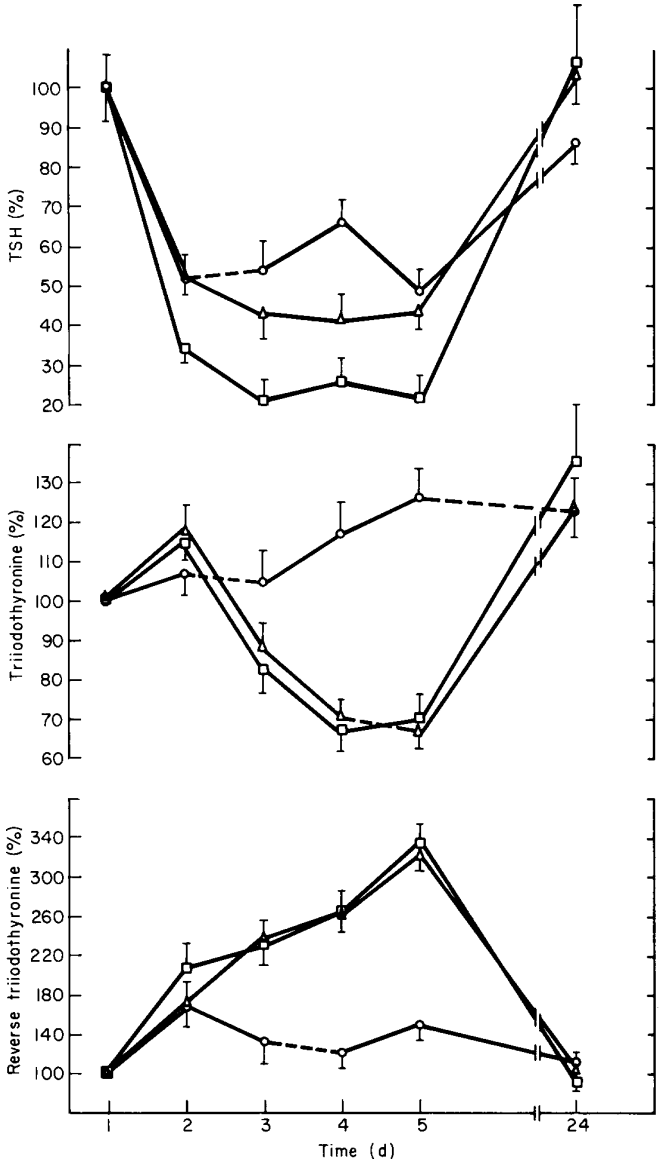

Fig. 10. The percentage changes in the serum concentrations of TSH, T3 and rT3 during a 5 d ranger training course with heavy physical activities, energy deficiency and sleep deprivation. The subjects are divided into 3 groups; Group 1 (n=9) (Δ) that was both sleep- and energy deprived, Group 2 (o) that wac compensated for the energy deficiency and Group 3 (□) that was allowed 3 hours of sleep each night. The day to day variation statistically significant at P<0.01 is shown by a thick line. The levels are given as means and the vertical bars represent the standard error of the mean.

Most hormones show circadian rhythm. The long half-life of thyroid hormones will mask a possible circadian rhythm. In the present work no significant circadian rhythm was demonstrated for thyroid hormones in spite of the presence of a circadian rhythm for TSH. The circadian rhythm of TSH showed a maximum level at midnight before the other hormones and the lowest level in the afternoon. In addition to decreased TSH levels in plasma, prolonged continuous stress also gave an extinguished circadian rhythm, which was re-established after 4-5 days of recovery. In light of the present results one might believe that some deterioration of mental and physical performance might be due to alterations in thyroid hormones and that these alterations might be reversed by adequate food supply during the course (Pasquini and Adamo 1994). There are indications that the conversion of T4 to T3 in the liver is dependent on carbohydrate metabolism, and that optimal nutrition during prolonged physical strain must contain a certain critical amount of carbohydrates to maintain thyroid hormones at a sufficient level in order to preserve mental and physical performance, and in our climate preserve the soldiers' cold tolerance.

The cadets' hypothyroidism may contribute to the explanation of many of the alterations in both mental and physical function during the course, since hypothyroidism may lead to slowing of all movements and mental function, decreased alertness and vigilance, loss of ambitions and impaired memory. There may be cognitive impairment which may reach dementia. Hypothyroid patients often sleep longer than normal, may become anxious and depressed (myxedema madness) (Swanson et al 1981). Speech is slow, hesitant and hoarse, and physical movements are clumsy. Contraction and relaxation phases of reflexes are prolonged. Paresthesia, sensorimotor neuropathies, cerebellar dysfunction, ataxia, intention tremor and nystagmus may also appear but are reversible when thyroid hormone levels are normalized (Swanson et al 1981, Beghi et al 1989, Osterweil et al 1992, Utiger 1995). Myalgia, muscle cramps, muscle stiffness, weakness and increased fatigability are common and pseudohypertrophy and pseudomyotonia of the muscles may develop with increased plasma levels of serum creatin kinase, lactate dehydrogenase and aminotransferase. Muscle fiber enlargements with oedema, loss of striation and sarcoplasmic degeneration, arthralgia, and joint stiffness due to synovial thickening are also described (Khaleeli et al 1983, Utiger 1995). The decreased thyroid function during the course may also contribute to impaired heart and lung function and to the gastrointestinal symptoms in the form of nausea, vomiting, decreased intestinal motility with constipation and abdominal distension (Ladenson et al 1992, Utiger 1995). The hypothyroidism may also contribute to the decreased haemoglobin levels during the course (Lindemann et al 1978, Tachman and Guthrie 1984) and may cause increased bleeding time, decrease in clotting factors and abnormal platelet function (Rogers et al 1982). The overall morbidity or mortality is not increased in hypothyroid patients, although some postoperative complications are more frequent, such as hypotension, cardiac failure, gastrointestinal dysfunction, and drug clearance is prolonged (Weinberg et al 1983, Ladenson et al 1984, Drucker and Burrow 1985). The decreased thyroid function during the course may also contribute to the decreased set-point temperature during the course.

INSULIN AND GLUCOSE METABOLISM

Glucose homeostasis is important for both human mental and physical performance. Plasma glucose is therefore strongly regulated by a variety of hormones. Insulin is, however, the only hormone able to reduce plasma glucose concentration, whereas a multitude of hormones may increase plasma glucose levels. These hormones are called the counterregulatory hormones and are the catecholamines, glucagon, human growth hormone, glucocorticoids and peptides such as VIP (for review see Kahn and White1995, Polonsky and O'Meara 1995). Plasma glucose is regulated both by a direct effect of glucose and its metabolites in the β-cells of the pancreas and via its influence on the hypothalamic structure via the autonomic nervous system. The neurotransmitters influencing the β-cells of the pancreas are acetylcholine, noradrenaline, GABA, and different peptides such as somatostatin, VIP, etc. Also circulatory catecholamines may influence insulin secretion from the pancreas since the β-receptors stimulate and α-receptors inhibit insulin secretion from the β-cells (Keiser 1995). At high catecholamine concentrations the α-receptor dominates over the β-receptor and this might be one of the factors explaining the decrease of plasma insulin during exercise. During a bicycle exercise test, plasma glucose was shown to increase, whereas a decrease was seen during the same exercise test during the ranger course (Opstad et al 1980, Rognum et al 1981, Opstad 1987). This is probably explained

by the depleted glycogen depots and that the glyconeogenesis is too slow to compensate for the absence of muscle and liver glycogen during the course.

An impaired glucose tolerance was also observed during the course mainly due to the physical strain, whereas extra sleep or extra food did not reverse the impaired glucose tolerance (Fonnum and Opstad 1983, Opstad unpublished). The mechanism for this decreased glucose tolerance is a combination of lower insulin responses in combination with peripheral insulin resistance. The insulin response to glucose is normalized within 3-5 hours, whereas the peripheral insulin resistance subsides well beyond this time. The decreased insulin response to glucose is not due to adrenergic inhibition of the insulin secretion since α-blockers did not reverse the decrease in insulin secretion (Opstad unpublished). The practical consequences of these findings is that the cadets during or after the course are advised to eat small meals particularly as carbohydrates are concerned.

PITUITARY HORMONES

Human Growth Hormone (hGH) stimulates protein synthesis as well as energy mobilisation. Energy mobilisation favours lipolysis, whereas the uptake of glucose in working muscles is inhibited by hGH, probably by decresing the insulin sensitivity. This effect of hGH is increased during starvation (Møller et al 1993). This also leads to an increased insulin response to glucose ingestion and impaired glucose tolerance. These changes may, however, also be mediated by the increased levels of plasma free fatty acids stimulated by hGH. Increased hGH levels may therefore contribute to the glucose intolerance observed during the course. The rapid metabolic actions of hGH, such as lipolysis, promotion of glucose and amino acid transport across membranes, are probably mediated trough the hGH receptor directly. In contrast, many of the growth promoting actions of hGH are mediated through the intermediate action of insulin growth factor 1 which is mainly synthesised in the liver (Vanderschueren-Lodeweyckx 1993, Weltman et al 1994, Daughaday 1995). HGH release from the liver is balanced between the stimulation by growth hormone releasing hormone (secreted from the nucleus arcuatus) and the inhibition by somatostatin (secreted from the paraventricular nucleus/ proptic nucleus) both released from the hypothalamus and transported to the pituitary gland by the portal circulation. Many stimuli of hGH secretion such as exercise, hypoglycaemia, proteins (arginine) and l-Dopa act through an α-adrenergic mechanism and are inhibited by α-adrenergic receptor blockers such as phentolamine, and are potentiated by the β-adrenergic blocker propranolol. Other neurotransmitters such as CCK, VIP, opioid peptides, γ-aminobutyric acid and acetylcholine, may also modify hGH secretion.

However, most of these transmitters seem to act via somatostatin and growth hormone releasing factor rather than directly on the somatotroph cells (Reichlin 1992, Parks et al 1993, Wass and Besser 1995). HGH is shown to increase during exercise already at low intensities and reaches its maximal response at 70 % of maximal oxygen uptake (Galbo et al 1977, Luger et al 1992). The release of hGH is increased during slow wave sleep, particularly in the beginning of the night, and is inhibited by sleep deprivation (Parker et al 1979, Radomski et al 1992, Pietrowsky et al 1994). Growth hormone releasing hormone promotes sleep in animals (Kerkhofs et al 1993, Krueger and Obal 1993). Whether growth hormone has any sleep promoting effect in humans is, however, debated (Mendelson et al 1980, Kern et al 1993).

The dramatic increase seen in the plasma levels of growth hormone during the ranger course, is reversed in the subjects given a high calorie diet. In contrast, 3 hours of sleep each night during the course did not influence plasma levels of hGH. However, the plasma levels of hGH were increased if the blood samples were drawn just after the sleep period (Aakvaag et al 1978, Opstad and Aakvaag 1981, 1983, Opstad 1991). The absolute hGH response to the bicycle exercise test after an overnight fast was not increased above the enhanced pre-exercise levels during the course (Opstad et al 1980). The high calorie diet prevented the hGH increase during the exercise test during the course. In the control experiment the exercise test was performed after 8 -12 hours of fasting, whereas during the course the subjects of the high energy group could not be kept fasting for more than 4 hours before the exercise test . This shows that nutrients ingested in the hours preceding the exercise test may abolish the hGH response to an exercise test even during constant strenuous exercise.

Prolactin is one of the most versatile hormones, and its membrane receptors have a wide distribution to very different tissues such as mammary gland, liver, kidney, adrenals, ovaries, uterus, placenta, testis, prostate,

seminal vesicles, Leydig cells, hypothalamus, choroid plexus, pancreatic islets, lymphoid tissue, peripheral mononuclear cells, brain, intestine and others. Many tissues are known targets for PRL action, others are not.

Comparative studies indicate that osmoregulation and modulation of growth and development may be the most fundamental actions of prolactin. In humans, functions of PRL are still incompletely delineated but seem mainly to be involved in reproductive functions. PRL increases or maintains the concentration of LH receptors on the Leydig cell membrane, thus increasing the sensitivity of the testis to LH and by that enhancing plasma testosterone levels (Aragona et al 1977). PRL also potentiates the effects of androgens on the growth and secretory activity of male accessory glands and may even have direct androgenic effects. PRL in the seminal fluid also stimulates glucose and fructose utilization and the sperm motility and fertilizing capacity (for reviws see Reichlin 1992).

The pituitary secretion of PRL is under the control of hypothalamic release- and inhibiting factors such as the thyroid-releasing hormone, dopamine, glucocorticoids, oestrogen and epidermal growth factor. The circadian rhythm of PRL, with the highest levels in the early morning hours, may be due to the effect of both sleep and the circadian rhythm of melatonin (Spiegel et al 1994). This nocturnal increase is due to increased amplitude of the secretion pulses which have a frequency of approximately 14 per 24 h (Veldhuis and Johnson 1988). PRL has been shown to increase during different types of stress such as general anaesthesia (halotan), surgery, insulin-induced hypoglycemia and medication with a dopamine blocking effect, such as haloperidol or metoclopramid (Røjdmark and Røssner 1991, Wass and Besser 1995).

During the ranger training course there is a decrease in the plasma levels of PRL which may contribute to the decrease in testicular androgen secretion and which may also contribute to the hypoandrogenisation during the course by more direct ways. The relatively high levels of PRL before the start of the course may indicate that PRL is sensitive to the cadets' anxious anticipation before a strenuous course (Aakvaag et al 1978, Opstad and Aakvaag 1983, Voigt et al 1990, Opstad 1991, 1992). This is in spite of the fact that academic examination stress did not affect the plasma levels of PRL or hGH (Malarkey et al 1991). No alterations were found during the submaximal exercise test at 50 % of the cadets' maximal oxygen uptake before or during the course. In contrast, others have shown that exercise induces a rise in the plasma levels of PRL (Galbo et al 1977). The explanation is probably that, in contrast to the plasma levels of hGH which increase also at low exercise intensity, PRL increases only during high intensity exercise, which is 70 % of VO_2 max or more (Luger et al 1992). Since the exercise intensity during the course has a mean of approximately 35 % of VO_2 max, and the bicycle exercise test was 50 % of VO_2 max, this was below the intensity threshold for PRL release during exercise. It is, however, interesting to notice that even the strenuous physical activities during the course did not affect this threshold for PRL release. The small alterations found in the prolactin response to TRH during the course, might indicate that decreased plasma levels of PRL were due to dopamine inhibition, since dopamine is known to inhibit synthesis as well as secretion of PRL.

The adrenals, the gonades and the thyroid gland are regulated by the hypophyseal hormones ACTH, LH/FSH and TSH respectively. LH/FSH decreased during the course and is a main reason for the decreased plasma levels of gonadal androgens. The increased LH/FSH responses to GnRH stimulation during the course may indicate that the decreased gonadotropin levels are due to decreased hypothalamic secretion of GnRH although the decreased levels of androgenic hormones may also contribute to an increased LH/FSH response to GnRH stimulation. GnRH which is detected in septum, preoptic area, amygdala, and midbrain has its highest concentration in the mediance eminence, nucleus arcuatus, and organum vasculosum of the lamina terminalis. Secretion of GnRH is inhibited by dopamine and GABA and stimulated by α-adrenergic agonists, histamine and glutamate. Besides olfactory and visual inputs, GnRH secretion is also influenced by the pineal body and the nucleus suprachiasmaticus. The feedback of gonadal steroids and inhibin both act at the hypothalamic secretion of GnRH and at the pituitary sensitivity to GnRH stimulation (Reichlin 1992, Hall and Crowley 1995, Lincoln 1995, Opstad 1990, Opstad et al 1994).

Thyroid hormones are mainly regulated by pitutary TSH which is stimulated by TRH produced in the paraventricular nucleus of the hypothalamus, inhibited by somatostatin produced approximately in the same area, the periventricular area, and by dopamine from the nucleus arcuatus. TRH is also influenced by the thermosensitive cells in the supraoptic nucleus, is stimulated by β-adrenergic agonists and may be inhibited by

serotonin. The negative feedback of thyroid hormones acts both at the hypothalamic and at the pituitary level (for review see Reichlin 1992, Scanlon and Hall 1995, Wondisford et al 1995)

The decreased TSH levels during the course would obviously contribute to the decreased thyroid secretion in spite of the fact that there was no direct connection between alterations in thyroid hormones and the decrease in TSH. The TSH response to TRH was reduced by 80 % during the course equally due to the strenuous physical exercise and energy deficiency, whereas sleep had minor significance (Opstad et al 1984).

Adrenal steroids are all stimulated by pituitary ACTH, which is under the control mainly of the hypothalamic CRH, but is also stimulated by AVP (vasopressin). CRH has also been localized to multiple brain areas, the spine and the gastointestinal tract but with the highest concentration in the hypothalamus, particularly the paraventricular nucleus. The CRH neurones receive excitatory inputs from many brain areas such as nucleus suprachiasmaticus, amygdala and the raphe nuclei and inhibitory inputs from the hippocampus and the locus coeruleus. CRH secretion/release is stimulated both by acethylcholine, serotonin, and interleukin 1 and inhibited by GABA and nor-adrenaline. The negative feedback regulation by glucorticoids may act at the pituitary as well as at the hypothalamic level, but there are also glucocorticoid receptors in various other parts of the brain such as in the amygdala and the hippocampus (for review see Reichelin 1992, Grossman 1995, Imura 1995).

The decreased ACTH levels measured during the training course could be due to the state of recovery just prior to the blood sampling since the subjects had only light physical activities 1-2 hours prior to blood sampling. The short half-life of ACTH compared to the long half-life for cortisol could explain the decreased ACTH levels during recovery (Opstad 1992a,b).

MENTAL PERFORMANCE AND CLINICAL SYMPTOMS

All mental performance and clinical symptoms have a biochemical or physiological basis, the disturbance of which will affect the soldiers' total performance. Sleep deprivation affects a series of mental functions, but does not affect the physical performance to any significant extent (Hill et al 1994, Reilly and Piercy 1994): First all subjective symptoms, such as the subjects' mood state, social well-being, ability to care for others, feeling of depression, and motivation will be affected. Then the most advanced mental performance tasks will be affected such as creativity, ability to solve complex mental performance tasks, or tasks requiring memorization and tactical abilities. With prolonged sleep deprivation the body's requirement for sleep increases so strongly that it becomes impossible to withstand sleep, and subjects will fall asleep even in the upright position. First, however, this will appear during the night-time, particularly in a period with rather low physical activities, in the form of extreme tiredness followed by balance disturbance, problems with straight walking along the roads, later with pseudo- or real illusions and hallucinations. In the beginning of the course the subjects take the hallucinations for real signs, whereas at the end of the course when they have got more used to them, they most often take all unexpected events as hallucinations. This might be a serious impairment of their function because they are not able to differentiate between real and not real signs and will be completely unfit for watchkeeping or surveillance tasks. These periods with intense feeling of sleepiness induce what has been called "microsleep" or lapses (Walter Reed Laps Hypothesis). The periods of microsleep increase in frequency and length almost proportionally to the length of sleep deprivation. The length might be from some seconds to several minutes and the frequency from some times a day to several times each hour, but still depending on the length of sleep deprivation and time of day (Johnson and Naitoh 1974, Opstad et al 1978, Haslam 1983, 1984, Angus et al 1987). During these periods the vigilance is so reduced that the subjects do not record even distinct signals in the environment. In the so called vigilance tests this gives error of omissions. In contrast, there are not many faults or wrong responses. The reaction time also increases and consequently all tasks therefore take more time than normal. On the third night of total sleep deprivation, the more serious signs appear, such as slow motions, balance disturbance, nystagmus, fog sight, disturbed distance vision, headache, visual hypnagogic hallucinations and physical exhaustion. In this state the soldiers are helpless and unable to manage their own situation. In stead of being a resource for the platoon, they become a problem. In such a state it is often observed that the cadets only disappear from the platoon or just sit/lay down and may fall into a state of sleep narcosis. They may be extremely hard to wake up. Due to

the internal sleep rhythm of approximately 90 minutes they may be much easier to wake up after only a few minutes. However, a sleep deprived person is possible to wake up for some seconds and then he will normally be oriented for time and site. This will make the difference to unconsciousness due to commotio cerebri which will be impossible to wake up by external stimulation.

Mental performance has a circadian rhythm with the highest performance in the afternoon and the lowest performance at night (±10-15%) (Bugge et al 1979, Czeisler et al 1980, Nickolson and Stone 1982, Folkard et al 1985). During the course there is a general decrease in mental performance; in addition the decrease is stronger during night-time than during day time leading to an increased amplitude of mental performance. This is not only due to the darkness of the night but also to endogenous circadian rhythms, since the same alterations are found in the courses organized in June, when the nights are rather light. All clinical symptoms are also more pronounced at night than at day-time. Almost all illusions, misperceptions, hallucinations, balance disturbances, and coordination problems are worse at night than during the day.

In contrast to the alterations in the circadian rhythm for mental performance which show increased amplitude during the course, the circadian rhythm of hormones are all extinguished during the course. The circadian rhythms are regulated from the nucleus suprachiasmaticus in the anterior hypothalamus (Van den Pol and Powley 1979, Rietveld 1992). This center is thought to regulate most of the known circadian rhythms. Previously it has been shown by Folkard (1985) that the period of the circadian rhythm for the feeling of alertness or drowsiness and deep body temperature dissociated when the period was shortened by 0.2 hours each day down to a “day” of 23 hours and then by 0.1 hour until a “day” of 22 hours which was run for the rest of the study. During the present military training course, it is shown for the first time that during continuous operations, a dissociation may appear between the amplitude of the circadian rhythm for mental performance which is increased, and the amplitude of circadian rhythm for steroid hormones which is extinguished. This indicates that these two rhythms are regulated by different mechanisms in the brain Opstad 1994.

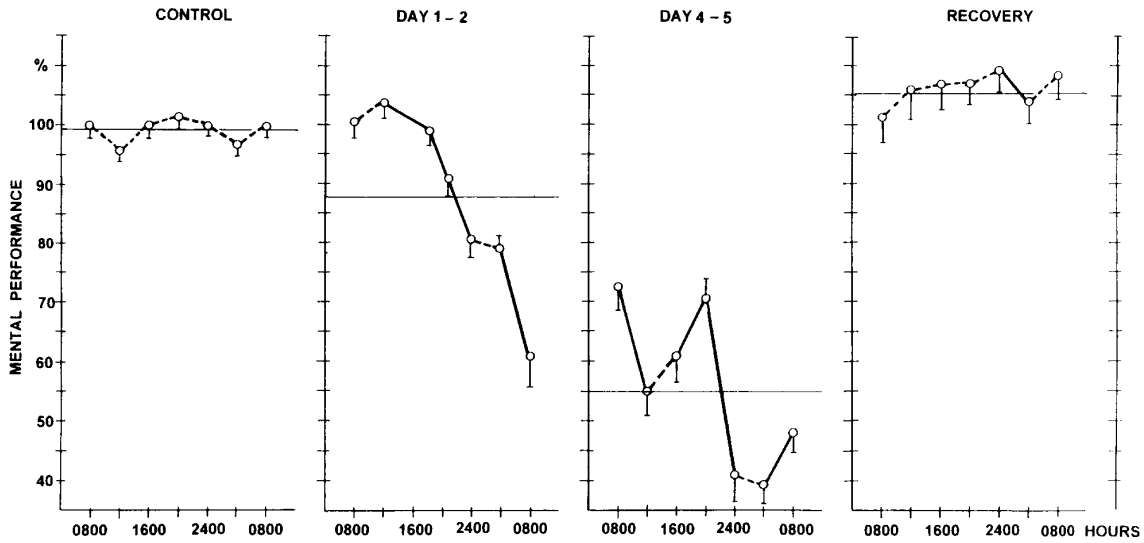

Fig. 11. The circadian rhythm for mental performance expressed as the mean of two mental performance tests; the code test and the logical reasoning test. The results are presented as per cent of the first test results (control at 08.00h) ± SEM. For details, see Fig. 8.

CONCLUSIONS

The present investigation shows rather large endocrine and metabolic alterations during a 5 day military training course with continuous physical activities combined with sleep and energy deficiency which might contribute to explain the accompanying alterations in both mental and physical performance. The main stress factors such as physical strain, lack of food and sleep all lead to an extreme catabolic metabolism with

similarities to the physiological state of the multitraumatized patients. One main finding is an adrenergic desensitization due to the physical strain, which is explained both by a decreased number and sensitivity of the adrenergic receptors and a decreased cAMP response to adrenaline stimulation. These alterations indicate that an important mechanism for reduced physical performance or exhaustion is target tissue desensitization.

The decreased levels of adrenal and testicular androgens and thyroid hormones may also contribute to alterations in both mental and physical performance. In addition the decreased thyroid hormones during the course may affect the cadets' cold tolerance. There is a dissociation between the circadian rhythm for mental performance, which is increased, and the circadian rhythms for steroid hormones, which are extinguished. This indicates that night activities are particularly strenuous and demanding for the cadets.

Some simple precautions or countermeasures, such as adjustment of the type and amount of food and sleep, may reduce the large impairment of the soldiers' mental and physical performance and prevent unnecessary health hazards.

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The Phagocyte Function during Multifactorial Military Stress, and Neuroendocrine Interactions with Phagocytes

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SUMMARY

The huminal-amplified chemiluminescence response of granulocytes to serum opsonized zymosan particles *ex vivo*, was investigated during a ranger training course lasting for 7 days with continuous strenuous physical activities, calorie and sleep deprivation. A priming for accentuated chemiluminescence response was observed during the first days the course with a maximum increase on Day 3 (+ 35 % of control levels). Thereafter, a reduction to below control values was observed, minimum value was observed on Day 7 (-28 %). One group (N = 8) receiving 6000 kJ/24 h of energy, showed a more pronounced priming during the first days compared to the other group receiving an average of 1000 kJ/24 h (maximum +57 % versus +21 %), and less reduction of the chemiluminescence compared to control on the following days. These data indicate that extreme physical activity for up to a few days primes the production of reactive oxygen species in granulocytes, while activity for a longer time results in a dowmmodulation.

INTRODUCTION

The neutrophil granulocytes provide the first line of defence against invading micro-organisms but activated granulocytes may also contribute to the damage of normal tissue during inflammation. Granulocytes constitute more than 50% of the leucocytes in peripheral blood, and exhibit a high degree of turn-over with a half life of less than 24 hours. Upon infection or inflammation, granulocytes react with chemotaxis towards e.g. formylated bacterial peptides and complement fragments, and particulate stimuli may be phagocytosed after adhesion to granulocyte surface receptors for complement fragments and for the Fc-portion of immunoglobulins. After phagocytosis, killing and digestion of the ingested material are provided for by production of reactive oxygen molecules and release of protolytic enzymes into the phagolysosome particle. Reactive oxygen molecules are produced by reduction of oxygen by a membrane bound enzyme (NADPH-oxydase) giving oxygen anion radical as the primary product, and other reactive oxygen molecules and oxygen radicals (like hydrogen peroxide, hydroxyl radical, hypoclorite and cloramines) are formed in spontaneous or enzymatic processes (Babior 1984).

It is often assumed that moderate physical activity stimulates the immune system, while more extreme exercise in terms of intensity is associated with immunosuppression (Fitzgerald 1988, Sharp and Koutedakis 1992). Exercise is known to strongly increase the number of granulocytes in peripheral blood due to the effects of catecholamines and cortisol (Loeper and Crouzon 1904, Bishop et al. 1968, Weicker and Werle 1991, Hack et al. 1992, Gabriel et al. 1992). For granulocyte function, however, quite variable effects of exercise have been reported. In one study, granulocytes from top athletes were less adherent, and exercise to exhaustion reduced the adherence and bactericidal capacity significantly more than in controls (Lewicki et al. 1987). In another study, no difference between athletes and controls were observed in phagocytic capacity and production of reactive oxygen species (Hack et al. 1992). In that study, a small reduction in the production of reactive oxygen species was observed immediately after exhaustive exercise. However, after 24 h of recovery, a significant increase both in the production of oxygen species and in the phagocytic capacity was observed (Hack et al. 1992). Intense (interval) running for up to several hours has been shown to result in post-exercise activation of granulocytes as indicated by degranulation and increased Fc- and C3bi-receptor numbers

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

(Gray et al. 1993) confirming the findings of Kokot et al. (1988) who found degranulation and reduced chemiluminescence to the phorbol ester PMA after (intense) 10 000 m running.

Little information is available about how granulocyte function is changed during continuous physical activity lasting for more than 2-3 hours. We therefore wanted to study the granulocyte function during a ranger training course lasting for 7 days with continuous, moderate exercise.

SUBJECTS AND METHODS

Subjects and blood sampling. As a part of their training program, the cadets of the Norwegian Military Academy take part in a ranger training course lasting for 7 days. During this course, the cadets are exposed to continuous physical activity corresponding to about 35% of their maximum oxygen uptake (VO_{2max}) around the clock or an energy consumption about 35 000 kJ/24 h. Cadets received no calories on Days 1-2, about 3000 kJ on Day 3, and about 4000 kJ on Day 4, no food on Day 5, 400 kJ on Day 6 and no calories on Day 7. The cadets were allowed no organized sleep during the course, but got short periods of sleep between activities estimated to a total of about 3 h.

Sixteen cadets were randomly selected to participate in the scientific part, and all volunteered. All cadets were males between 20-30 years of age, all exercised regularly, were in good health and used no regular medication. Each day, at 08:00 a.m., venous blood was drawn with EDTA an anticoagulant. The participants were randomly selected into two groups. Group 1 (N = 8) got no extra food, while Group 2 (N = 8) received additional 5000 kJ/24 h of energy throughout the course.

Separation of granulocytes from venous blood. EDTA-blood was mixed with 10% (v/v) of 6% (w/v) dextran in 0.9% (w/v) NaCl. After sedimentation, the leucocyte-rich plasma was layered onto Lymphoprep^R (Nycomed, Oslo, Norway) and centrifuged (15 min, 600 x g) to obtain suspensions of granulocytes (Bøyum 1968). The granulocyte suspension was incubated with 0.83 % (w/v) NH_3Cl to induce hypotonic lysis of the contaminating erythrocytes and was then washed twice in 0.9 % NaCl (400 x g, 7 min, 4^{o}C). Then, granulocytes were suspended in Earle's Balanced Salt Solution (EBSS) without phenol red and supplemented with 50 mmol/l HEPES.

Opsonization of zymosan and the chemiluminescence assay. The procedure was carried out as previously published with small modifications (Wiik 1989). Serum opsonized zymosan was produced by incubation (37 ^{o}C, 30 min) of 1 ml of 20 mg/ml zymosan with 5 ml of mixed serum from 6 healthy donors. The opsonized zymosan was then washed twice in sterile water and diluted (2.5 mg/ml) in EBSS.

Opsonized zymosan (0.5 mg/ml) was used to activate the granulocytes (0.5 x 10^6 cells) and 0.1 mmol/l luminol (5-amino-2,3-dihydro-1,4-phthalazindione; Sigma, St. Louis, MO, USA) was added to a total volume of 0.25 ml. Measurement was done in triplicate, and each sample was measured every 5 minutes for 25 minutes. All buffers and reagents were from the same batch, and care was taken to keep all procedures constant on each day during the course.

Luminol is converted to an excited amonophthalate ion in the presence of oxidizing compounds, and this reaction emits blue light which was measured at 425 nm in a LKB-Wallac1251 luminometer. The chemical basis of the chemiluminescence reaction is not known in every detail, but superoxide anion and the myeloperoxidase product hypoclorite (HOCl) are necessary for generating luminol amplified chemiluminescence (Dahlgren et al. 1991).

Cortisol. Plasma cortisol was measured with radioimmunoassay (RIA) kits from ImmunoDiagnostic Systems Limited, Bolden, UK.

Calculations and statistics. Results are presented as mean (with SD) of N determinations. For analysis of the chemiluminescence data, maximum control value for each person was set to 100%, or given as absolute numbers. Statistical analyses were performed with SPSS^R software program. The statistical significance of

differences was tested by analysis of variance (repeated measures design) with *Day* as the repeated within subject factor. For the chemiluminescence also another within subject factor (*kin*) was included in the model to represent the activity at the five different time points after activation. For testing of *group effects*, control values were included as covariates. Correlations were calculated by the Pearsons product-moment formula.

RESULTS

The numbers of peripheral blood leucocytes and granulocytes during the ranger training course are shown in Fig. 1. An overall increase in the total number of leucocytes ($p < 0.001$) as well as granulocytes ($p < 0.001$) was observed, the maximum increase from control (C1) was observed on Day 1. After this initial maximum, a decrease was observed, but higher leucocyte(s) counts than control levels were observed throughout the course.

An overall significant effect of additional food supplement (Group 2) was observed in the leucocyte and granulocyte counts in peripheral blood during the course (*group x day* interaction; $p \leq 0.05$) (Fig. 1). The strong increase to Day 1 was similar in the two groups, while Group 2 had somewhat lower leucocyte counts during the rest of the course.

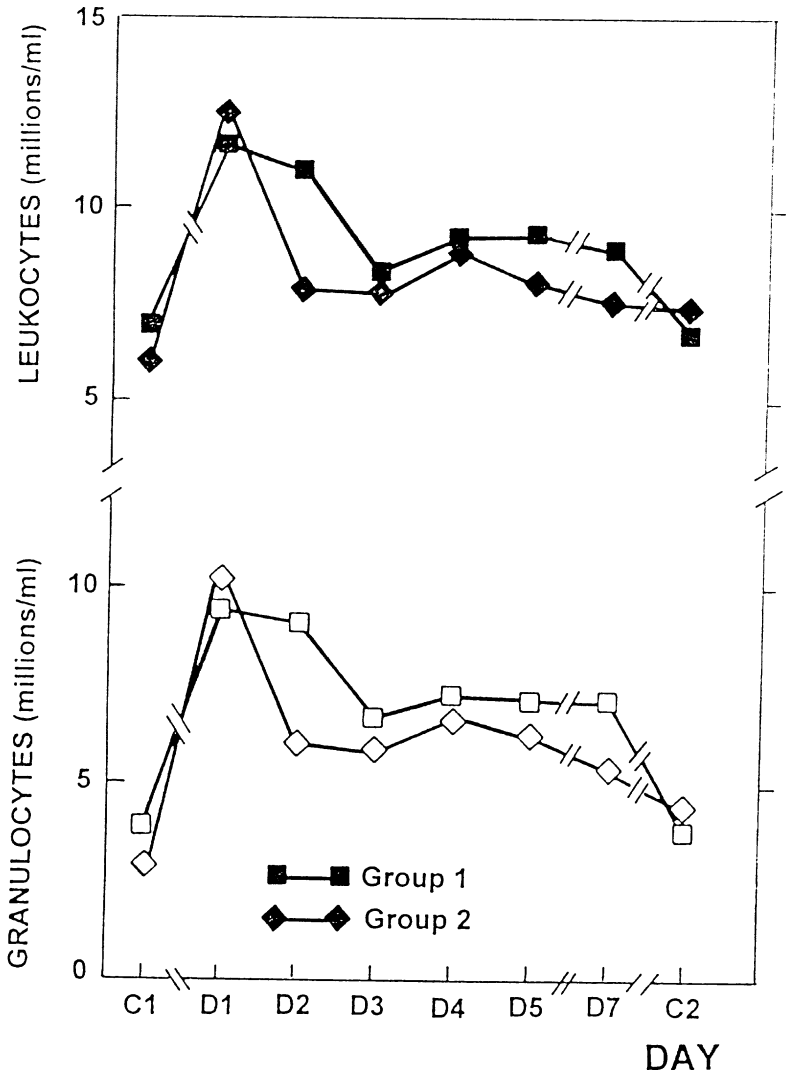

Fig. 1. The total numbers of peripheral blood leucocytes (upper) and granulocytes (lower) are shown in the control situation (C1 and C2) and on different days (D1 to D7) during the ranger training course. Subjects were exposed to continuous exercise around the clock, with energy expenditure about 35 000 kJ/24. Group 1 (N = 8) received an average of 1000 kJ/24 h of energy while Group 2 (N = 8) received 6000 kJ/24.

The chemiluminescence response to serum opsonized zymosan on different days is shown in Fig. 2. There was an overall difference between days ($p < 0.001$). During the first days an increase was observed and the maximum chemiluminescence response was on Day 3 ($p = 0.044$).

The effect food supplement on granulocyte chemiluminescence during the ranger training course is shown in Fig. 2. There was an overall significant effect of additional food supplement on granulocyte chemiluminescence (*group x day x kin* interaction, $p = 0.006$). In Group 2 receiving 1000 kJ/24 h, the initial increase was larger (Day 3, $p = 0.047$), and, although not significant, the reduction seemed to be somewhat less pronounced on Days 4-5 for Group 2 while no difference between the Groups was observed on Day 7.

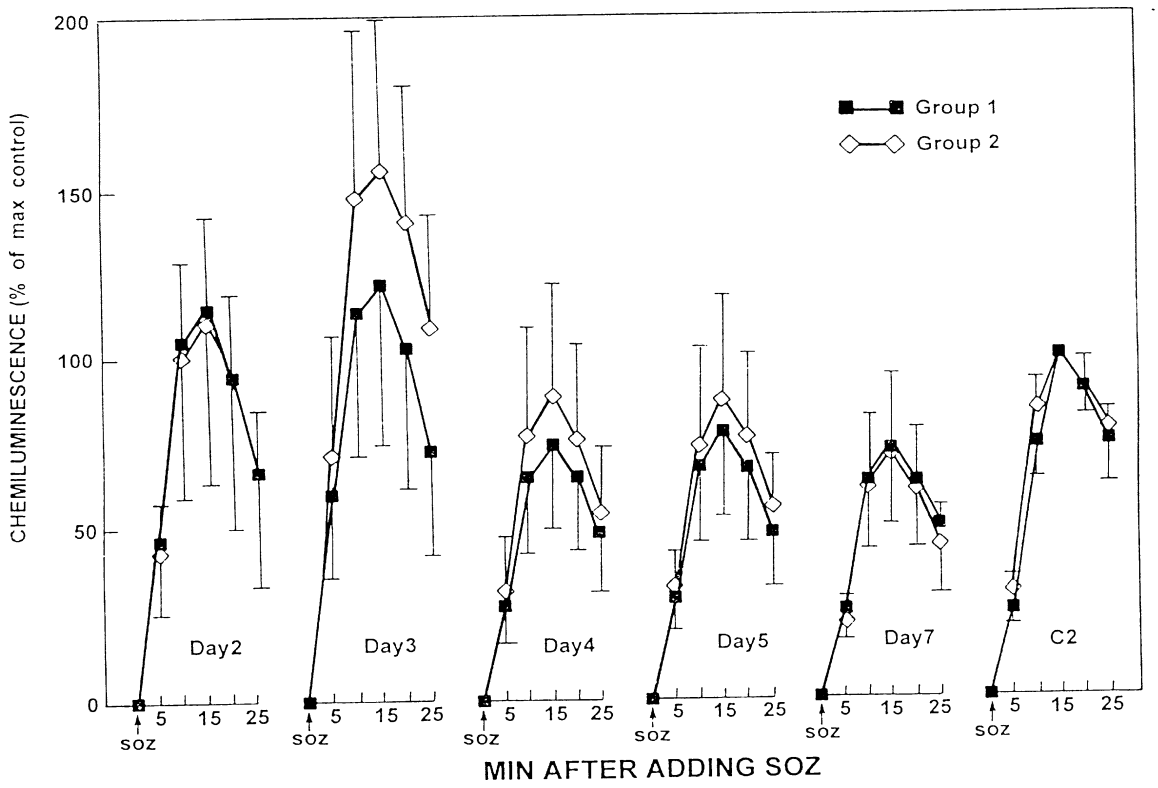

Fig. 2. The luminol-enhanced granulocyte chemiluminescence response (with SD) to serum opsonized zymosan (SOZ) at different days during. Groups as in Fig. 1. Results are expressed as per cent (with SD, N = 8) of max control (C2) value.

Serum cortisol concentrations are shown in Fig 3. Cortisol increased significantly ($p < 0.001$) to a maximum (800 nmol/l) on Day 1 and then submaximal levels (700-800 nmol/l) were observed until Day 6 when a drop to normal values was observed.

The effect of food supplement on serum cortisol levels throughout Course A is shown in Fig. 3. There was an overall difference between the groups (*group x day interaction*, $p < 0.001$). For Group 1 (which did not receive additional food), high serum cortisol levels were observed on Days 2-6 (730-800 nmol/l) with a maximum on Day 4 (800 nmol/l). In contrast, the cadets receiving extra calorie supplement (Group 2) showed a stronger and earlier increase from control with a maximum on Day 1 (920 nmol/l), then moderate values on Days 2-3 (740 and 790 nmol/l), and normal plasma cortisol levels on Days 4-7.

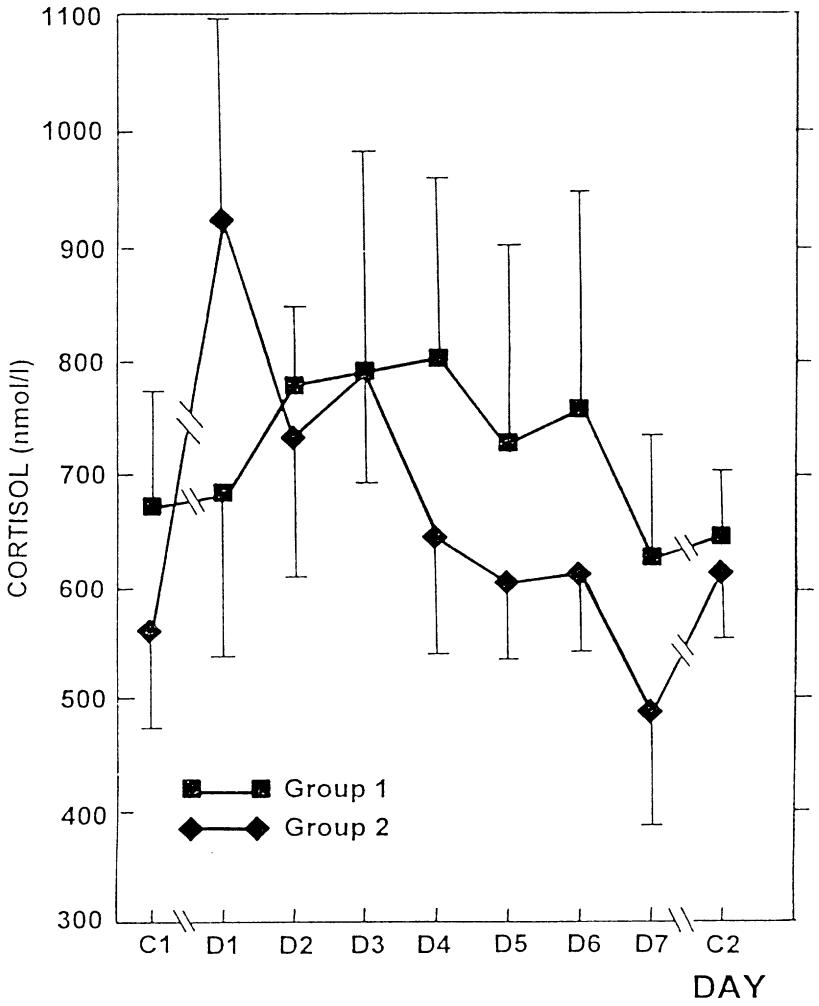

Fig. 3. Plasma concentration of cortisol (with SD) in the control situation (C1 and C2) and at different days during the ranger training course. Groups as in Fig. 1.

Correlation of cortisol with granulocyte numbers and chemiluminescence is shown in Table 1. There was a significant negative correlation between serum levels of cortisol and chemiluminescence in the control situation as well as during the training course. However, the *change* from control to the different days did not correlate for cortisol and chemiluminescence (data not shown). For serum cortisol and the number of granulocytes, a significant positive correlation was observed on Day 1 (Table 1) when the granulocyte number was maximal, but not on the other days (data not shown). The *change* from control to the different days correlated for Day 1 (r=0.49, p=0.05), not for the other days (data not shown).

Table 1. Correlation of serum cortisol with SOZ-activated granulocyte chemiluminescence and with peripheral blood granulocyte numbers on different days during the course

	Day 2	Day 3	Day 4	C2
Chemiluminescence (15 min value)	–0.55**	–0.58**	–0.37	–0.63**
Granulocyte numbers	0.13	0.30	0.13	0.34

**p < 0.01

After the observed increase in chemiluminescence during the first days, a reduction to below control values was observed. Significantly lower values than control were found on Days 4, 5 and 7 ($p < 0.05$).

DISCUSSION

This study demonstrates a biphasic granulocyte response to exercise: During the first 1-3 days of continuous physical activity an *in vivo* priming of peripheral blood granulocytes was taking place, which is in accordance with e.g. Gray et al. 1993. When the physical activity continued for a longer time, a reduction to below control values was observed for the zymosan stimulated production of oxygen species. Furthermore, fasting during the first days (Group 1) was shown to result in lower priming of granulocytes compared to Group 2 with a small energy supply (1000 kJ/24 h).

Reduced granulocyte function may contribute to immune suppression, susceptibility to infections, and may also be part of an overtraining syndrome. On the other hand, the observed priming during the first days may be beneficial for the defence against e.g. bacterial infections, but activated granulocytes are also involved in tissue damage associated with a broad range of pathophysiological conditions. For the musculo-skeletal system this includes exercise-induced muscle-fiber injury, ischemia-reperfusion injury (e.g. joint during exercise) and inflammation (e.g. rheumatoid arthritis, tendinitis) (Babior 1984, Armstrong et al. 1991). However, it is unknown whether granulocytes from blood are representative for granulocytes from local inflammatory processes in the tissues.

The mechanism for the increased chemiluminescence during the first days is not known. However, several factors are known to prime granulocytes, e.g. endotoxin, interleukines, interferons and growth factors (Al-Mohanna and Hallet 1992). The concentration in plasma of several of these factors are changed during and after exercise (Sharp and Koutedakis 1992). During the ranger training course, we have measured a significant increase in the plasma concentrations of GM-CSF (3-4 fold), no change in interleukin 1 and interleukin 2, and a 10-20% reduction in interleukin 6 (Bøyum et al. 1993). These (and other) factors can be involved in the *in vivo* priming mechanism observed.

Cortisol is known to cause immunosuppression, and granulocytes were exposed to high levels of cortisol during several days of the ranger training course as demonstrated in this and previous reports (Opstad and Aakvaag 1981, 1983, Opstad 1991). An inhibitory effect of cortisol on the chemiluminescence is supported by the significant negative correlation observed for cortisol and chemiluminescence. However, other factors also seem to be important for the regulation of granulocyte function during stress, since *change* in cortisol (from control values) did not correlate with *change* in chemiluminescence on the different days.

During an extreme situation like the ranger training course, the subjects are also exposed to significant psychological stress which may as well affect the immune system (Khansari et al. 1990). Further studies are necessary in order to clarify the effect of psychological stress on the granulocyte function.

In summary, this study demonstrates a priming for accentuated production of reactive oxygen species of granulocytes during the first days of continuous physical activity, while physical activity for a longer period of time resulted in suppressed granulocyte function. However, the physiological implications of the presented data are difficult to outline since activation of granulocytes can be beneficial for the host defence against invading microorganisms, but may on the other hand increase the inflammatory damage to normal tissues like e.g. tendons, joints and muscle fibres during exercise.

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The Effect of Strenuous Exercise, Calorie Deficiency and Sleep Deprivation on White Blood Cells, Plasma Immunoglobulins and Cytokines

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ABSTRACT

Moderate exercise appears to stimulate the immune system, but there is good evidence that intense exercise can cause immune deficiency. In the present study we examined the effect of continuous physical exercise (~35 % of VO_2 max), calorie deficiency and sleep deprivation on the immune system of young men participating in a 5-7 days military training course.

There was a 2-3 fold increase of neutrophils from day 1, the values remained high and decreased slightly at the end of the course. Monocyte counts also increased with a pattern similar to that of neutrophils. Eosinophils decreased to 30 % of control and lymphocyte numbers decreased by 30-40%. All the major subgroups (CD4 T cells , CD8 T cells, B cells, NK cells) were reduced.

Neutrophil function, as tested by measuring chemotaxis, was significantly stimulated during the first days of the course, in particular in the group with the lowest calorie intake. The mitogenic response of lymphocytes to PHA and Con A was variable, ranging from stimulation during one course to no effect in another course.

Serum levels of immunoglobulins decreased significantly during the course. IgG was reduced by 6-7%, IgA by 10-20% and IgM by 20-35%.

We found no changes of interleukin 1 , 2 and 4 during the course, but a (12-20%) reduction ($p<0.01$) of interleukin 6, and an increase ($p<0.01$) of granulocyte-macrophage colony stimulating factor.

Altogether the results from the ranger course present a mixed-up picture. The non-specific phagocyte-related immunity was enhanced. On the other hand, our data indicate that even a moderate physical activity, around the clock, caused significant suppression of a number of parameters reflecting the status of the specific, lymphocyte-related immunity. Still, it is noteworthy that there was no significantly increased infection rate during the course or in the first 4-5 weeks thereafter.

INTRODUCTION

In recent years considerable interest has been directed to the effects of exercise on immune function. As demonstrated in animal experiments [1,2], moderate exercise appears to stimulate the immune system. However, several studies indicate that intense training increases susceptibility to illness. These illnesses range from persistent colds, sore throats to flu-like illnesses and post-viral fatigue syndrome [3]. Clearly the immune system may be a limiting factor in human performance. There are however, distinct individual variations.

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

Some athletes can withstand rigorous training without problems, others are very susceptible to colds and infections. The mechanisms for these effects are only partly known. Reduced levels of salivary IgA has been observed in well-trained Nordic skiers [4], and it has been speculated that this might reduce the resistance to infection. Following intensive exercise the in vitro response to T and B cell mitogens is variable [5], but mostly a suppression has been observed [6,7]. This could be due to increased levels of cortisol or catecholamines [8,9], both of which are generally immunosuppressive [10,11]. However, epinephrine may either suppress or stimulate the immune response, depending upon the experimental set-up [12]. Reduced bactericidal activity of neutrophils has also been observed, but otherwise the effect of exercise on neutrophil function is quite variable [13].

In the present work, published previously in Scandinavian Journal of Immunology [14], we have studied young army cadets before and during strenuous exercise lasting for 5-7 days, combined with sleep deprivation and calorie supply deficiency amounting to 35-40 000 kJ/24 h. The intent was to examine whether the severe strain of this ranger course leads to suppression of the immune system and thereby constitutes a health hazard.

MATERIALS AND METHODS

The ranger training course. Well-trained cadets of the Norwegian Military Academy participated in the courses, which lasted for 5-7 days, usually in june, july or august. The mean age of the cadets varied from 22 to 24 years (range 21 to 27), mean height 183 cm and mean weight 78 kg. During the course they slept for only 2-3 h and were exposed to continuous physical activity around the clock of about 35% of their VO_2 max with a calorie consumption of 35,000-40,000 kJ per 24 h. In general the daily intake of food represented less than 3000 kJ, which lead to a weight loss of 4-5 kg, mostly fat, in a 5 day period [9]. The intake of water was free. The results are from 8 different courses (87 cadets), each comprising 8 cadets or more. Most of the data are from 3 courses denoted I,II and III. In course II the cadets were split in group IIA and IIB, with average daily energy intake of 1000 kJ and 6000 kJ, respectively, which implies an energy deficiency of approximately 97 or 85 %. Otherwise they were treated similarly except that group IIB received 3 hours of extra sleep/rest on day 5. The cadets were under medical surveillance throughout the courses. During the courses (two), and 4-5 weeks thereafter, the cadets self-evaluated their health condition daily, based on a detailed standardized questionnaire.

Dextran 500 (Pharmacia) was dissolved in water and used as a 6% solution. **Lymphoprep** and **Metrizoate 32.8%** were provided by Nycomed (Oslo). The osmolality of Lymphoprep was increased from 300 to 320 mOsm/kg by adding 60 mg NaCl per 100 ml. Dextran-Metrizoate was made by mixing 10 parts Metrizoate with 25 parts dextran 6 % .

Blood (20-80 ml) was collected in vacutainers, with or without anticoagulant (EDTA), between 06.00 and 07.00 h in the morning. Plasma and serum were sampled from blood to which Trasylol 500 IU/m (Bayer, Leverkusen, Germany) had been added immediately. Leucocytes (EDTA-blood) were separated 5-6 h later and enumerated in an electronic counter. Erythrocytes and platelets were counted microscopically after appropriate dilution with 0.9% NaCl (erythrocytes) or 1% ammonium oxalate (platelets). Reticulocyte counting was done in smears made after staining with brilliant cresyl blue (0.25%).

Smears were made as follows: Two ml EDTA-blood was layered over 2.5 ml Dextran-Metrizoate. The leucocyte-rich plasma was collected when the red cells had sedimented to the bottom, centrifuged (5 min at 600 g), resuspended in a small volume and smears were made. The differential count of these smears corresponds to that in whole blood.

Cell separation. Equal parts of EDTA-blood and 0.9% Nacl were mixed and 30-35 ml of the mixture was layered over 12-15 ml of Lymphoprep and centrifuged for 17 minutes at 600 G. Mononuclear cells (MNC) were collected from the interface between plasma and Lymphoprep, and granulocytes from the bottom fraction [15]. Contaminating erythrocytes in the granulocyte suspension were removed by NH_4Cl (0.83 %) lysis for 7 minutes at room temperature (22-24^o C).

Flow cytometry. MNC were incubated with optimal concentrations of fluorescein- (FITC) or phyco-erythrine (PE) -labeled monoclonal antibodies (Becton Dickinson) directed against the following lymphocyte subsets: T cells (CD3, CD4 and CD8), natural killer cells (CD16) and B cells (CD19). The distribution of lymphocyte subgroups was then determined in a flow cytometer (Argus, Scatron, Drammen, Norway). Irrelevant isotype-matched controls were run for each antibody, and at least 10000 cells were analysed for each sample.

Phytohemagglutin (PHA) and Concavalin A (Con A) cultures. MNC (10^5) were suspended in 200 ml RPMI 1640 containing 5% fetal calf serum (FCS) and 10 mg/ml of PHA or Con A. The cells were cultured for 72 h in a humified atmosphere with 7.5% CO_2 at 37^o C. After 48 h 1 mCi of ^{3}H-thymidine was added and 24 h later the cells were harvested onto glass fibre filters with a semiautomatic microculture harvester (Skatron, Lier, Norway). ^{3}H-thymidine incorporation was determined with a liquid scintillation counter.

Chemotaxis. Neutrophil chemotaxis [16] was measured by the ability of cells to migrate towards a chemotactic peptide (n-formyl-methionyl-leucyl-phenylalanine). The net distance migrated by cells in the leading front was measured after incubation for 90 minutes at 37^o C.

Immunoglobulins and acute phase proteins. Serum immunoglobulins, a_1-antitrypsin and orosomucoid were measured with a Behring nephelometer, and C- reactive protein (CRP) was measured with a Cobas Bio turbidimeter.

Plasma cytokines. Plasma concentrations of interleukin 1,2 and 6 were determined with radioimmunoassays (RIA) (Amersham, UK). Interleukin 3 and 4, granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) were quantified with Enzyme-linked Immunosorbent Assay (ELISA) kits (Genzyme Corp. Boston).

Statistics. Alterations during the course were analysed with an analysis of variance for repeated measures. Student's t test was used to identify differences by comparing each individuals test value at different time points to their own baseline value.

RESULTS

There were no indications of increased infection rate during the course or the following weeks. Some complaints of rhinorrhea were noted.

Cell numbers

Cell numbers in blood changed significantly during the course (Fig. 1). The highest number of neutrophils, a 3 fold increase, was observed 24 h after start, and the values remained high throughout the course. The majority of these cells were mature polymorphonuclear granulocytes, but there was a slight ($p<0.05$) increase of band forms during the course (Table I).

The monocyte numbers increased by in parallel with granulocytes. Eosinophils decreased to approximately 30% of control, and the lymphocyte numbers decreased by 30-40%. A moderate increase of energy intake (IIB) did not change this pattern (not shown). Flow cytometric measurements showed that all major subgroups (CD3, CD4 and CD8 T cells, B cells, NK cells) were reduced (Fig. 2). The CD4/CD8 ratio increased during the first 24 h (Table II, $p<0.01$), followed by a decrease on day 2 ($p<0.05$). Otherwise the relative proportions of different cell types did not change appreciably, although there was a percentual decrease of all lymphocyte subgroups during the course. Thus the total sum of CD4, CD8, CD16 and CD19 cells, which amounted to 120% at start, decreased to 77 % on day 4 (Table II).

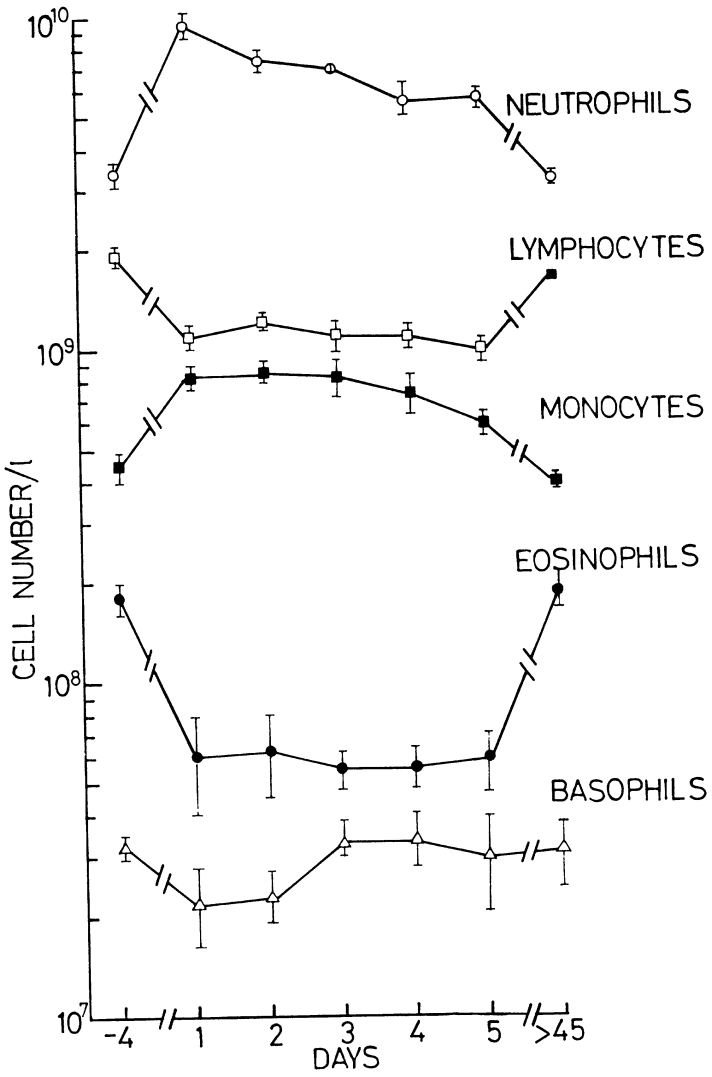

Fig. 1. Cell numbers (per liter) in blood during the training course. The number of days from start are indicated on the abscissa. Mean values (±SE) from five separate courses, each comprising 8-10 individuals.

Table I. Number of band forms of granulocytes in blood

Control	Day 1	Day 2	Day 3	Day 6
49 ± 19	263 ± 71	118 ± 24	204 ± 38	130 ± 59

The table gives the number of band forms per ml blood on different days of the ranger course. Mean values (± SE) from 10 individuals.

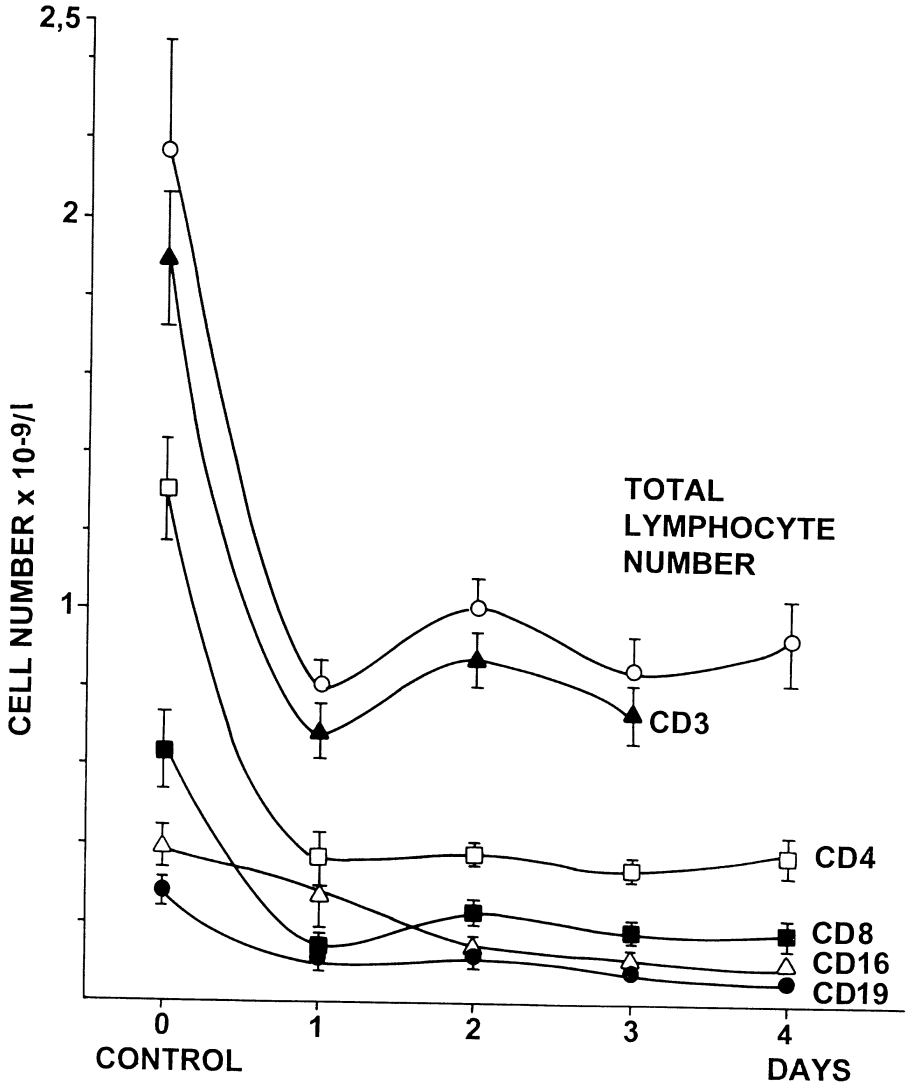

Fig. 2. The number of lymphocytes (per liter) in different subgroups from course I, as determined by flow cytometry. The antibody against CD8 cells was PE-labeled; the other antibodies were FITC-labeled. Mean values (± SE) from 10 cadets.

Table II. Percentages of lymphocyte subsets during course I

	CD4	CD8	CD19	CD16	CD3	CD4/CD8
Contr	60	29	13	18	88	2.1
Day 1	42*	17*	14	29	87	2.7*
Day 2	37*	22*	12	13	87	1.7*
Day 3	42*	22*	9*	10*	87	2.1
Day 4	40*	19*	7*	11*		2,5

The percentages of lymphocyte subsets in blood as determined by flow cytometry. Mean values from 10 cadets. CD16 values varied considerably, in particular during the first two days, with SE up 30% of the mean, as compared to £13% (mostly below 10%) for other subtypes. *denotes values that are significantly different ($p<0.05$) from control.

Erythrocyte numbers (Table III) decreased by 15-20% during the course, and there was a corresponding decrease of hemoglobin values, and a gradual increase of reticulocytes. Platelet numbers were not affected (Table III). All blood cell counts were normal 1-2 months after the course.

Table III. Hemoglobin, erythrocytes reticulocytes and platelets during the training course

	Control	Day 1	Day 2	Day 3	Day 4	Day 5
Hgb g/100 ml	15.3 ± 0.6	15.2 ± 0.6	14.5 ± 0.3	13.7 ± 0.3	13.5 ± 0.3	12.9 ± 0.6
Eryth $l \times 10^{-12}$	5.3 ± 0.1	5.0 ± 0.2	4.7 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	4.3 ± 0.2
Retic (%)	0.32 ± 0.1	0.57 ± 0.1	0.79 ± 0.1	0.99 ± 0.3	1.60 ± 0.6	0.91 ± 0.2
Plat $l \times 10^{-9}$	235 ± 14	303 ± 29	259 ± 29	271 ± 30	273 ± 30	270 ± 10

Average values (± SE) from 11 individuals (5 for reticulocytes).

Cell function

Neutrophil chemotaxis was stimulated ($p<0.01$) on days 1 and 2 and 7 after start of the course (Fig. 3), in group IIA (1000 kJ/day), which confirmed the results observed during course I (not shown). Stimulation was observed also in the energy-supplemented group (IIB, 6000 kJ/day, $p<0.01$, day 1), but less than in the low calorie group ($p<0.01$, day 7).

The mitogenic response by MNC to PHA and Con A (Fig. 5) was not consistent, varying from significant stimulation (course II) on day 1 and 2 ($p<0.01$), to no change of response during course III.

Immunoglobulins and acute phase proteins

Serum IgG was slightly reduced (6-7%, $p<0.05$) on days 3 and 6 (course III, Table IV), IgA decreased by 10-20 % from day 1 ($p<0.05$) and IgM by 20-35 % ($p<0.01$). The same effect ($p<0.01$) of stressful training on IgM was observed in course II (EDTA-plasma), and group IIA (1000 kJ/day) and IIB (6000 kJ/day) did not differ. A slight reduction ($p<0.05$) of orosomucoid was observed on days 3 and 6, whereas a_1-antitrypsin and CRP were not significantly affected (Table IV).

Table IV. Immunoglobulins and acute phase reactants during the training course

		Control	Day 1	Day 2	Day 3	Day 4	Day 5
IgG	g/l	13.5 ± 0.6	13.7 ± 0.8	13.5 ± 0.9	13.7 ± 0.3	12.5 ± 0.8*	12.6 ± 0.8*
IgM	g/l	1.7 ± 0.2	1.3 ± 0.2*	1.2 ± 0.2*	4.3 ± 0.1	1.1 ± 0.1*	1.2 ± 0.2*
IgA	g/l	2.7 ± 0.3	2.4 ± 0.2*	2.3 ± 0.2*	0.99 ± 0.3	2.2 ± 0.2*	2.3 ± 0.2*
Orosomuc	g/l	0.9 ± 0.07	0.9 ± 0.04	0.9 ± 0.05	271 ± 30	0.8 ± 0.1*	0.8 ± 0.1*
CRP	mg/l	0.9 ± 0.07	10.8 ± 0.8	18.4 ± 4.7	0.9 ± 0.07	11.8 ± 3.7	13.0 ± 2.5
Alpha-1 antitr	g/l	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	0.9 ± 0.07	2.3 ± 0.1	2.4 ± 0.1

The table shows average values (± SE) in sera from 10 individuals (course III). Control values are pooled data obtained 7 and 2 days before start of the course, and * indicates values that are significantly different ($p<0.05$ or better) from control. For C-reactive protein 10 mg/l was the minimum detection level. Three out of 10 cadets had increased CRP values on days 2 and 3.

Cytokines

Plasma levels of interleukin 1 (1a and 1b) and interleukin 2 did not change. There was a 12-20 % reduction of interleukin 6 ($p<0.05$) on days 4-7 (Fig. 5). There was no difference between the two groups with different energy intake.

Plasma concentrations of interleukin 3 and G-CSF remained below detectable levels in the observation period. A significant increase of GM-CSF ($p<0.01$) was found on days 1,2 and 7 (Table V).

Table V. Concentration of GM-CSF in plasma

Control	Day 1	Day 2	Day 7
2.8 ± 0.5	9.5 ± 1.5	10 ± 1.3	8 ± 1

Plasma concentration (pg/ml) of granulocyte-macrophage colony stimulating factor during the training course. There was no difference between the group IIA (1000 kJ/24 h) and group IIB (6000 kJ/24 h) and the data were pooled. Mean values (± SE) from 16 individuals.

DISCUSSION

The immediate effect of physical exercise of short duration is an increase of blood granulocytes, monocytes, lymphocytes and lymphocyte subgroups [17-20]. The response to prolonged physical activity (Fig. 1) was different in some respects. Neutrophil and monocyte numbers remained at elevated levels, whereas there was a lasting decrease of eosinophils and lymphocytes (Fig. 1) and lymphocyte subsets (Fig. 2) from day 1. Short term exercise has yielded different results, and a typical finding is a decrease of CD4 T cells and an increase of NK cells [21,22].

The mechanism for the changes of blood cells counts (Figs. 1 & 2) are not clear. In previous studies [8,9] a significant increase of cortisol, epinephrine, norepinephrine and dopamine plasma levels was found during the training course. Thus, a combined effect of corticosteroids and catecholamines may account for the observed changes. The leucocytosis-inducing effect of epinephrine was demonstrated [23] already in 1904. After an initial increase of granulocytes and lymphocytes, there may be a secondary decrease of lymphocytes [24,25]. Catecholamines may cause an increase of cells by inducing a wash-out of cells from different organs [26,27].

The increase of neutrophils may not only be due to a redistribution of cells within the vascular system. The increased number of band forms (Table I) indicates an increased influx of cells from the bone marrow, probably caused by cortisol [28]. Short term exercise mostly leads to elevated numbers of lymphocytes in blood [29]. The lymphopenia and eosinopenia during more long-lasting strenuous exercise (Figs. 1 & 2) can probably also be ascribed to an effect of cortisol. Many studies have shown that cortisol depresses the lymphocyte number [30,31]. It appears that cortisol causes a selective depletion of the recirculating portion of the intravascular lymphocyte pool [30,32]. The first step in lymphocyte migration from the blood stream into the lymph nodes involves a specific binding between the lymphocytes and the endothelial cells lining nodal postcapillary venules. In a recent work [33] it was shown that the expression of adhesion proteins (L-selectin) on circulating lymphocytes was decreased during the first 8 hours following prednisolone administration. Furthermore, other investigators have shown that steroids cause a redistribution of cells to other organs, such as the bone marrow [34]. By large the number of different lymphocyte subtypes decreased approximately to the same extent during the course (Fig. 2). It is noteworthy that the subtypes with different markers (CD4, CD8, CD16 and CD19) amounted to 120 % at start (Table II), indicating that some cells had more than one marker. However, on day 4 only 77 % of the cells expressed these markers, suggesting that a significant fraction of the surface markers had been lost in response to the multifactorial strain. An alternate explanation is that the selection of cells in blood changes during the course. It has otherwise been speculated that the eosinopenia, like lymphopenia, also somehow is related to alteration of cell adherence [35].

Neutrophil chemotaxis was significantly enhanced during the course (Fig. 3), in particular on days 1 and 2. This can hardly be attributed to increased GM-CSF levels (Table V), since GM-CSF injections may inhibit

chemotaxis when separated cells are tested in vitro [36,37]. Still, in some respects GM-CSF is an activator [36,38,39] of granulocytes (phagocytosis, antibody-dependent cytolysis).

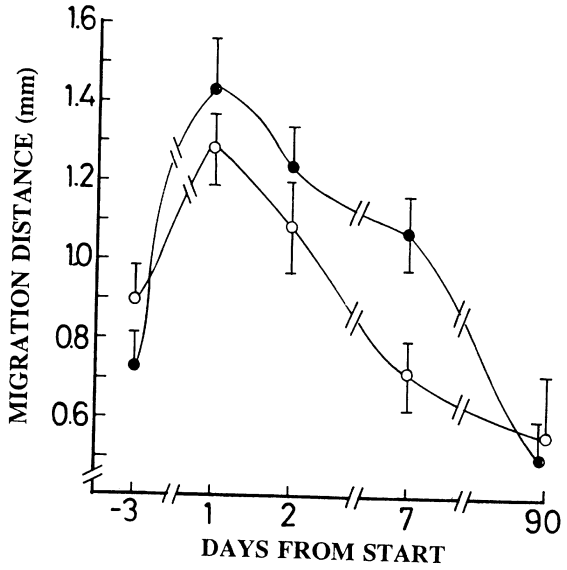

Fig. 3. Neutrophil chemotaxis with cells from the low (IIA, 1000 kJ/24 h, closed circles) and higher calorie group (IIB, 6000 kJ/24 h, open circles). Both groups had a caloric deficiency of more than 80%. Control samples were taken 3 days before, and 90 days after the start of the course. Mean values (±SE) from 8 individuals in each group.

The mitogenic response of lymphocytes to PHA and Con A was not consistent. Stimulation was observed during the first two days of one training course, whereas no effect was found in another course (Fig. 4). A varying physical activity in different courses may also account for these results. A particular problem here is that the differential distribution of the mononuclear cells changed during the course, with an increase of monocyte/lymphocyte ratio. A striking variability has also been found in response to short-term (1-3 h) exercise. Mostly this exercise suppresses the cell proliferation [40], but stimulation has also been observed [1, 29,40]. In long-lasting (~ 60 days) ranger courses the mitogenic response was almost consistently suppressed [41]. However, the results from a consecutive course indicated that the suppression could partly be prevented by increasing the food intake [42]. Based on previous studies [43-45] one should expect suppressed lymphocyte function due to increased levels of cortisol and epinephrine [8,9] found during the present training courses. A short-term immunosuppression has been observed after epinephrine injections [10], but altogether rather conflicting results have been found [12, 29]. Further, it has been shown that glucocorticoid inhibits the synthesis of interleukin 2 [11], a vital regulator in the immune system. However, the complexity of changes in response to strenuous exercise [8,9] makes it difficult to predict the effect on blood cell function based on values of a few parameters.

The reduced immunoglobulin levels (Table IV) during the course, may suggest impaired immune function. No such effect on immunoglobulins was observed during long-lasting (~60 days) endurance exercise [42] with daily energy expenditure of 16-18000 kJ and a significant caloric deficit. In general, heavy short term exercise tends to be associated with increased levels of immunoglobulins (5,46). A decrease of immunoglobulins (IgA and IgG more than IgM) was found following 45 and 75 km runs [47]. However , these results were not reproduced in another marathon run, presumably due to lower intensity as a results of high air temperature. Altogether, many studies have yielded contradictory results [reviews, 5,29,46].

Immune dysfunction has been observed in chronically undernourished subjects [48]. Starvation itself for 5-10 days does not affect immunoglobulin levels [49-51] and an increase has been observed in obese subjects during fasting [52]. However, it remains to be shown whether reduced immunoglobulins (Table IV) is due to a synergistic effect of exercise and starvation. It is also necessary to consider whether a predominant catabolic state of the cadets may affect immmunoglobulin production. However, the decrease of IgM was observed

repeatedly already after 24 h, before they were exposed to maximal metabolic stress. Hormonal changes [review 53] during the course may affect immunoglobulins. It has been reported that high doses of methylprednisolone [54] reduced serum IgG and IgA (but not IgM), and it is possible that increased levels of cortisol during the course [9] have a similar effect. This issue may be further evaluated by measuring serum immunoglobulins in group a well-fed of cadets, since cortisol increases less among participant on a isocaloric diet [9], whereas extra sleep (3 h/24 h) has no such effect [9]. Catecholamines increased consistently during the course, unaffected by extra sleep or energy intake [9], but it is difficult to evaluate their effect on immunoglobulin synthesis. As regards the primary antibody response available reports have yielded conflicting results [12], ranging from inhibition [55,56] to stimulation[57].

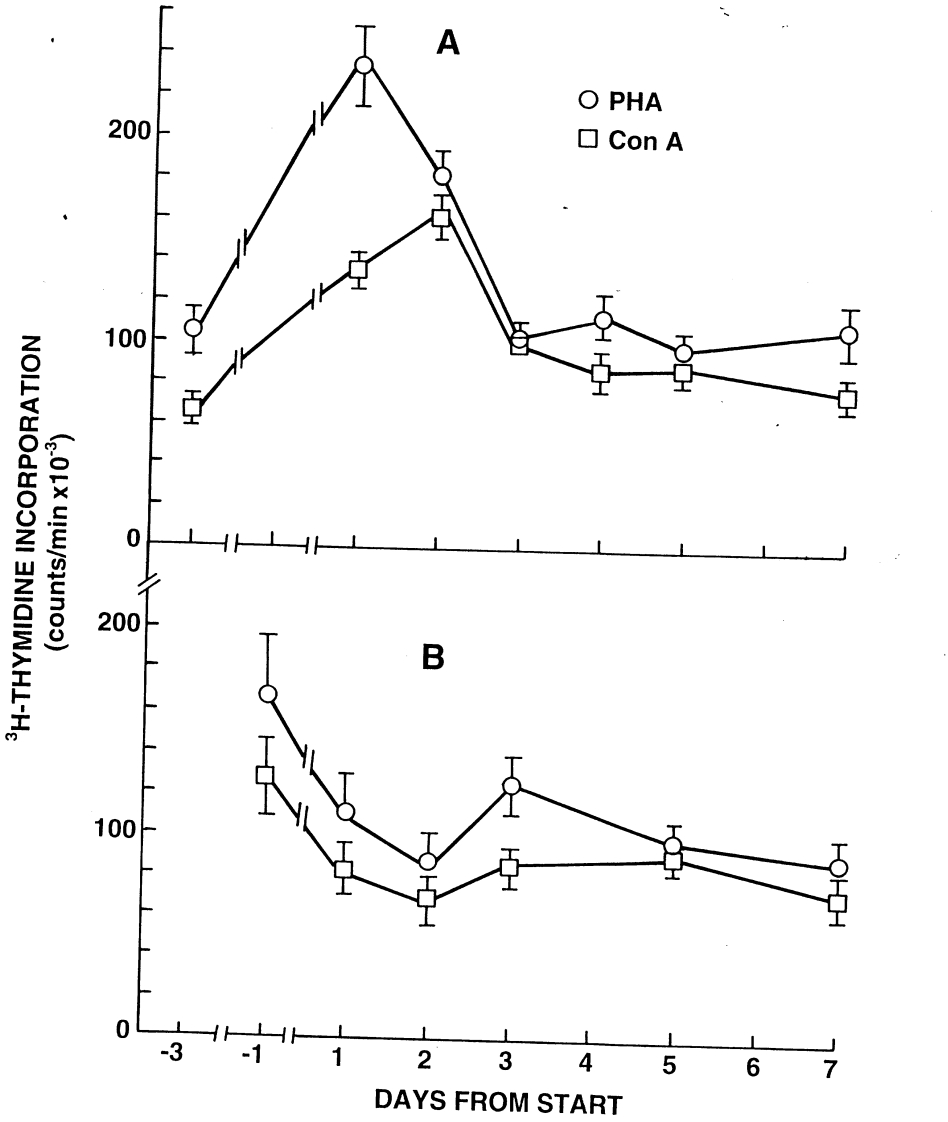

Fig. 4. The response of mononuclear cells to mitogens (PHA and ConA). The cells were cultured 2 days and then ^{3}H-thymidine was added, and the uptake was measured on day 3. Stimulation was observed during course II (A), whereas there was no change of response during course III (B).

Among the interleukins, a significant (15-20%) reduction was observed for interleukin 6 (Fig. 5). Il-6 is a multifunctional cytokine that stimulates B and T cells by different mechanisms, and also acts in synergy with colony stimulating factors. This decrease of Il-6, although small, may impair immune function.

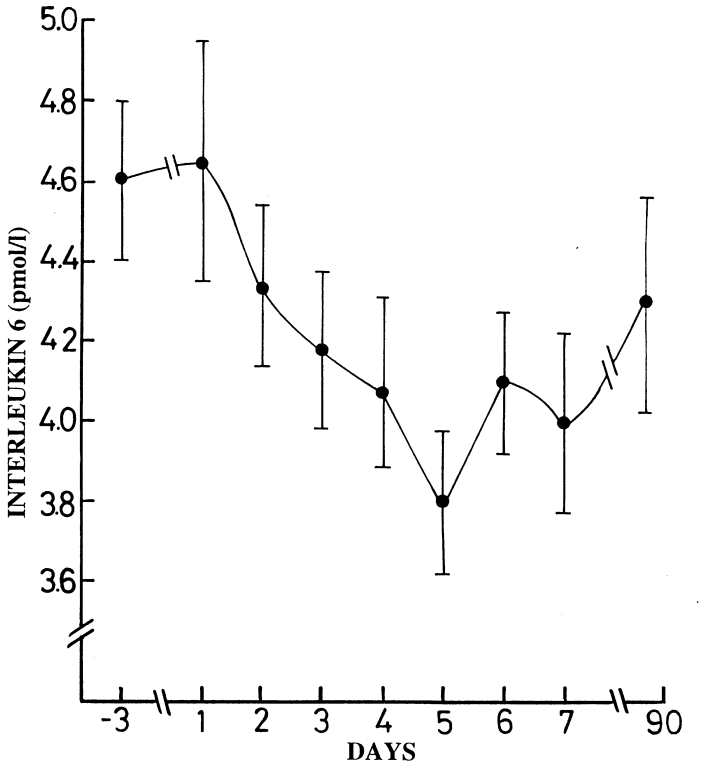

Fig. 5. Concentration of interleukin 6 in plasma from course II (A + B) , determined with radioimmuno-assay. Mean values (±SE) from 15 individuals.

The decrease of Hgb values (Table III) from day 2 ($p<0.05$) is in agreement with previous studies, with a decrease already on day 1 [58]. There was a corresponding decrease of erythrocytes, which coincided in time with an increase of reticulocytes (Table III). These changes can hardly be totally due to the amount of blood (30-40 ml) taken per day. Reduced Hgb, immunoglobulin or Il-6 levels could be explained by plasma expansion due to excessive water intake. However this may not seem likely since alpha-1-antitrypsin remained constant (Table III), and total serum protein was also constant and decreased only slightly (6-7%) from day 4 [58]. Reduced haptoglobin values [58] rather suggests that the drop in Hgb is caused by mechanical damage of red cells.

the intensity, energy supply and the duration of the exposure, and is affected by a large number of molecular participants [59]. The response to acute exercise is transient and quite variable and may also differ in trained and untrained subjects [6]. In our study we found some indication of impaired immune function without any increased infection rate, possibly due to a relatively short duration of the course. The infection rate was increased in a more long-lasting (62 days) ranger course, with daily energy expenditure of 16800 kJ and energy intake of about 6000 kJ (41). The majority of these infections involved cellulitis of the lower extremities, possibly due to injuries (blisters) and environmental factors (contaminated water), together with suppressed immune function. However, the prevalence of infections was lower when the caloric intake was increased [42]. This improved resistance is also consistent with the effects on cellular immune function [42]. Increased infection rates were observed in a even more long-lasting ranger course (30 weeks), with marked periodicity for viral infections of the respiratory system [60]. In any case, in studies of this type it appears important to define the actual physical stress program properly. This may be fairly easily achieved for energy supply and duration. However, conceivably it is more difficult to maintain a constant intensity level for courses arranged at different times. Still, our data indicate that even moderate physical exercise may be suppress the immune system within a few days, when the activity is continuous, around the clock. However, it remains to elucidate more thoroughly the role of energy and sleep deprivation in this multifactorial stress situation.

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Fluid regulation and time course of erythropoietin during multifactorial strain of Austrian Special Forces survival training

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Abstract

The aim of this study was to provide data on fluid-regulating mechanisms with special regard to the role of plasma proteins in the control of plasma volume (PV), and to investigate erythropoietin production and release during a period of prolonged multifactorial strain. 29 male subjects, with a mean age of 22.2±2.8 years, were studied during a 5 day lasting survival training including restricted water (1| H_2O· day^{-1}) and food intake (628 kJ· day^{-1}) additionally to physical exercise and sleep deprivation (20 h within 5 days). Under field conditions heart rate was monitored continuously, and body mass, body composition and blood parameters were measured at (T1), after 72 h (T2), after 120 h (T3) and in the recovery period after 48 h (T4) and 72 h (T5). The estimated energy expenditure was approximately 24000 kJ day^{-1}. The mean decrease of body mass was 6.77 kg (9.5%) at T3 ($p<0.001$). A reduction of total body water of 3.8| was estimated at T3. Serum creatinine ([Cr]) was raised at T3 by 18.5% ($p<0.0001$). The PV decreased by 3.7% ($p<0.0001$) at T2, increased by 1.6% ($p<0.0001$) at T3 and was not different to baseline at T4 (+0.2%; n.s.). Plasma proteins shifted into the intravascular space at T2 and T3 and moved out of the intravascular space at T4 and T5. Our data provide evidence that this mechanism assists PV-homeostasis efficiently over a period of 120 h even under conditions with a fluid loss of almost 8% of the total body water. EPO controls at T1 were 15.2 ± 8.8 $mU.ml^{-1}$. EPO was decreased during the course (T2: 8.7 ± 7.9 $mU.ml^{-1}$; $p<0.01$ and T3: 11.6 ± 6.7 $mU.ml^{-1}$; $p<0.01$) and showed a significant increase in the recovery period. Serum iron increased from 13.5 ± 4.5 µ $mol.l^{-1}$ at T1 to 24.5 ± 4.1 $\mu mol.l^{-1}$ at T2 ($P<0.01$) and decreased during recovery. Haptoglobin (HAPTO) decreased from 165.4 ± 55.3 $mg.dl^{-1}$ at T1 to 85.8 ± 51.7 $mg.dl^{-1}$ at T3 ($P<0.01$). Thereafter HAPTO increased (T4 132.0 ± 52.2 $mg.dl^{-1}$, $P<0.01$) and remained below control level at T5 (131.6 ± 58.3 $mg.dl^{-1}$, $P<0.01$). Transferrin decreased continuously from 303.3 ± 65.3 mg . dl^{-1} at T1 to 256.8 ± 58.8 $mg.dl^{-1}$ at T5 ($P<0.01$). Ferritin increased from 70.2 ± 50.2 $ng.ml^{-1}$ at T1 to and 150.1 ± 60.2 at T3 ($P<0.01$) and returned to control level at T5 (85.7 ± 44.8 $ng.ml^{-1}$, $P<0.01$). [Hb] increased from T1 (15.3 ± 0.7 $g.dl^{-1}$) to T2 (16.6 ± 0.7 $g.dl^{-1}$, $P<0.01$) and decreased significantly thereafter (T5 14.6 ± 0.6 $g.dl^{-1}$, ($P<0.01$). Haematocrit increased from 44.2 ± 2.1 % (T1) to 46.8 ± 1.9 %, $P<0.01$) at T2 and remained afterwards below the control (T5 41.4 ± 1.8 %, $P<0.01$). It seems that EPO production and release is diminished by nutritional factors, i.e. mainly caloric intake, during prolonged physical strain. In the recovery period a rapid EPO increase took place to normalise red cell mass. These data shade new light upon the changes of erythropoiesis in astronauts observed during and after space flight. Since astronauts also show body mass losses and decreased EPO levels in a similar range during and immediately after space flights the question arises whether this might be due to a lowered caloric and/or protein intake rather than an effect of micro-gravity per se.

Introduction

Several kinds of sustained military operations with prolonged exercise can lead to a negative energy balance (16) and can induce dehydration even under ad libitum fluid intake (27,28,40). This study addressed specific questions posed to us by the Command of the Austrian Special Forces due to the fact that their survival training is designed to create multifactorial stress to evaluate the trainees under physically and psychologically demanding conditions. The course schedule included fluid restriction and food deprivation combined with prolonged exercise. Long-term physical exercise is known to have strong influences on the fluid-regulating mechanisms of the human organism which can endanger the size of the plasma volume (PV) and the extracellular sodium concentration. Beside fluid regulating hormones (2), the control of PV depends on

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

plasma protein kinetic (21,23,33). In most studies dealing with long-term physical exercise, a decrease of PV was found during or immediately after exercise (2,13,19,18,33). It is still unclear to what extent such exercises lead to changes of PV, thus, being haemoconcentration at least partly responsible for the increase of various parameters of metabolism. The first aim of the present study was to investigate the time course of PV and the influence of plasma proteins as regulators of PV.

Since short-term protein deprivation can impair erythropoiesis in healthy individuals (10) and a severe negative energy balance occurs during food deprivation of the five day lasting survival training of the Austrian Special Forces the second aim of the field study reported here was designed to investigate the time course of serum EPO and related haematological parameters.

The data of the present report are currently under evaluation of reviewed journals and preliminary results were partly presented at the international symposium on “The Physiology and Pathophysiology of Exercise Tolerance”, September 1994 in Ulm, Germany (41). This study received a grant of the Austrian Bundesministerium für Landesverteidigung, BMLV ZL.65.505/43-5.2/92 and of the German Bundesministerium für Forschung und Technik, BMFT (DARA) 01QV8712-50QV87120.

Subjects and Methods

The subjects were participants of a ranger training unit of the Austrian Army Special Forces. The data of 29 subjects (22.2±2.8 years; range 18-28 years) were collected during a survival training course in August 1993 after informed written consent was obtained. The anthropometric data and physiological variables of the subjects before the course are shown in Table 1.

Table 1. Anthropometric data and physiological variables of the subjects

n=29	Mean	SD	Range
Age (years)	22.2	2.8	18 – 28
Heigh (cm)	178.0	6.3	162 – 193
Body mass (kg)	73.5	8.6	60 – 96
Lean body mass (kg)	64.4	7.4	52 – 86
Body fat (kg)	10.6	2.4	7 – 16
Total body water(l)	48.0	4.9	40 – 63
HR at rest (beats· min^{-1})	53.5	1.6	38 – 60
$HR_{max,CE}$ (beats· min^{-1})	186.3	10.4	162 – 207
Relative maximal work capacity (W· kg^{-1})	4.5	0.46	3.75 – 5.71
Relative $\dot{V}O_{2max}$ (ml· min^{-1}· kg^{-1})	52.7	5.55	43.8 – 67.3

Protocol: After a laboratory testing program to determine anthropometric data, health status and physical performance, the subjects abstained from strenuous physical work in the 24 h-period before the course but had performed physically strenuous military training during the preceding weeks.

The five day survival training course took place in a woody area 430 m to 570 m above mean sea level. Meteorological data were measured at 0600 hours, 1200 hours, 1800 hours and 2400 hours. The mean temperature was 19.9 (SD4.4)° C with a range from 10.1° C to 28.6° C. The relative humidity was 61.1 (SD19.7) % with a range from 35% to 95% and the sky was clear. The wind speed was 3.0 (SD2.2) m· s^{-1} with a range from 1.1 m· s^{-1} to 12.0 m· s^{-1}. During a period of 120 h the subjects had to perform 90 km of marching, partly with tactical missions, during which 22.3 (SD 3.7) kg of clothes and military equipment had to be carried around. The subjects slept only 20 h without tent and sleeping bag. They daily received 1l of water and additionally approximately 1l in the morning of the first day and 1l in the afternoon of the fourth day. After a breakfast on the first day of about 6250 kJ (1500kcal) the mean food intake during the five day course

was only 628 kJ· day^{-1} (150 kcal· day^{-1}). Body mass, body composition, tissue thickness and blood parameters were measured early in the morning on day 1 before the course started (T1), after 72 h (T2), after 120 h at the end of the course (T3) and in the recovery period after 48 h (T4) and 72 h (T5). Food and fluid intake in the recovery period were ad libitum and could not be controlled.

Physical work capacity: The maximal oxygen uptake ($\dot{V}O_{2max}$) was estimated for each subject from the maximal work achieved during cycle ergometry (CE) as described in detail in a previous report (42).

Monitoring of heart rate: The method has already been described in detail previously (42). Briefly, the heart rate (HR) was monitored continuously with a sport-tester PE 4000 (Polar Electro, Kempele, Finland) in representative subgroups of subjects and was based on beat-by-beat ECG measurement with HR transmission by telemetry. From the HR - readings the mean intensity of the physical activity during the course was calculated as a percentage of $HR_{max,CE}$ (%$HR_{max,CE}$). Around 80% of the physical activity during 120 h was Δ50% of the $HR_{max,CE}$. During the last 12 h of the course until T3 (marching at night) the subjects had a mean HR around 50% of the $HR_{max,CE}$. The energy expenditure, estimated by analysing HR-recordings, and calculations by references for energy expenditure during physical work (37), was approximately 24000 kJ· day^{-1} (5760 kcal· day^{-1}).

Body composition was measured at T1 by a bio-electrical impedance analysis (5) using a BIA 101- S analyser (RJL, Detroit, Mich., USA). The values are shown in Table 1.

Blood samples were drawn by repetitive venipuncture with a 20-G needle from different cubital veins in the same sitting position with identical positioning of the arm at T1,T2,T3,T4 and T5. All samples were transported immediately to the laboratory after collection. Metabolic and haematological parameters were analysed within 3 hours after collection. For all other parameters serum or plasma was obtained from whole blood in a centrifuge and stored at Δ30°C until analysis within 2 weeks. Haematocrit (Hct) and haemoglobin concentration ([Hb]) were measured with an auto-sampler T890 (Coulter Electronics, Luton, England). The intra-assay coefficient of variability (CV) was <1.5% (n=20) for these methods. The serum concentrations of uric acid ([UA]; CV<2.0%) and blood urea nitrogen ([BUN]; CV<1.3%) were determined enzymatically, total protein ([TP]; CV<2.6%) using the biuret method, and creatinine ([Cr]; CV<2.7%) by the Jaffé reaction, employing an BM/Hitachi 747 *spectrophotometer* (Boehringer Mannheim GmbH, Mannheim, Germany). The sodium concentration ([Na^+]) was analysed from serum by flame-photometry (KLiNa, Beckman). The intra-assay CV was <1%. The colloid osmotic pressure (COP) was measured with an BMT-921-onkometer (Thomae, Germany), the CV was 0.44%. Plasma osmolality (Osm_{pl}) was measured by freezing-point depression by a digital micro-osmometer type 5B (Roebling Company, Berlin, Germany). The CV was 0.6%.
EPO was measured by using a commercial available ELISA (IBL, Hamburg, Germany). The coefficient of variation (CV) for this method was 4.8 %.
Serum iron ([Fe^{++}]) was measured with atomic absorption spectrophotometry (Philips SP9) without dilution (CV <1%). Ferritin (FER) was analysed with a commercial available IRMA (Company Bio-Rad) (CV 3.4%).
Haptoglobin concentration (HAPTO; CV<3%) and Transferrin (TRANS; CV <5%) was measured by nephelotomy (Nephelometer 100, Behring Werke, Germany). HAPTO was measured to estimate haemoglobin loss by intravascular haemolysis. The values are shown in Table 1. Based on the stoechiometric relation between haemoglobin and HAPTO the haemoglobin consumption by haemolysis was calculated according to the literature (35). Assuming a PV of approximately 3.5l, the total intravascular [Hb] loss via haemolysis was 1,8g. This cannot account for relevant changes of [Hb] and Hct in the calculation of PV.
The percentage changes in plasma volume (%ΔPV) were calculated from [Hb] and Hct according to the equation given by Strauss et al. 1951 (38).
To obtain more information about the maintenance of the colloid osmotic capacity by plasma proteins it was necessary to consider changes of [TP] and PV simultaneously. Of the two it is possible to appraise the change of the intravascular plasma protein mass ($IVTP_{pl}M$), which is mostly responsible for the water-binding capacity of the intravascular space. As described previously (33) we related the percentual changes of [TP] (%Δ[TP]) to the percentual changes of PV (%ΔPV) by calculating the difference of % ΔPV and % Δ[TP]. This difference describes the relationship between % Δ [TP] predicted by % ΔPV compared to measured % Δ[TP]. If there is no increase or decrease of $IVTP_{pl}M$, the expected value (E) of the above-mentioned difference is not

significantly different from zero (E=0). This can be interpreted as pure haemoconcentration or haemodilution. A significant value E>0 can be interpreted as a gain of $IVTP_{pl}M$ into the vascular space, whereas a significant value E<0 can be interpreted as a loss of $IVTP_{pl}M$ out of the vascular space.

Statistics

The data showed normal distribution determined by Chi Square test and Kolmogorov-Smirnov test. The hypothesis of differences in repeated measures was tested by t-test. The Bonferoni technique was used to protect significance level. Interaction effects between variables were tested by ANOVA. Dependencies between variables were tested by linear regression. The results are presented as mean ΔSD. If not otherwise indicated changes of percentage values are presented as the means of individual, percent changes. Statistical significance was attributed if the probability of error was less than 5% ($p<0.05$).

Results

1. Time course of body mass

The observed decrease of mean body mass was 5.12 kg (6.8% ; $p<0.001$) at T2, 6.77 kg (9.5%; $p<0.001$) at T3, 0.95 kg (1,3%; $p<0.05$) at T4 and 0.68 kg (0.9%; n.s.) at T5. The decrease of the body mass was not correlated to the baseline values of physical work capacity, body mass, total body water, body fat, or weight of clothes and military equipment which had to be carried around by the subjects.

2. Time course of parameters measured from blood samples (Table 2)

$[Na^+]$ was within normal range over the whole time course, though it was slightly decreased at T2 by 2.24 $mMol \cdot l^{-1}$ ($p<0.05$) and at T5 by 3.0 $mMol \cdot l^{-1}$ ($p<0.01$) compared to T1. Osm_{pl} was within normal range and did not change over the whole time course. COP (Table 2) was increased at T2 and T3 and decreased at T4 and T5 compared to T1.
[TP] had increased by 11.7% ($p<0.0001$) at T2, 2.6% ($p<0.01$) at T3 and was decreased ($p< 0.0001$) at T4 (8.2%) and T5 (5.7%). The changes of the [TP] at T2 (Fig. 2) and T3 (Fig. 3) correlated with the changes of COP .
[BUN] and [UA] were increased ($p<0.0001$) at T2 by 57.3% and 48.8%, and at T3 by 98.7% and 88.6%. At T4 [BUN] and [UA] had already returned to values within normal range. No correlations or dependencies were found between [BUN] and [UA] and the changes of PV.
[Cr] was increased ($p<0.0001$) at T3 by 18.5% compared to baseline values, and was not changed significantly at any other time. No correlation or dependency was found between [Cr] and changes of the PV, [BUN] or [UA].

Table 2. Time course of parameters measured from blood samples.
T1= before the course, T2= after 72 h of strain, T3= end of the course after 120 h of strain, T4= 48 h of recovery, T5= 72 h of recovery. Not significant (ns) changes are indicated. All other changes of parameters were significantly different to the initial value at T1 by at least $p<0.05$. Abbreviations for demonstrated parameters are explained under methods.

n=29	T1		T2		T3		T4		T5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$[Na^+]$ (mmol· l^{-1})	138.2	4.2	136.0	3.6	^{ns}139.0	5.4	^{ns}137.9	3.5	135.2	2.1
Osm_{pl} (mmol· l^{-1})	290.1	5.8	^{ns}289.7	7.4	^{ns}290.2	5.1	^{ns}286.6	11.7	^{ns}290.9	6.1
COP (mmHg)	26.8	2.0	32.7	2.2	30.2	2.2	23.7	2.1	24.0	2.8
[TP] (mg· dl^{-1})	73.2	3.4	81.7	4.9	75.1	3.9	67.2	3.4	69.0	2.4
[BUN] (mg· dl^{-1})	15.3	2.9	23.6	3.7	29.4	3.7	^{ns}16.3	2.8	^{ns}16.3	2.9
[UA] (mg· dl^{-1})	5.5	0.9	8.1	1.0	10.3	1.3	5.2	0.8	5.3	1.0
[Cr] (mg· dl^{-1})	1.09	0.10	^{ns}1.09	0.10	1.29	0.11	^{ns}1.11	0.07	^{ns}1.09	0.09

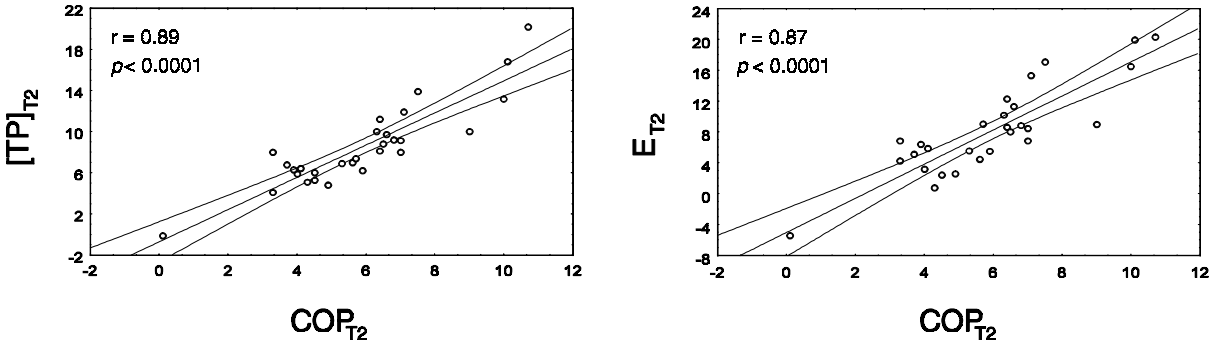

Fig. 1. Relationship between changes after 72 h (T2) of strain. Colloid osmotic pressure (COP) vs total protein concentration ([TP]) and COP vs the E-values. The definition for E-values, which indicate protein shifts, is given under methods.

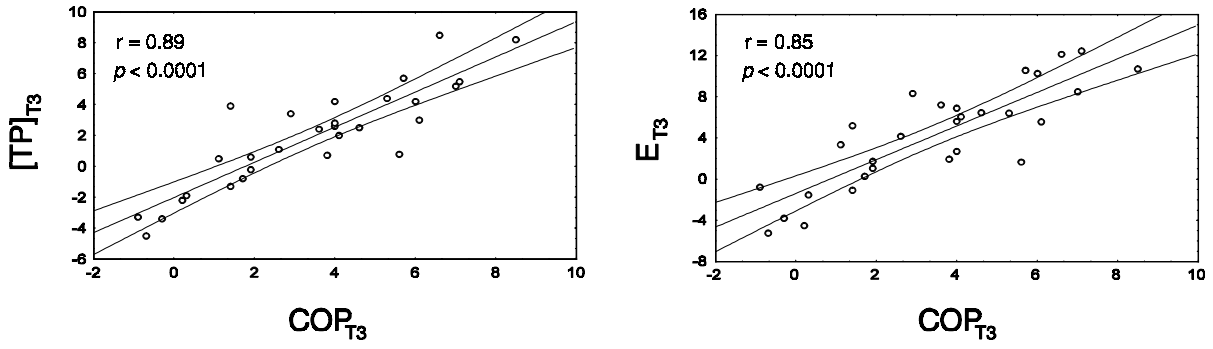

Fig. 2. Relationship between changes after 120h (T3). For definitions see Fig.1

3. Percentage changes of PV and protein shifts (Fig.3)

The level of statistical significance of the changes is shown in Fig. 3. PV was decreased at T2 by 3.7%, raised above baseline value at T3 by 1.6%, was statistically indistinguishable from baseline at T4, and was decreased at T5 by 2%. There was a significant gain of intravascular [TP] seen at T2 (E=8.0) and T3 (E=4.2). A significant loss of [TP] out of the intravascular space appeared at T4 (E= Δ8.4) and T5 (E= Δ7.7). Between COP and the E-values a significant correlation was found at T2 (Fig.1) and at T3 (Fig.2). No correlations or dependencies were found between E and $[Na^+]$.

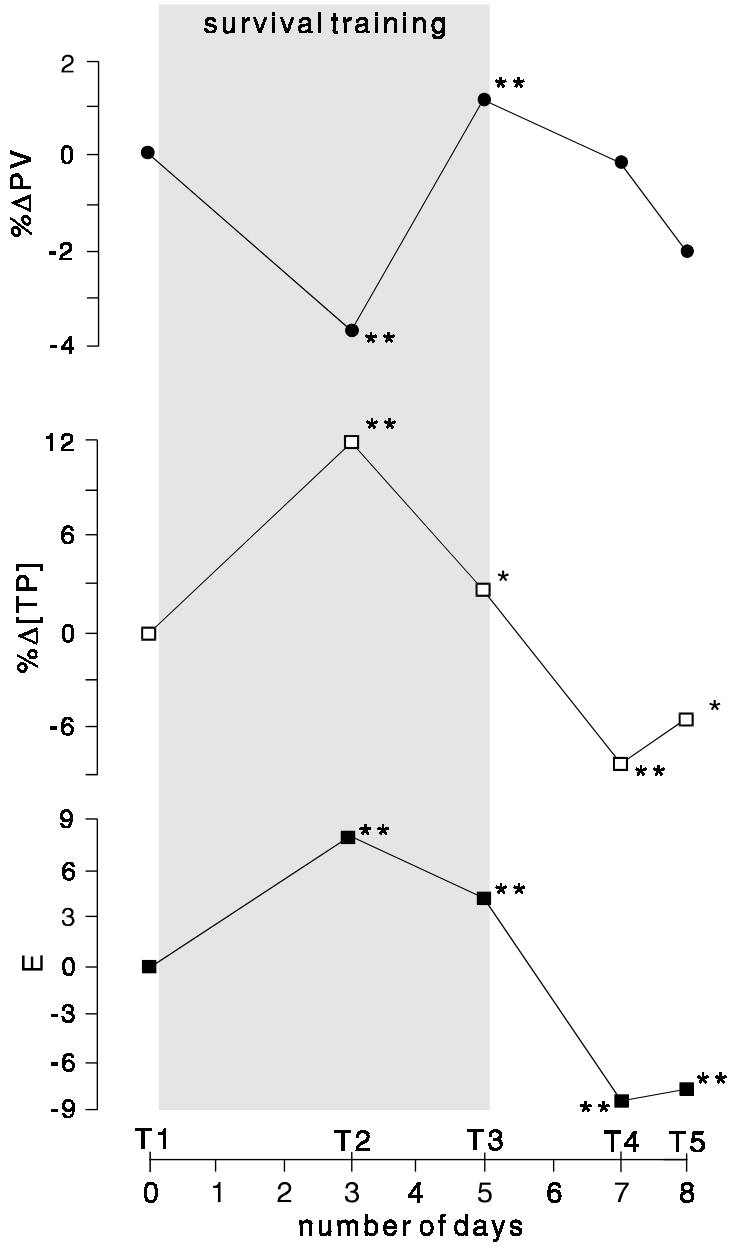

Fig. 3. Time course of percentage changes of plasma volume (%ΔPV), percentage changes of total protein concentration (%Δ[TP]) and the mean E-values (a detailed definition of E-values is given under methods) before, during and after one week with food and fluid deprivation. A significant value E>0 can be interpreted as a gain of the intravascular total protein mass into the vascular space, whereas a significant value E<0 can be interpreted as a loss of the intravascular total protein mass out of the vascular space. The level of significance of either parameter in relation to the initial value was: * p<0.05; ** p<0.0001

4. Serum EPO and related haematological parameters

The main results are summarised in figure 4,5 and 6.

EPO controls at T1 were 15.2 ± 8.8 mU· ml^{-1}, T2 8.7 ± 7.9 mU· ml^{-1} (P<0.01), T3 11.6 ± 6.7 mU· ml^{-1} (P<0.01), T4 23.4 ± 12.0 mU· ml^{-1} (P<0.01) and at T5 18.7 ± 11.3 mU· ml^{-1} (P<0.05).

[Fe^{++}] increased from 13.5 ± 4.5 µmol· l^{-1} at T1 to 24.5 ± 4.1µmol· l^{-1} at T2 (P<0.01), decreased at T3 20.5 ± 6.7 µmol· l^{-1} (P<0.01) and T4 14.5 ± 4.4 µmol· l^{-1} and increased towards T5 again (17.0 ± 5.4 µmol· l^{-1}, P<0.01).

HAPTO decreased from 165.4 ± 55.3 mg· dl^{-1} at T1 to 117.4 ± 55.5 mg· dl^{-1} at T2 (P<0.01) and 85.8 ± 51.7 mg· dl^{-1} at T3 (P<0.01). Thereafter HAPTO increased (T4 = 132.0 ± 52.2 mg· dl^{-1}, P<0.01) and remained below control level at T5 (131.6 ± 58.3 mg· dl^{-1}, P<0.01).

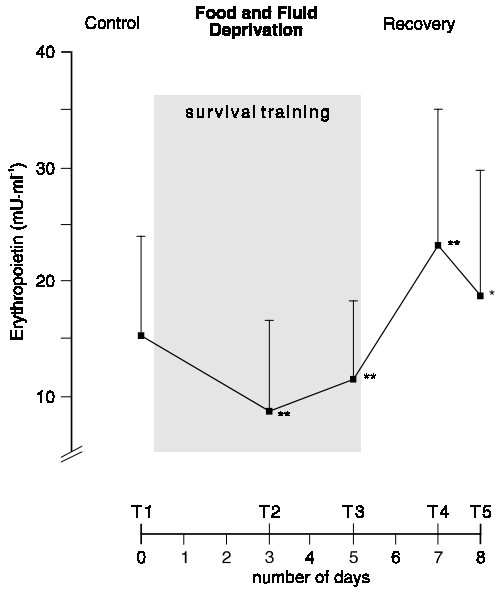

Fig. 4. Time course of erythropoietin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: *P<0.05, **P<0.01.

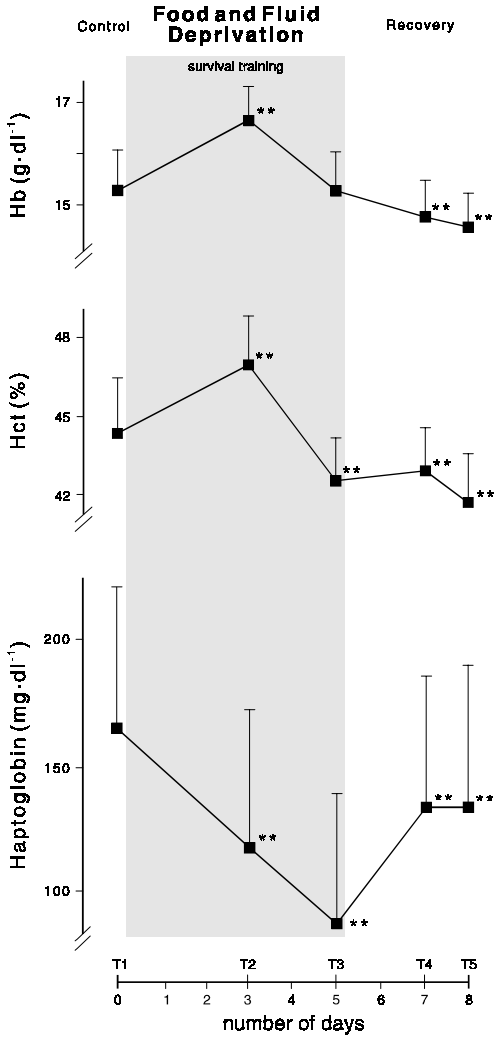

Fig. 5. Time course of haemoglobin (Hb), haematocrit (Hct) and haptoglobin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: **P<0.01.

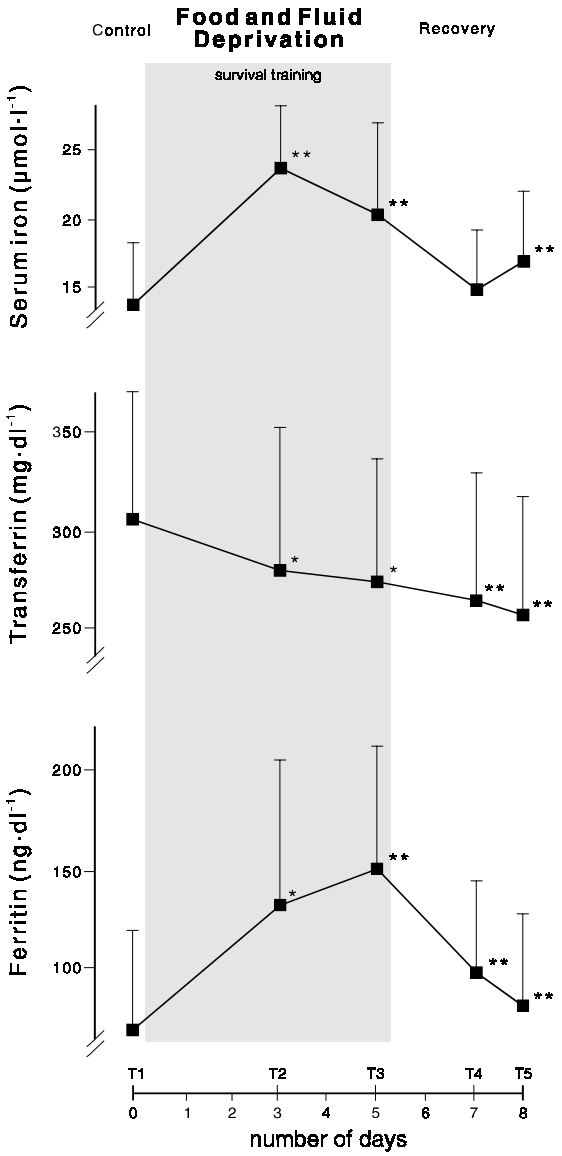

Fig. 6. Time course of serum iron, transferrin, and ferritin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: *P<0.05, **P<0.01.

TRANS decreased continuously over the week from 303.3 ± 65.3 mg· dl^{-1} at T1 to 272.4 ± 63.1 mg· dl^{-1} at T3 (P<0.05) and 256.8 ± 58.8 mg· dl^{-1} at T5 (P<0.01).
FER increased from 70.2 ± 50.2 ng· ml^{-1} at T1 to 134.4 ± 70.4 ng· ml^{-1} at T2 (P<0.01) and 150.1 ± 60.2 at T3. Thereafter FER decreased (T4 101.7 ± 45.1 ng· ml^{-1}, P<0.01) and reached control level at T5 (85.7 ± 44.8 ng· ml^{-1}, P<0.01).
[Hb] increased slightly from T1 (15.3 ± 0.7 g· dl^{-1}) to T2 (16.6 ± 0.7 g· dl^{-1}, P<0.01), showed no significant changes compared to the controls at T3 (15.2 ± 0.7 g· dl^{-1}), and decreased significantly at T4 (14.8 ± 0.6 g· dl^{-1}) and T5 (14.6 ± 0.6 g· dl^{-1}) (P<0.01).

Discussion

From the HR-monitoring and calculations (37) based on the pattern of the physical activity it can be estimated that the average intensity of exercise was 35% to 40% of the $\dot{V}O_{2max}$. The calculations of energy expenditure during the 120 h of strain leads to an estimation of approximately 3 kg loss of body mass by catabolism.

The mean decrease of body mass at the end of the course was 6.77 kg, which was partly due to fluid loss. It was estimated that at this time a reduction of total body water by 3.8| had occurred.

According to prior results under comparable conditions (28), although confounded in the present study by a restriction of fluid intake, it could be observed that the extracellular [Na^+] was not altered in a biologically relevant amount. Although the [Na^+] was within normal range over the whole time course, there was a slight decrease of 2.6% after 72h of exercise. This was in parallel with a slight decrease of PV. It can be assumed that the mechanisms responsible for water retention and production of increased plasma solute concentration during exercise have not been activated sufficiently at this time to counterbalance completely the net water loss. Convertino et al.(11) described a PV decrease of > 3.7 % as one of the threshold stimuli to produce increased plasma solute concentration during exercise. After 120 h the [Na^+] was unchanged whereas PV was 1.6% above baseline values. The slight increase above base line values of PV after 120 h is in accordance with a prior report of seven consecutive days of hill-walking (40). Williams et al. (40) assumed, that the mechanisms responsible for water retention took longer than one day to become fully operative, and subsequently more than counterbalanced the net water loss. The present study could show, that the PV can be maintained over a period of several days of exercise even under conditions with a net fluid deficit of almost 4| .

During the course [TP] was markedly higher than predicted by ΔPV. An E-value of +8 after 72 h and +4.2 after 120 h indicated a significant shift of plasma proteins into the intravascular space during the period of exercise, similar to changes found immediately after a marathon run (33). Additionally to previous studies we found a significant relationship between COP and [TP] after 72 h and 120 h of strain. If this relationship had been based only upon haemoconcentration, ΔCOP and %ΔPV should have been correlated. This was not the case, but COP and the E values correlated significantly at 72 h and after 120 h, indicating that changes of the $IVTP_{pl}M$ lead to the changes in COP.

These results supported the hypothesis that one important factor for the maintenance of PV during prolonged exercise is provided, beside other complex regulating mechanisms (7), by protein shifts from the extravascular into the intravascular space (4,33).
In a critical evaluation of the used formula given by Strauss (38) for the calculation of PV changes reference should be made to the fact, that this formula includes Hct and [Hb]. Reliable results can only be achieved in the absence or with knowledge of the amount of intravascular haemolysis. Therefore we measured HAPTO providing an objective marker for intravascular haemolysis, which occurred only in a negligible amount in the present study (see below).

The elevated values of [BUN] and [UA] during the course are indicating at catabolism and reduced excretion. Ad libitum rehydration led to normal values of these parameters within 48 h. Also, the highly significant increased [Cr] at the end of the course was not due to haemoconcentration. This could be proved by an increased PV of 1.6% at this time and by the fact that [Cr] showed no statistical relationship (regression analysis, Student´s *t*-test, ANOVA) with [BUN] and [UA]. Thus, this increase could be interpreted as an independent factor indicating at a reduced renal excretion of creatinine. This points out a reduced creatinine clearance which correlates with a decrease in urine flow mainly due to a reduction in glomerular filtration rate (9).

The most prominent findings of the second question addressed in the present study were the decreased EPO levels during survival training and a rapid increase post exercise (Fig. 4). HAPTO concentrations decreased during and increased after the survival training (Fig. 5), TRANS decreased continuously (Fig. 6). [Fe^{++}], [Hb] concentrations and Hct were inversively related to EPO concentration. Data on the EPO response during comparable conditions of prolonged physical strain are scanty available in the literature. Most likely is the study done by Lindemann et al. (24) who measured haematological changes during a Norwegian combat course. Some of the EPO related haematological parameters ([Fe^{++}], HAPTO, TRANS) in this study behaved similar to our findings but Lindemann et al. (24) found in contrast to our findings an increased erythropoietic activity during the course. Unfortunately, at that time only pooled plasma from a group of subjects could be used and that method cannot serve reliably as a reference anymore. De Paoli Vitali et al. (14) measured EPO

concentrations in 11 well-trained athletes before and immediately after a 50-km cross-country ski race. They found an increase immediately after the race. Vedovato et al. (39) analysed EPO concentrations in 18 athletes before and immediately just after a 20-km long-distance run. They found as well a marked increase in EPO concentrations. Schwandt et al. (36) analysed the influence of prolonged physical exercise in marathon runners on EPO concentration before and several hours after a race. It was found that EPO values increased significantly 3 hrs, and more impressively 31 hrs after the race.

It is well-known that [Hb] and iron stores are affected by physical exercise which might have influence on a later stimulation of EPO, therefore these data were of special interest. Our soldiers showed normal [Hb] and FER concentration at T1. The [Hb] remained rather stable during the course, which is in contrast to the findings of Lindemann et al. (24). In our study HAPTO decreased from 165.4 ± 55.3 $mg \cdot dl^{-1}$ at T1 to 85.8 ± 51.7 $mg \cdot dl^{-1}$ at T3 and the mean plasma volume was approximately 3.5 l. Then the [Hb] loss by haemolysis would have been only 1.83 g haemoglobin. Thus, it could be shown, that haemolysis occurred only in a negligible amount. This finding can be explained by the fact, that all marching exercise had to be performed in a terrain with soft ground, wearing special combat boots with soft and thick rubber soles.

On the other hand, the biochemical constellation of an increased serum [Fe^{++}], a decreased TRANS and an increased FER is a typical sign for a haemolytic anaemia with impaired erythropoiesis (20) which would be in line with decreased EPO values found during the survival training program. However, the interpretation of the given data is complicated by the fact that while Ferritin is a form of storage protein for iron, it is also an acute phase reactant (12). As pointed out by Moore et al. (25) some acute phase-like-disturbances may occur as transient normal adaptations to the multifactorial stress of comparable military conditions. Thus, the serum concentration of Ferritin can rise sharply in the absence of any change in total body iron stores. The classical biochemical constellation of an haemolytic anaemia might be misleading under the described conditions.

The body mass changes found in our group are similar to those of astronauts. Astronauts usually show after space flights lasting longer than one week a body mass loss of about 7% and sometimes a negative inflight nitrogen balance up to -4.5 $g \cdot day^{-1}$ as it is known from the six crewmembers of the first two Skylab Missions (23,26). Furthermore, the astronauts regularly show a decreased red cell mass (26) and decreased EPO levels inflight whereas postflight a rapid increase of EPO production and release was observed (22). These results were recently confirmed by EPO data from one cosmonaut of the German MIR´92 Space Mission (32) and from four astronauts of the German D-2 Space Mission (17).

The reason for the decreased EPO level is not yet known. It is interesting to note that the time course and extent of the EPO response are similar to findings of the present group. The multifactorial strain of work (except the physical work load) of the astronauts of the German D-2 Space Mission was comparable to the present group. It is known that especially during the first days under micro-g-conditions vomiting occurs and food and fluid intake are usually reduced. It might well be that the reduced EPO production observed during space flights is influenced by an overall lack of caloric and/or protein intake, which was described for astronauts and cosmonauts in the literature (26). This conclusion is in line with the findings of Caro et al. (8) and Rosenberg et al. (34). Caro et al. (8) investigated the effect of the thyroid hormone T3 and glucose supplementation on EPO production in rats and found that a 48 h fasting significantly reduced the circulating levels of thyroid hormones and the production of renal and extrarenal EPO in response to hypoxia. In their opinion the caloric deprivation is primarily responsible for the decreased EPO levels induced by fasting in rats and that this effect is probably mediated by a decreased level of T3 and a decreased responsiveness to it. Although in contrast, Jelkmann et al. (19) who investigated the effects of fasting on EPO production in rats found that a reduced food intake cannot account for the fall in EPO during continuous hypoxia. However, in the present study near sea-level (430-570 m altitude) lowered T3 levels in the subjects can be expected as shown by Aakvaag et al. (1). Furthermore, Opstad and Aakvaag (29) investigated under comparable conditions of strain well-fed subjects and a group of soldiers with food deprivation, revealing significantly higher levels of T3 in the well-fed subjects. It is interesting to note that those subjects of our group who had the most impressive EPO increase during the recovery period reported that they had eaten mainly sweets, cakes, carbohydrates during the recovery period. This observation would support the results from Caro et al. (8) that it is mainly the caloric intake which influences the responsiveness of the erythropoietic system during food deprivation. These findings are not in line with those studies in rats and man from Bethard et al. (6),

Reissmann (31), Anagnostou et al. (3), Catchatourian et al. (10) and Rosenberg et al. (34) who concluded that protein intake is more essential for maintenance of normal erythropoiesis than total caloric intake.

Concluding Remarks

1. The results of the present study provide evidence that, physical exercise of moderate intensity lasting 120 h confounded by food deprivation and a fluid loss of almost 8% of the total body water did not change PV in a biologically relevant amount. Important counterregulatory mechanisms were, at least in part, protein shifts into the intravascular space, accompanied by a potentially dangerous reduction of the glomerular filtration rate. This result of the study already lead to a cancellation of the fluid restriction during survival training of the Austrian Army ranger course.

2. The knowledge of the mechanisms behind EPO production and release under terrestrial conditions is not only essential for the humans' well-being during and after prolonged physical strain with food and fluid deprivation on earth but as well as for astronauts' long-term space flights, i.e. to develop effective countermeasures against the "astronauts' anaemia" and a general understanding of the factors being relevant for EPO control under physiological and pathophysiological conditions.

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Sleep Recovery from Physical Exercise: A New Understanding of Brain Responses to Stress

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Abstract

The effects of physical exercise on human sleep (exercise in temperate conditions, in the cold and in hot climates) are analysed and discussed in comparison to studies on sedentary sleep in extreme environments (tropical and polar climates), and on sleep in rats after stressful events (sleep deprivation). An attempt to interprete the stress-induced sleep changes is developed, involving a "central" response and a "general" stress response. These responses ("diachronic" or "synchronic") are also examined in relation to chronobiological mechanisms.

INTRODUCTION

Most studies on physiological recovery after physical exercise concern immediate reactions. The number of studies dealing with long-term recovery, especially with the restorative functions of sleep after exercise is indeed limited. The effects of physical exercise on sleep patterns are still subject to controversy (4). The association of the secretory peak of growth hormone with sleep onset (39, 48) initiated the restorative theory of sleep which implied that physical exercise would induce an increased need for slow-wave sleep (38). However, although Horne (19, 20, 21, 22) agrees with the coexistence of growth hormone and the increase mitotic activity during nocturnal sleep, he denies the role of physical restoration to slow-wave sleep. Horne and Staff (23) attributed the changes in slow-wave sleep to a modification in the cerebral function. Furthermore, the after-effects of exercise on sleep depend on the fitness level of the subject, the exercise programme, and the degree of strain imposed on individuals. Sleep changes were attributed to stress-induced sleep disturbances or a "hyperthermia"-induced increase in slow-wave sleep (4). When the strain due to exercise provokes a diurnal stress reaction, marked for example by increased adrenal cortical activity, slow-wave sleep may be impaired. If the stress reaction extends through to the following night, rapid-eyemovement (REM) sleep will also diminish (8, 9). The hyperthermia effect is characterized by increased total sleep time and slow-wave sleep (23). The latter can be counteracted by body cooling during the exercise (24). This may explain why marathon runners show either no variation in slow-wave sleep after exercising in a cold climate (49) or increased amounts of slow-wave sleep, when running in a warm climate (44, 45). However, recent development in studies on sleep deprivation in animals tend to relate the rebound in sleep during recovery to a stress response modulated in the central nervous system.

Our purpose was to further examine the implication of this hypothesis on sleep changes after exercise, through experiments conducted in our laboratory on sleep in extreme environments.

METHODS

Although sleep can be approached through interviews, questionnaires and sleep diaries, the only objective method to record sleep patterns remains the polysomnographic technique. Polysomnography requires carefully placed electroencephalographic (EEG) electrodes, following the 10-20 electrode system. Other electrodes are fixed near the eye sockets to record electrooculogram, and at the tip of the chin to record electromyography, as the EEG and these two parameters are necessary for the scoring of the states of vigilance. Polygraphy may also include the recording of heart rate by electrocardiography, respiration to

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

detect sleep apneas, body temperature, or even the pH of the lower cesophagus to examine gastro-cesophageal reflux. The subject can be recorded directly through a connecting cable attached to an EEG machine, or an ambulatory system such as the portable Oxford Medilog 9000 series system, which is able to record 8 channels of electrobiological signals for 24 hours on a C-120 audio cassette. The traces are scored using the international classification (42) in order to produce a hypnogram, which represents the distribution of the states of vigilance throughout the night. The states of vigilance are represented by wakefulness, REM sleep and non-REM sleep. The latter is constituted of 4 stages: stage 1 being a transitory stage, stage 2 being characterized by the occurrence of sleep spindles and K complexes, and stages 3 and 4 containing an increasing proportion of slow-waves of high amplitude (delta waves, 0.5 to 4 Hz) and are thus called slow-wave sleep. The night of sleep is rhythmic, due to the succession of 3 to 6 REM sleep episodes, equally spaced at 90 min intervals, determining 3 to 6 REM-non-REM sleep cycles. The first sleep cycle starts with sleep onset till the end of the first REM sleep episode; the second extends from the end of the first to the end of the second REM sleep episode, and so on. Under temperate conditions, in young healthy sedentary subjects, slow-wave sleep occurs during the first half of the night and mostly during the first two sleep cycles (Fig. 1). Contrarily, REM sleep episodes are longer during the second half of the night. Calculations regarding each stage of vigilance are made versus sleep period time (from falling asleep to the last awakening) for wakefulness and versus total sleep time (sleep period time minus intercurrent wakefulness) for the stages of sleep (10).

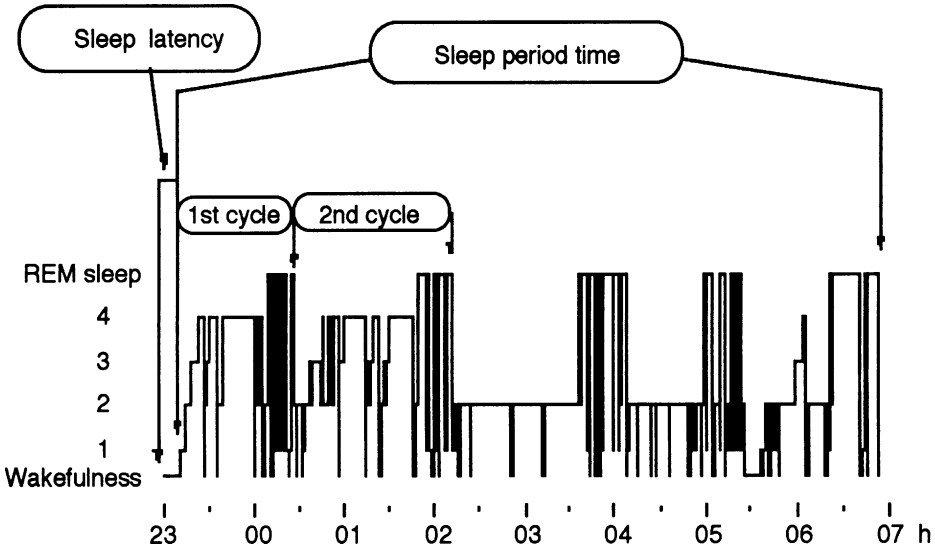

Fig. 1. Hypnogram showing the distribution of the stages of vigilance (wakefulness; REM sleep; non-REM sleep, stages 1 to 4) throughout night sleep in a young healthy subject living in a temperate climate.

In all our experiments, polysomnographic recordings were taken for 3 consecutive nights, the first night being used for subject adaptation to avoid the well known first night effect sleep disturbances attributed to the laboratory environment (1).

Under temperate conditions and in young healthy subjects, the proportions of the stages of vigilance are approximately as follows: 1-5 % of wakefulness, 1-5 % of stage 1, 45-55 % of stage 2, 16-23 % of slow-wave sleep and 18-23 % of REM sleep.

INDIVIDUAL SLEEP PATTERNS AFTER EXERCISE IN A TEMPRATE CLIMATE

Sleep patterns were studied after moderate exercise in temperate conditions under a joint experiment with the Franco-Canadian Accord for Defence Research (8). The experiment took place in the fall near Lyon. The subjects marched for 6 hours at 6 km.h-l during 6 consecutive days, between 09:00 h and 17:00 h. Each subject carried a back pack to adjust energy expenditure to 40 % of individual tO2maxX The experiment was

divided into three epochs, with a 5-day baseline, 6 days of exercise and 5 days of recovery. Sleep was recorded every night. Urines were collected from 09:00 h to 17:00 h, from 17:00 h to Z:00 h and during the night from 22:00 h to 06:00 h, and 17-hydroxycorticosteroids (17-OHCS) were analysed to serve as an indicator of adrenocortical activity.

Fig. 2 demonstrates that individuals experienced differences in sleep patterns after exercise, in relation to adrenal cortical activity. Subject S5 was a farmer for whom the march did not represent a s train, as s how n by a decrease of adrenocortical activity. He showed an increase in both stages 3 and 4 after exercise. The next 3 subjects showed an increase in stage 3 without any modification in 17-OHCS excretion during the march. Subject S4 had an increased excretion of urinary 17-OHCS, and also showed no change in stage 3. On the contrary, subject S6, who exhibited the largest increase in 17-OHCS excretion during the march, had a decrease in stage 3 and stage 4. The increased adrenocortical activity extended into the night: he was the only subject with a decrease in REM sleep.

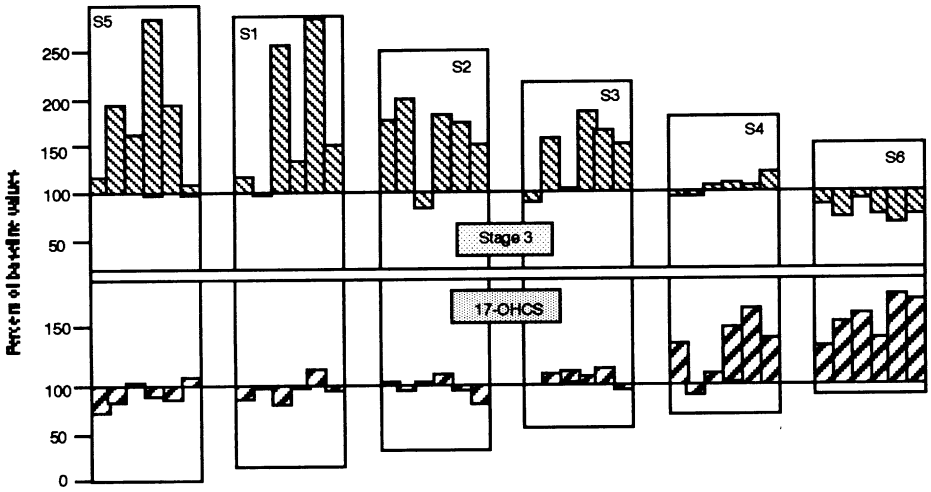

Fig. 2. Sleep pattern changes (Stage 3) in the 6 subjects (S 1 -S6) during the 6-day exercise period in relation to the daytime urinary excetion of 17 hydroxycorticosteroids (17-OHCS) while marching. The values obtained during the 6-day exercise period were expressed as a percentage of baseline values for each individual. The subjects were ranked in decreasing order of slow-wave sleep increase, which corresponds to the increasing order of 17-OHCS secretion.

These results can be interpreted in terms of the presence or the absence of a "classical" stress reaction, as evidenced by an increased heart rate during night sleep (43).

These results can also be interpreted in terms of chronobiology. In S4 and S6, two types of effects were observed. There was a decrease in total sleep time and in slow-wave sleep which was distant from exercise. This type of delayed reaction has been called "diachronic" by Jouvet (28). The second effect was the decrease in REM sleep in S6, which was concomitant with an increase in adrenocortical activity at night, i.e. a "synchronic" reaction. In the other 4 subjects, the absence of any stress reaction during the exercise led to a diachronic increase in slow-wave sleep.

This diachronic increase in slow-wave sleep could also be refered to as an effect of the exercise-induced hyperthermia, not counterbalanced by the stress effect. It has been reported that sauna exposure (41) or a hot bath (32) induce an increase in slow-wave sleep the following night.

To answer these questions, sleep was studied in sedentary subjects and in athletes with and without physical exercise in a dry tropical climate in Africa.

SLEEP IN SEDENTARY SUBJECTS IN A DRY TROPICAL CLIMATE

The study took place in Niger over a period of 12 years, in a sahelian climate, during the dry season, because of power failure in the rainy season. In the Sahel, the dry season is divided in a cool season in January and February and a hot or very hot season in April, May and June. During the month of March, the ambient temperature increases steadily. The experiments took place at the Faculty of Medicine of Niamey. The polysomnographic recordings were realised in an airconditioned laboratory, at ambient temperatures of 23-24 °C. Sleep patterns were analysed in 34 African students in sedentary conditions and in 6 French military expatriates. All subjects were recorded by direct polysomnography during 3 consecutive nights in each of the cool and hot seasons.

The hypnograms of African subjects (Fig. 3), as well as those of European expatriates (unshown data, 35), showed that sleep architecture differed from that of people living in temperature countries(l2, 16).

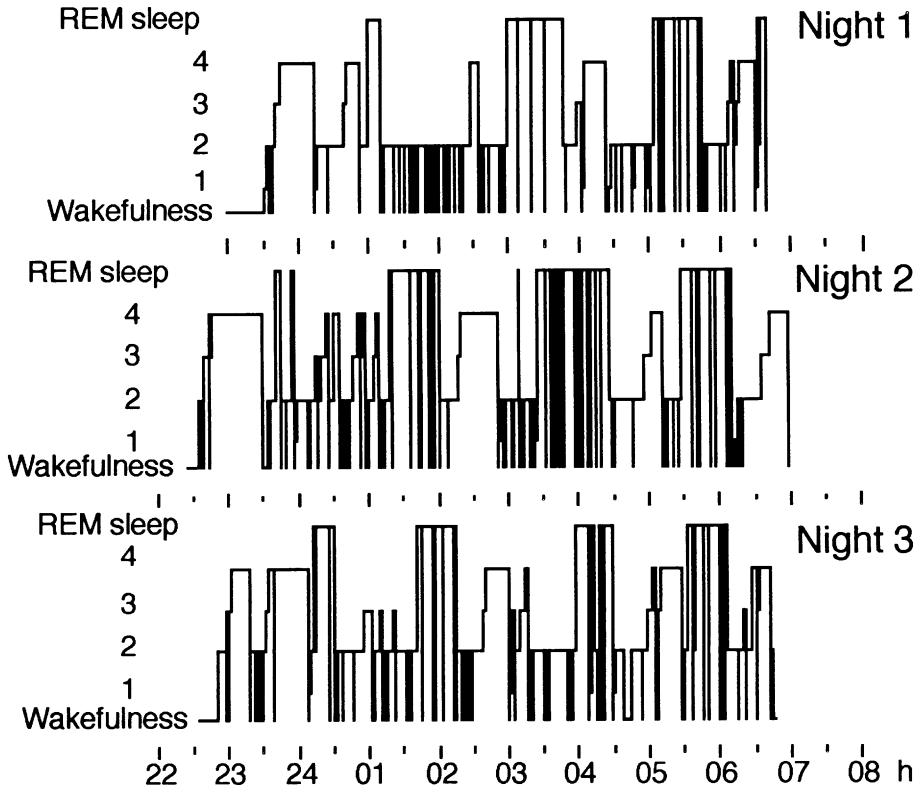

Fig. 3. Hypnograms showing the distribution of night sleep in an African subject during three consecutive nights. The states of vigilance are represented by wakefulness, REM sleep, and non-REM sleep (stages 1, 2, 3 and 4).

The modifications in the amount of slow-wave sleep were related to the ambient temperature and slow-wave sleep increased in the hot season compared to the cool season. The increase in slow-wave sleep during the hot season occurred at the expense of stage 2. This was well demonstrated in the European expatriates who were also studied during the transitory March period (Fig. 4).

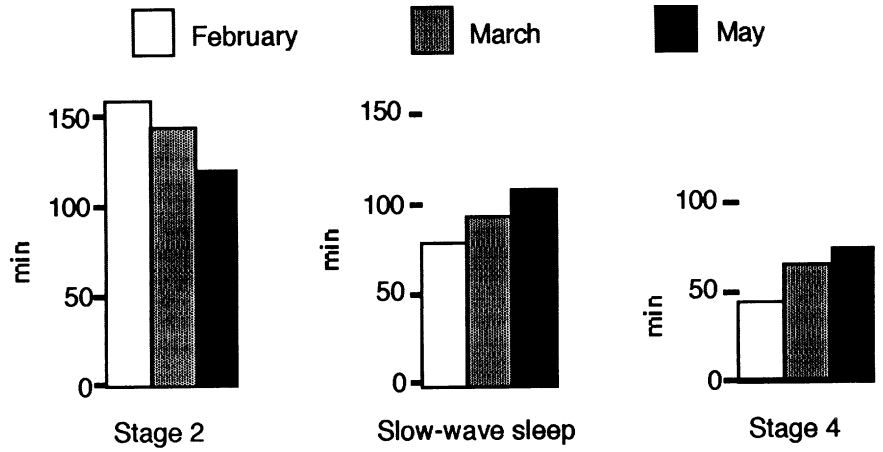

Fig. 4. The effect of the seasonal heat on sleep patterns of European expatriates during the cool (February) and hot (May) seasons and during the intermediary month of March.

In conclusion, daytime exposure to climatic heat led to diachronic changes in sleep patterns, especially an increased amount of slow - wave sleep proportional to the environmental heat. To determine whether heat-induced sleep pattern changes would be modified by endogenous heat production, the effect of exercise under such climatic conditions was analysed.

SLEEP AFTER EXERCISE IN SUBJECTS LIVING IN A DRY TROPICAL CLIMATE

The effects of progressive training were analysed in sedentary subjects (13), using a square wave endurance exercise test performed on an ergometer. The physical fitness level of the subjects (maximal aerobic power, MAP) was evaluated from a triangular maximal test with 25 W increments every 2 min performed before training. VO_{2max} was estimated from MAP values and confirmed the sedentary quality of the subjects (between 2.1 and 2.8 $L.min^{-1}$)

The training programme consisted of a 6-week square-wave endurance exercise test (SWEET) performed 3 times a week. The test was made of a repeated 5-min sequence with a 4min submaximal plateau followed by a 1-min maximal peak. The number of sequences was increased from 5 in the first week to 7 in the second week, then to 9 during the following 4 weeks. The improvement of physical performance was judged using triangular maximal tests after the third and sixth weeks of training. The third week triangular test served to adjust the work load to the improved level of MAP. All the exercise tests were performed at laboratory temperatures of 24-25 °C. The training programme was realised twice, during the cool season and the hot dry season of the sahelian dry tropical climate, with an interval of 10 weeks during which routine sedentary activity was resumed. Physical fitness improved during each training session, but had returned to baseline values after the 10-week training interruption. Baseline polysomnographic recordings were taken during 3 consecutive nights, preceding the training session. During the fourth and sixth weeks of each exercise period, sleep recordings were performed during the last 2 consecutive nights following day exercise.

After exercise (Fig. 5), slow-wave sleep increased during the cool season, compared to baseline values. This increase was enhanced during the hot season. Therefore, the conjonction of muscular heat production and external climatic heat load led to a diachronic increase in slow-wave sleep. REM sleep was not influenced by exercise nor by the season.

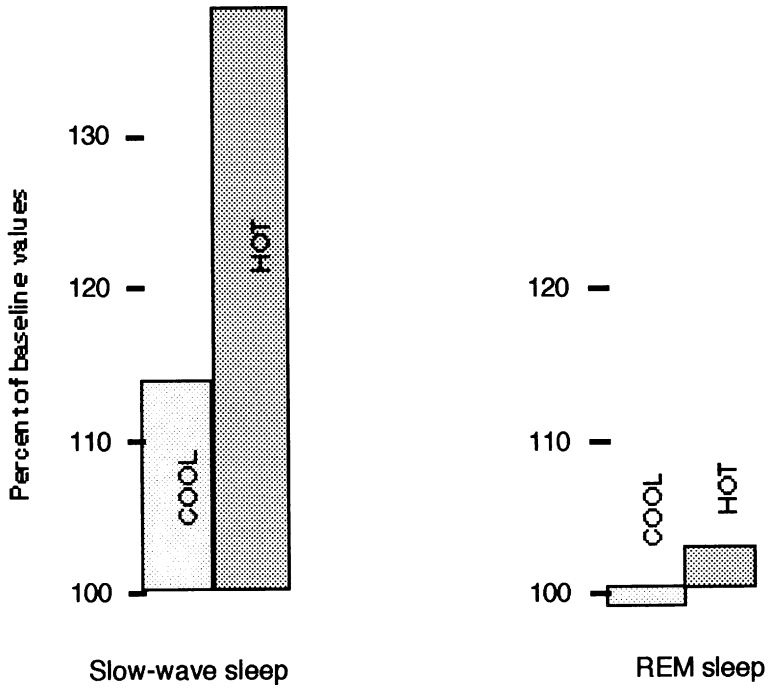

Fig. 5. Combined effect of physical training and seasonal heat variations on slow-wave sleep (SWS) and REM sleep.

Sleep patterns were also studied during the two seasons in African sportsmen in baseline sedentary condition and after exercise with and without rehydration (36). All conditions were randomly assigned. The exercise programme consisted of 3 sequences of cycling, beginning with 10 min at 30 % of MAP, followed by an exhaustive supramaximal effort (1 to 2 min at 130 % of MAP), and ending with a 10 min recovery at 30 % of MAP. Polysomnography was recorded for 2 consecutive nights in each condition. The first session however, regardless of the exercise condition, included one additional familiarization night.

Baseline slow-wave sleep and REM sleep were high (Fig. 6). In the hot season, there was an overall increase in slow-wave sleep, due primarily to an increase in stage 4. Conversely, stage 2 decreased. This effect was also observed after exercise with rehydration, but was absent in the non-hydration condition. This distinction between the rehydrated and non-rehydrated condition is thought to be due to the greater stress which may accompany an exercise-induced relative dehydration. This would also lead to a relative increase in body temperature, as drinking while exercising limits this increase (33). The effects observed were diachronic in baseline and exercise with rehydration conditions. They may have been synchronic in the heat after exercise without rehydration.

Compared to baseline values, REM sleep (Fig. 6) was lower in the cool season after exercise in both hydration conditions due to shorter phases. This decrease in REM sleep could also be related to a stress reaction occurring or persisting during the night (9).However, it did not occur in the hot season. This difference may be related to the interaction of exercise and heat acclimatization on water balance regulation. Born et al. (3) demonstrated recently that REM sleep is reduced by vasopressin. Both heat exposure and exercise are known to increase plasma vasopressin (34) whereas vasopressin secretion is lowered in heat acclimated subjects (15).

These data emphasise the complexity of the sleep-wake modifications after exercise, with interactions between hyperthermia, water balance and stress.

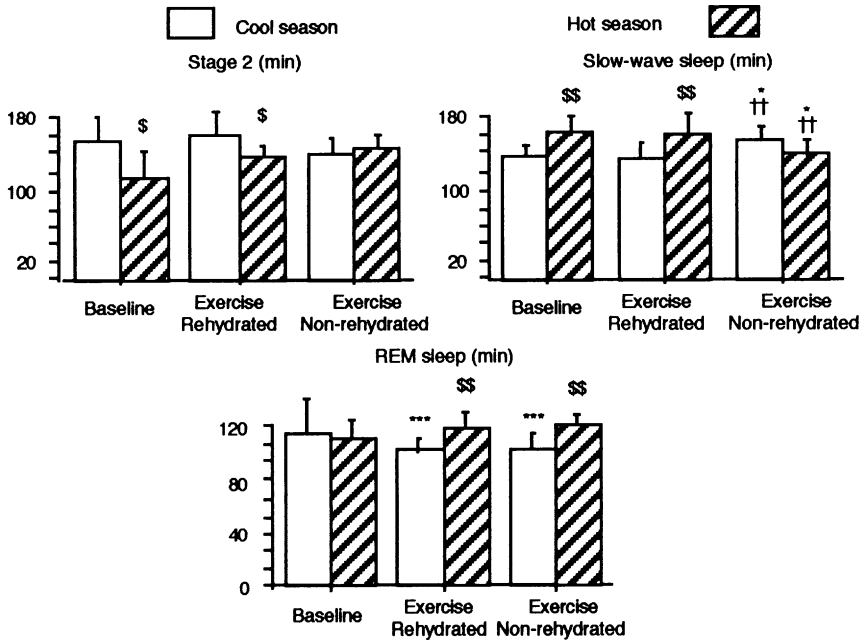

Fig. 6. Combined effect of exercise with and without rehydration and seasonal heat variations on slow-wave sleep and REM sleep in African sportsmen. Significant variations between seasons ($: p<0.05 $$: p<0.02, $$$: p<0.01), between exercise and baseline data (*: p<0.05) and between the two hydration conditions (†: P<0.05; ††: p<0.02; †††: p<0.01) are indicated.

SLEEP IN POLAR CLIMATES

In Antarctica, polysomnograms were recorded in 8 men wintering on the French base of Dumont d'Urville, which is situated under the antarctic circle (10). The base enjoys a microclimate and has been called the "Côte d'Azur" of the Antarctic and was our "island in the sun". As soon as the sea ice permitted, the winterers performed daily outings to visit the magnificent glacier of the Astrolabe, or our neighbours, the Emperor pinguins in winter, and the seals in the spring. This led to a progressive increase in physical fitness accompanied by a progressive increase in slow-wave sleep. These results differ from those of other antarctic polysomnographic studies. At the south pole (27, 46), the base is situated at an altitude of 2,804 m, ambient temperature averages -51 °C and the polar night lasts for 6 months. Slow-wave sleep is highly disturbed and even disappears during the winter. REM sleep is also decreased due to altitude periodic breathing. At Halley Bay (39), situated on an coastal iceshelf, far south of the antarctic circle, slow-wave sleep decreases during the wintering months with the polar night. At Mirnyy (2), under the arctic circle, the Russians reported no change in sleep patterns in winterers living on their coastal base. We attribute the differences with our study to the exceptional attractiveness of Dumont d'Urville's surroundings, which lead to an improvement of physical fitness due to daily outings.

Polysomnography and body temperatures were also recorded in the Arctic, where subjects slept under unheated tents for 10 to 16 consecutive nights (5, 6, 7). Compared to thermoneutral conditions, sleeping in the cold occasioned a large decrease in rectal temperature, which reached 34.9 °C in the middle of the night. Slow-wave sleep was preserved, as it occurred during the first half of the night. However, when the subject was in hypothermia, he could not maintain sleep anymore. Many awakenings interrupted lighter sleep made of stage 1 and stage 2. REM sleep episodes occurred at the same time as they did in the thermoneutral condition. However, REM sleep could not be maintained. This may be related to the fact that body movements and shivering are suppressed during REM sleep.

REM sleep deprivation, expressed as a percent of baseline values, was proportional to the intensity of cold exposure and also proportional to the excretion of 17-OHCS during the night. Therefore, REM sleep shortening was synchronic of the stress reaction.

Three of our subjects had been preacclimated to cold by 9 cold bath sessions of one hour in water at 10 °C. Contrary to our subjects, they did not show any increase in nocturnal diuresis, in 17-OHCS excretion or in noradrenalin excretion. They also did not have any change in their sleep patterns, especially no REM sleep deprivation. This demonstrated that acclimation can prevent the synchronic stress reaction to occur.

SLEEP AS A STRESS REACTION

Jouvet and his group (14, 29) proposed recently an explanation of the intervention of the nervous system in stress reactions following sleep deprivation. In the rat and in the cat immobilization induces sleep deprivation with a stress followed by a rebound in REM sleep and slow-wave sleep. When sleep deprivation is obtained by cuddling the animal, there is no stress and no rebound phenomenon. Stressful sleep deprivation induces an increase of axonal release of serotoninergic neurons ending in the arcuate nucleus (26), one of the main hypothalamic structures which produce proopiomelanocortin. After successive cleavages, this large protein gives ACTH and its two derivatives, a-MSH (melanostimulin) and CLIP (cotic tropin-like intermediate lobe peptide) which represent somnogenic peptides. Such peptides are in turn released in the raphe nuclei where they induce a dendritic release of serotonin and an autoinhibition of serotoninergic neurons, leading to a rebound in REM sleep (14).

Therefore (Fig. 7), when animals are sleep deprived using a stressful technique, a central response involving the arcuate nucleus induces a diachronic REM sleep rebound. When sleep deprivation is gentle, there is no such "central" response and no rebound phenomenon occurs. This is also observed after a lesion of the arcuate nucleus in hypophysectomised rats (50). Such a mechanism could be involved in the changes in sleep patterns in man, not only after sleep deprivation but also after exercicse or exposure to extreme environments.

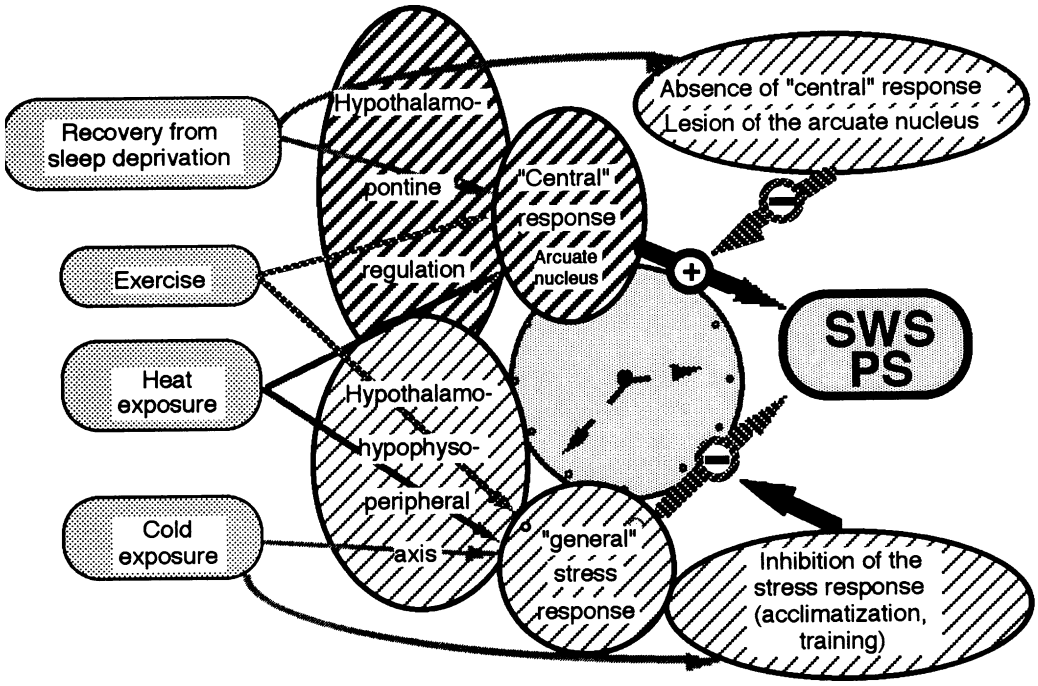

Fig. 7. Recapitulative interpretation of the stress-induced sleep changes after sleep deprivation, exercise and exposure to extreme environments (see text for legend).

Exercise has two effects. The diachronic enhancement of slow-wave sleep is observed in the absence of activation of the adrenal cortical glands and could therefore use the central POMC-serotonin pathway. This effect is observed in moderately trained subjects and in athletes performing a tolerable exercise. A diachronic (such as a decrease in total sleep time and in slow-wave sleep) and synchronic (such as a decrease in REM sleep) sleep disruption would occur when this pathway is overloaded or bypassed, leading to the activation of

the hypothalamo-pituitary-adrenal axis. This is observed when the exercise load is too important for the subject or when the subject is not used to the exercise conditions.

A similar regulation is observed after daytime heat exposure. The diachronic increase in slow-wave sleep would be due to the activation of the central pathway. The synchronic decrease in slow-wave sleep is observed when the subject sleeps in an unusually hot environment.

Cold exposure at night leads to synchronic stress reactions with the activation of stress hormones. The synchronic stress reaction can be limited by previous acclimation or training.

In conclusion, when the brain can deal with the stressful situation, the diachronic increase in slow-wave sleep and/or REM sleep occurs. When this pathway is overloaded, the classical stress reaction occurs with diachronic and synchronic disruptions of sleep architecture. In any case, we believe that the enhanced slow-wave sleep after exercise in temperate climates and in people living in a tropical country, both in sedentary conditions or after exercise, is beneficial. It may serve to lower energy expenditure, as oxygen consumption is at its lowest during slow-wave sleep (17, 46), but also to lower body heat content. This is achieved by an increase in evaporatory heat loss concomitant with slow-wave sleep (17, 18, 37) and by a lowering of core temperature, as was demonstrated in a patient with an aplasia of sweat glands (11). The lowering in metabolic brain activity during slow-wave sleep would allow the occurrence of REM sleep, a state of high energy consumption (29). Furthermore, REM sleep occurs preferentially when core temperature is low (30). In man, it is a state of poor thermal regulation, with an inhibition of sweating in the heat (18) and shivering in the cold (6), leading to increases in skin and core temperatures (18). Therefore, body core cooling during slow-wave sleep may be essential in permitting REM sleep to occur after external or internal heat load.

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Acute Recovery of Physiological and Cognitive Function in U.S. Army Ranger Students in a Multistressor Field Environment

by

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1. ABSTRACT

The time course for soldier recovery and reentry to the battlefield following high intensity direct action missions is of importance to unit commanders and military planners. It also provides a critical background understanding for medical researchers investigating approaches to extend the limits of soldier physical and psychological endurance. This report summarizes findings for soldiers followed through two recovery periods, during and after, a prolonged exhaustive military activity, the U.S. Army Ranger course. Within the course, with a modest increase in sleep and energy intake for 7 days, the soldiers demonstrated recovery of some cognitive function (e.g., decoding and pattern analysis) and acute metabolic/stress markers (e.g., serum testosterone, IGF1, and triiodothyronine). More complex intellectual processes (e.g., reasoning), other biochemical indicators (e.g., hyperlipidemia, serum cortisol), and cell-mediated immune function (e.g., *in vitro* PHA-stimulated T-lymphocyte proliferation) demonstrated progressive changes in response to the cumulative stress and were unaffected by the partial cessation of stressors during the course. Five weeks after the course, all of these parameters demonstrated recovery, or even overshoot. These data illustrate the remarkable resilience of fit young soldiers and demonstrate that a brief period of increased sleep and feeding partially restores soldiers before reentry to combat.

2. INTRODUCTION

The multiple stressors in a combat environment produce an overall stress burden with consequences similar to those reported in athletic "overtraining." Overtraining is defined here as unusually high intensity effort with inadequate physiological recovery, leading to degraded military performance. Problems associated with such prolonged exhaustive military activities, or "military overtraining," include a reduction in physical capabilities and cognitive function, and possibly a reduced ability to recover from infection and injury. This overtraining phenomenon occurs even in the absence of an infectious disease or traumatic injury overlay.

The earliest functional recovery of soldiers following intensive and stressful missions is of great importance as a force multiplier. The combination of stressors such as high energy flux, energy deficit, inadequate restorative sleep, anxiety, ambient heat etc., have been shown to produce critically important physiological consequences which can produce critical functional impairments (1,2). The stress and fatigue responses produce neuroglycopenic symptoms marked by suboptimal cognitive function. Resultant errors in interpretation and judgement can produce catastrophic failures in combat, such as misidentification of friendly forces and selection of a poor course of action. Stress-induced reductions in immunocompetence increase susceptibility to infectious disease, with the possibility of performance degradations or incapacitation of entire units. Reduced immune function may also be an early indicator of other impairments in soldier stress

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Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

defenses. Thus, cognitive and immunological status are critical factors in the decision to return soldiers to duty after an intensive mission.

This report describes the pattern of recovery of measures that reflect these two critical capabilities and compares them to patterns of change in metabolic/stress markers. This type of descriptive study is important to subsequent pursuit of safe and effective strategies to prevent decrements or enhance recovery of performance such as identification of critical timepoints for sleep and refeeding, and use of pharmacological enhancers, neurotransmitter precursors (e.g., tyrosine and choline), and immune function boosters (e.g., anti-oxidants).

3. THE RANGER COURSE AS A MODEL OF MILITARY OVERTRAINING

3.1 Course description

The U.S. Army Ranger course is designed to train young infantry leaders through discovery of their strengths and weaknesses under stress and development of strategies to cope with leadership challenges in this environment. This course deliberately limits food intake and sleep and includes other environmental stressors which are components of realistic tactical training (Table 1).

Table 1. Principal categories of environmental stressors encountered in summer Ranger training.

Stressor (reference)	Description & method
Food restriction (2)	Deficit=1000 kcal/d for 8 wks, from body composition change
Sleep restriction (3)	Sleep=3.6 h/d for 8 wks, from 24 h wrist activity monitors
Physical exertion (4)	Energy expenditure=4000 kcal/d, up to 6000 kcal/d, from $^{2}H_{2}{}^{18}O$
Weather (1)	Avg daily maximum temperature=30 C; relative humidity=~80%
Medical problems (5)	Blisters, insect bites, sprains & strains, cellulitis and respiratory infections
Evaluation anxiety	(unquantified)

The Ranger Course is divided into 4 phases of approximately 2 weeks each and exposes soldiers to training in four different environments: temperate forest, desert, rough "mountain" terrain, and coastal swamp. Each phase begins with a few days of adequate feeding and increased sleep while soldiers are taught new skills. This is followed by 7-10 days with one meal per day and more constrained opportunities for sleep, during realistic small unit operations. These typically involve 8-12 km patrols with loaded rucksacks (~35 kg).

3.2 Course stressors

The energy deficit is actually produced with a repeated food restriction and refeeding (a mixed diet, with caloric content divided into approximately 50% carbohydrate, 35% fat, 15% protein) in the four phases through the course (Figure 1). This repeated semi starvation and refeeding produces a profound hunger stressor but without the gastric upset which occurs with fasting in stressful conditions. Energy expenditure fluctuates throughout the course, with the highest measurements by doubly-labeled water (6,000 kcal/d), occurring in the first half of the mountain phase training, when abseiling and climbing techniques are taught. The lowest energy expenditure occurs towards the end of the course, reflecting behavioral and physiological efficiencies induced by the energy deficit.

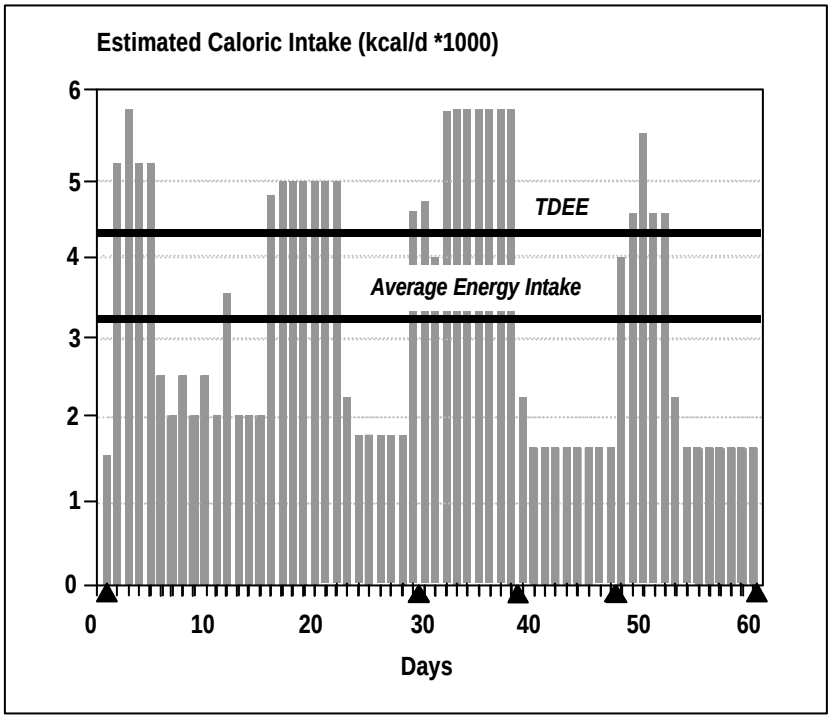

Figure 1. Daily energy intake provided to Ranger students in Class 92-11. Arrows mark data collection points in this study.

Based on data from wrist-worn activity monitors, sleep averaged 3.6 h/d in two separate studies (3). This increases slightly during the first half of each of the four course phases (4.0 vs 3.2 h)(3). Increased amounts of sleep in the last two-week phase of training (4.3 □□0.8 h/d) probably reflect an unavoidable increase in napping during training.

3.3 Functional deficits

Some of the key consequences that have been described in Ranger training are summarized in Table 2. Although there is a substantial and prolonged energy deficit, nutritional biochemistry and physical exams at the end of the 1991 study demonstrated no deficiencies in any key vitamins or nutrients; only retinol concentrations were marginally reduced from baseline and below normal limits (1,2). Protein intake was at least 50 g/d on days with only a single meal. Thus, the primary consequences of the nutritional stressor are an uncomplicated energy deficit. The approaching depletion of available fat energy stores produces an increase in cortisol and an increased utilization of body protein to meet energy requirements (8).

Table 2. Physiological/functional consequences of multistressor exposure in summer Ranger training.

Function (reference)	Description & indicators
Energy metabolism (1)	↓ glucose availability (fasting glucose -20%, insulin -40%); ↓metabolic rate (↓T3); ↑ counterregulatory hormones (growth hormone & cortisol)
Protein status (2)	↑ protein catabolism (-6% of total FFM) without protein deficiency (↓ prealbumin, ↔ total proteins,↑ RBP)
Physical (2,6,7)	↓ max aerobic capacity (-14%); ↓ max lift strength & vertical jump (-20%)
Cognitive (2)	↓ speed in decoding -33%, pattern analysis -15%, and reasoning -20%
Immunological (1,2)	↓ T- & B-lymphocyte proliferation response *in vitro* to mitogens; ↓ IL6

Cumulative stress responses and fatigue near the end of the course produce a mental drowsiness referred to as "droning." Cognitive deficits have been studied in several Ranger studies (2,9). In the 1992 study, rote memorization of a short list of code words was relatively unaffected by severe food and sleep restriction. However, other tests indicated that accuracy on tasks such as decoding map coordinates or analyzing maps could be sustained under severe food and sleep restriction, but predicted that soldiers would take 1.5 times longer than normal to complete the tasks. The ability to make logical inferences or develop a course of action, would also take longer than normal (2).

Immunological function has been primarily assessed in Ranger students using *in vitro* proliferative activity of lymphocytes. No consistent results were obtained in the 1991 study using delayed hypersensitivity tests with antigens to 7 microorganisms. However, phytohemaggutinin (PHA)-stimulated T-lymphocyte activity demonstrated marked and reproducible declines in both the 1991 and 1992 studies, with the largest attenuation in response associated with periods of highest energy expenditure and deficit. Interleukin-2 receptor and IL2 secretion from stimulated lymphocytes demonstrate recoveries before the end of the course and in advance of recovery of the T-lymphocyte proliferative activity (1).

Ranger students are more susceptible to infectious diseases, including problems with cellulitis, uncommon in other soldiers (5). Another problem that is uncommon in healthy adults, Streptococcal pneumonia, has been a significant problem for winter Ranger students (5). A limited trial was made with administration of prophyllactic bicillin to entire classes in an effort to reduce the infectious disease problems (5). A modest increase in food intake appears to attenuate the infection rate and some of the observed decrements in immune function measures (2). The consequences of this multistressor environment can themselves further contribute to the total stress burden of the individual student (e.g., injuries which occur through errors made by fatigued soldiers and illnesses which result from reduced immunological defenses).

4. DESCRIPTION OF RECOVERY STUDY

This report summarizes findings from new data analyses of the 1992 study (2) concerning recovery within one phase of the course and following the course at one and five weeks. Originally, there was concern that Rangers may have lasting impairments stemming from the rigors of the training. Six months after the 1991 study, eight Rangers were reexamined with detailed physical exams and recent medical histories and for physical assessments (1). These soldiers were all fully restored to baseline health and strength; however, they described profound impairments in the first few weeks after training. This recovery study design was built into the 1992 study to further examine the pattern of acute functional recovery within and following the course.

Data for the 10 soldiers participating in the 1992 study are shown in Table 3. Limited data was obtained from blood samples collected from another 9 course finishers at one week after the end of the course; no other data was available on these soldiers. Table 3 illustrates the large reduction in body fat stores, supplying 85-90% of the energy deficit. It also shows the large rebound in fat stores representing 2000 kcal/d excess energy after the course, with unrestricted rest and food intake (9).

Table 3. Body weight and body composition changes for 10 soldiers participating in the Ranger recovery study (means±sem).

----R a n g e r T r a i n i n g----					
Measure	**B/L**	**4 wk**	**5 wk***	**8 wk**	**+5 wk***
Body wt (kg)	73.9±2.9	68.1±2.3	73.9±1.5	65.5±2.3	76.3±3.0
#Fat wt (kg)	9.5±1.5	----	----	5.3±0.9	13.7±1.2
#FFM (kg)	64.4±1.6	----	----	60.2±1.9	65.5±2.2

*Recovery periods; # calculated from body weight and % body fat assessed by dual-energy x-ray absorptiometry

During the first 5 days after the end of the course, soldiers only increased sleep duration to 6.5 h/d. They also demonstrated a reduced quality of sleep over that measured during the course, with greater sleep fragmentation and greater movement during sleep (3).

5. RECOVERY OF COGNITIVE FUNCTION

Soldiers were tested on four tasks that are commonly used in cognitive assessment batteries: information processing, perceptual processing, reasoning, and memory (11). Although the tasks were all administered as timed pencil-and-paper tests and all yielded dependent measures of speed and accuracy, the cognitive processes measured by these tasks differ in complexity, level of processing, and simplicity of response.

Decoding. The decoding task was used to measure speed of information processing. It was a straightforward task, requiring the soldier to translate geometric figures into numbers using a code key. This task could be compared to the task of authenticating military communications. Soldiers could check their work for accuracy as they completed the test.

It is clear from Figure 2 that there was substantial recovery on the mid-mountain phase test and on the post-training test, relative to the desert and jungle phase tests, respectively, and that the degree of recovery was similar in both cases. Soldiers completed approximately 1.4 times as many problems on the post-training test as they had on the jungle phase test. This is supported by the ANOVA, which showed that only the training intensity was statistically significant.

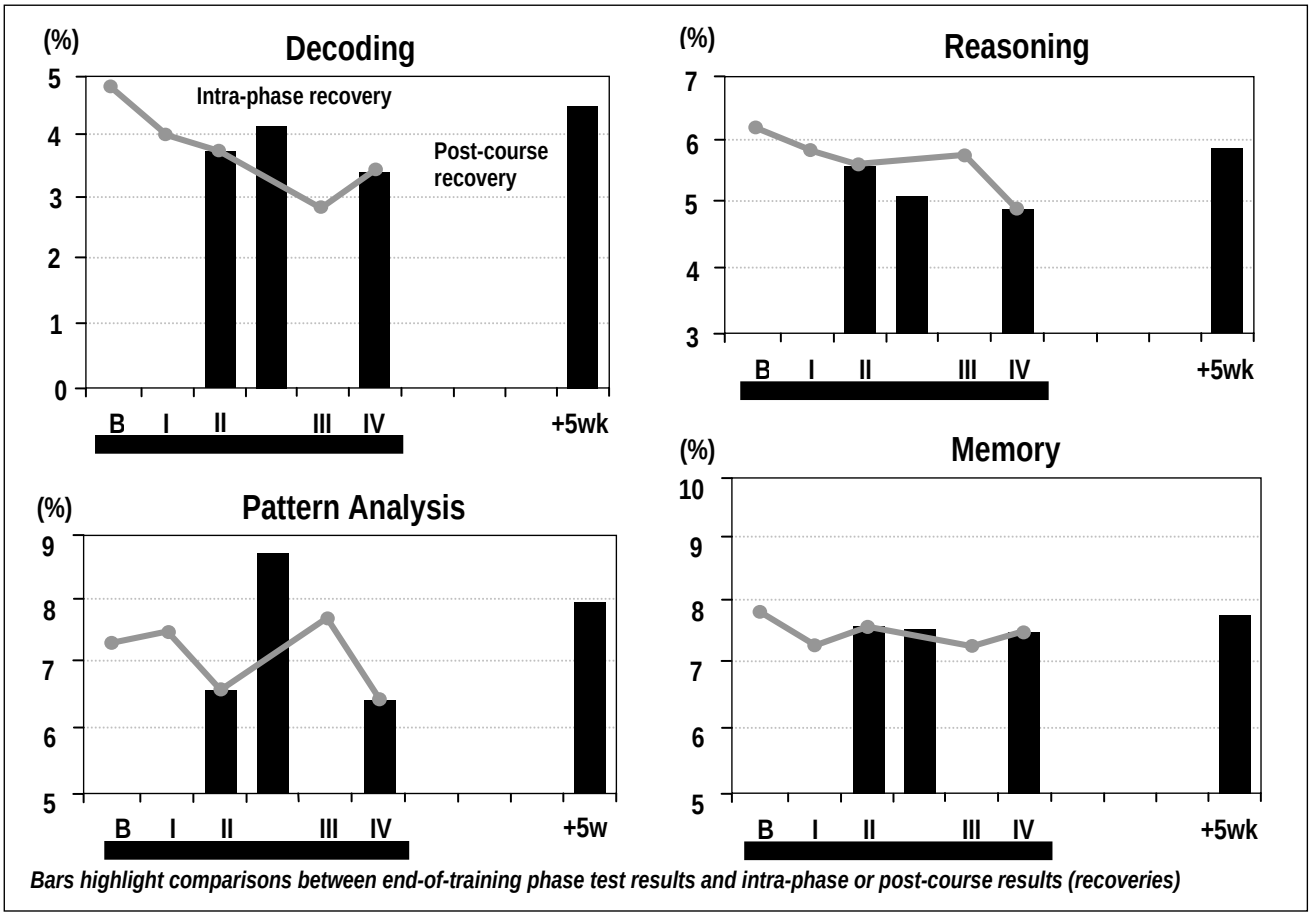

Figure 2. Results of the cognitive function tests for 10 soldiers followed through the Ranger course and subsequent recovery. (Results are shown as % correct responses).

Pattern analysis. The pattern analysis task was used to measure speed of perceptual processing. It was similar to the decoding task; however, the task required a higher order skill. Soldiers were required to analyze complex geometric figures to determine if they contained a simpler figure found in the code key. This task could be compared to the task of analyzing unit symbols on a military map.

Performance on the pattern analysis task was similar to that on the decoding task. Figure 2 shows the same pattern of recovery as for the decoding task. Soldiers completed approximately 1.25 times as many problems on the post-training test as they had on the jungle phase test. Only the main effect of training intensity was significant in the ANOVA.

Reasoning. The reasoning task was used to measure speed of inferential logic. It required soldiers to rapidly determine whether a one-sentence description of the order of a simultaneously presented pair of letters was logically true or false. For example: AB, A is not followed by B (true or false?). This task could be compared to the task of analyzing a plan of action to determine its agreement with a field operations order. An interesting interaction effect was seen in the reasoning data. As shown in Figure 2, performance on the reasoning task did not recover on the mid-mountain phase test, but did recovery on the post-training task. Soldiers completed approximately 1.2 times as many problems on the post-training test as they had on the jungle phase test. The interaction effect was significant but the two main effects were not significant. Pair-wise comparisons showed a statistically significant recovery of performance only on the post-training test, compared to the jungle phase test ($p<0.02$). These data suggest that the more complex intellectual process of reasoning recovers only when food and sleep are unrestricted.

Memory. The memory task was used to measure accuracy of memorization. It required soldiers to rapidly memorize nine pairs of words. Military operations often require memorization of seemingly random lists of word, such as challenge and pass words, codenames for objectives, landmarks, operations, etc. The memory task was divided into two parts: presentation and recall. In the presentation portion, soldiers were given a pair of words in a mnemonic sentence (i.e., nine sentences - 18 words). In the recall portion, soldiers were given a list of 15 words and asked to mark "true," if the word were from the previous set of 18 words and "false," if it were not. Among the 15 words, nine were always from the previous list and six were not, although soldiers were never told of this relationship. The reasoning task was administered between the two parts of the memory task, preventing soldiers from rehearsing words during the delay between presentation and recognition testing.

Performance on the memory test was apparently unaffected by severe food and sleep restriction. There were no statistically significant differences in the ANOVA (Figure 2).

6. RECOVERY OF CELL-MEDIATED IMMUNE FUNCTION

PHA-stimulated T-lymphocyte activity declined significantly from baseline to the beginning of the mountain phase but did not increase in response to refeeding within the course (Figure 3). This response was restored to baseline by the end of the course and was not significantly different at one week post, but it was substantially higher than baseline at 5 weeks post (2-way ANOVA; $p<0.05$). This suggests that other factors in the course beside energy and sleep deficits, such as total energy expenditure, are responsible for the suppression of cell-mediated immune function. There may also be a compensatory recovery which would occur despite continuation of the stressors during the course. The hyperresponsiveness noted at 5 weeks post supports this.

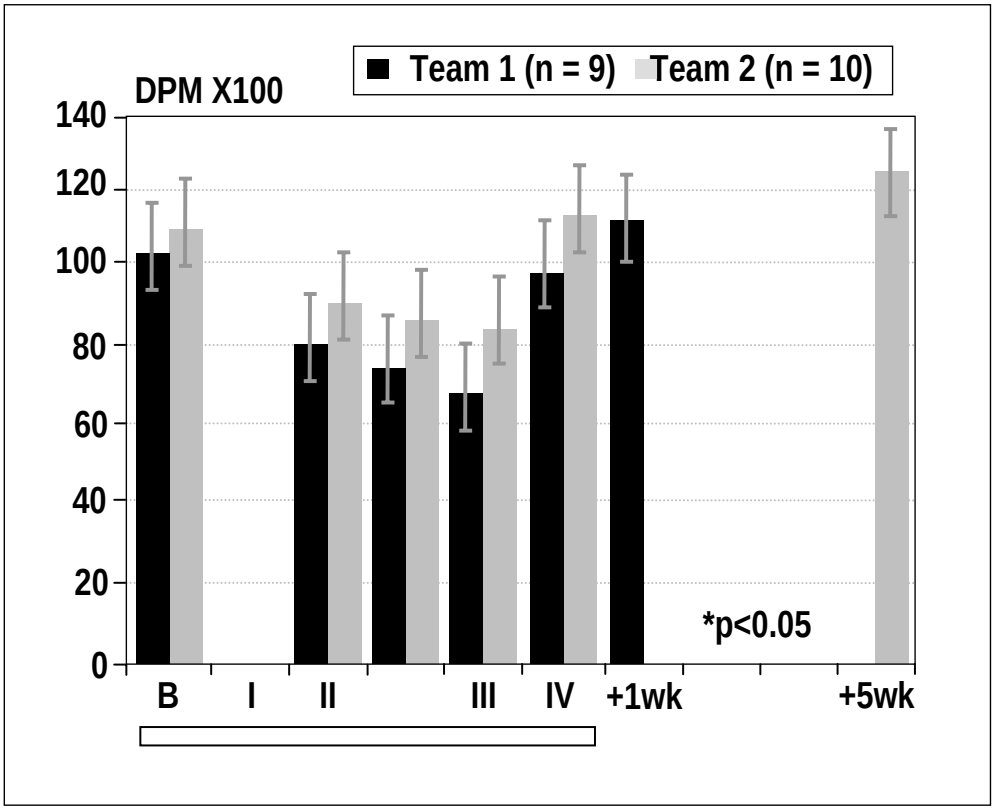

Figure 3. T-lymphocyte proliferative response following *in vitro* stimulation with phytohemagglutin.

7. RECOVERY OF METABOLIC MARKERS

Morning blood samples were drawn from soldiers following overnight fasting, processed and frozen onsite for later analysis, with all samples for individuals analyzed in the same assay. Biochemical markers of stress and metabolic status were used to assess recovery during and after the course.

Thyroid axis & other metabolic markers. Classical markers of protein-energy malnutrition, such as IGF1 (12) and triiodothyronine (13), demonstrated prompt and complete return to baseline with refeeding in the mid-mountain phase, and a significant rebound in the 5 week post-recovery sample. These differences in the magnitude of recovery were reflected in significant interactions between training intensity and course phase (2-way ANOVA, $p<0.05$). Reduction in thyroid activity is at least partially adaptive in the hypocaloric setting (14) and with increased risk of infection (15). Other aspects of thyroid function responded as expected in energy deficiency, with only a modest reduction and slower recovery of thyroxine. Prompt reduction in TSH following refeeding periods indicated regulation by higher centers but this differs from short-term studies (16).

Ferritin and binding proteins (TBG, SHBG) demonstrated the same pattern of acute recoveries, with significant changes in both recovery periods. Insulin is known to be a key regulator of the binding proteins (17), and prompt suppression of these two proteins during recovery indicates a return to normal carbohydrate metabolism.

Gonadal axis as a generalized stress marker. Testosterone promptly returned to baseline in both refeeding periods, supporting the concept that energy deficiency is a most critical stressor in this course. Although sleep was increased during these recovery periods, sleep deprivation is not as important in the suppression of testosterone (18). Anxiety stress produces a sustained suppression of testosterone during military training but ambient heat stress has no effect (Friedl, unpublished, 1985). As with the thyroid axis, the observed suppression of LH reflects a higher level regulation (19).

Table 4. Serum hormone levels (means±SEM) during and after Ranger training (n=10).

----R a n g e r T r a i n i n g----					
Parameter	**B/L**	**4 wk**	**5 wk***	**8 wk**	**+5 wk***
IGF1 (ug/L)	205±16	101± 7	**185±21**	88± 7	**253±11**
T3 (nmol/L)	1.84±0.16	1.55±0.11	**1.96±0.07**	1.47±0.08	**2.46±0.10**
T4 (nmol/L)	101±5.9	84±4.3	**77±3.1**	82±5.0	**101±5.8**
TSH (mU/L)	2.4±0.2	3.7±0.5	**3.0±0.3**	4.5±0.5	**2.7±0.4**
TBG (nmol/L)	20.3±1.0	22.9±0.8	**20.7±1.1**	24.0±1.5	**18.4±0.9**
T (nmol/L)	16.3±1.6	4.3±0.5	**14.6±0.8**	2.2±0.9	**19.3±3.2**
LH (U/L)	8.0±0.7	6.1±0.6	**6.3±0.5**	4.2±0.9	**8.6±0.7**
SHBG (nmol/L)	24.6±2.4	48.5±3.9	**28.0±2.4**	44.0±4.0	**19.7±1.2**
F (nmol/L)	441±22	417±31	**550±50**	706±34	**507±51**
HDLC (mmol/L)	1.3±0.1	1.7±0.1	**1.9±0.1**	2.3±0.2	**1.2±0.2**
Ferritin	82±14	128±17	**91±20**	163±17	**47±8**

*Recovery periods

Note: IGF1=insulin-like growth factor 1; T3=triiodothyronine; T4=thyroxine; TSH=thyroid-stimulating hormone; TBG=thyroid binding globulin; T=testosterone; LH=immunoreactive luteinizing hormone; SHBG=sex hormone binding globulin; F=cortisol; HDLC=high density lipoprotein cholesterol.

Markers of more severe metabolic stress. Cortisol demonstrated the largest rise in the second half of the course, as did other markers such as HDL-cholesterol (and the other cholesterol fractions), indicating the cumulative metabolic stress as body fat stores become substantially reduced (20). The period of refeeding during the course was inadequate in restoring levels to baseline but, 5 weeks after the course, all values were returned to initial concentrations (2-way ANOVA, $p<0.05$). In the alternate subsample of students, cortisol was restored within the first week after the end of the course. This indicates a difference between the two recovery periods with respect to adrenal activation, possibly reflecting the difference of high intensity activity during the course and virtual cessation of physical exertion in the first week after the course.

8. CONCLUDING REMARKS

The most important observation in this study was that a period of refeeding, along with a small increase in sleep, provided only a transient restoration of some measures or functions. Even with continuation of other course stressors, simple cognitive tasks were restored to baseline, as were generalized stress markers such as serum testosterone and metabolic markers such as triiodothyronine and IGF1. Thus, the acute improvement in metabolic status produced some short term functional restoration; however, these measures promptly returned to their place in a pattern of progressively larger change from baseline, following this within-course recovery period. Other measures were unchanged by this partial relief of course stressors, including indicators of cell-mediated immune function, reasoning capabilities, and metabolic markers such as ferritin and cholesterol subfractions.

By the end of the 8 week course, T-lymphocyte response was returned to baseline, even with continued stress; this demonstrated an overshoot from baseline by 5 weeks after the course. Cessation of stressors and a large positive energy balance produced a larger-than-baseline lymphocyte responsiveness when added to the compensatory recovery. Acute metabolic markers including triiodothyronine and IGF1 also demonstrate substantial rebound recoveries 5 weeks after the course, in concert with a body fat increase of 150% over baseline.

In summary, these findings suggest that 1) simple aspects of cognitive function may be susceptible to partial relief of the multiple stressors including increased feeding with acute metabolic recovery, 2) more complex cognitive functions such as reasoning are not, 3) the large reduction in immune function is either not

susceptible to refeeding or has a longer latency period than the 5-7 days in this recovery period, and 4) compensatory mechanisms correct the immunological deficit by the end of the course.

Acknowledgements

We gratefully acknowledge the technical assistance of Mr. Lou Marchitelli, SGT Robert Toyota, and SGT Bradley Nindl, the graphics/layout by Ms. Janet Reese, and the generous cooperation of the volunteers who participated in this study.

This study was conducted in accordance with the principles of the Declaration of Helsinki and the explicit directives of Army Regulation 70-25, "Use of Volunteers as Subjects of Research." This study was approved by an Institutional Review Board at the U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts, and by the Human Use Review Office, Office of the Surgeon General, Department of the Army.

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Managing Fatigue in Long Duration Airlift Operations 1994

by

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Abstract

During September, 1994 the operational tempo for US Air Force C-5 transport crews was at a record high. Support flights were routinely sent to assist international efforts to bring peace to warring factions in Rwanda, Somalia and, in addition to their normal full time responsibilities, there were additional flights needed to reinstate the elected government in Haiti. I interviewed crews at Dover AFB to learn their perspectives of the sources and the extent of fatigue on these sustained missions. Many of these crews had participated in Operation Restore Hope II to Somalia which involved multiple 25 + hour flights from the US to Somalia before crew resting in Cairo. I learned the pace of C-5 operations has remained at record levels since the Gulf War. Important issues identified by the crews were cumulative sleep debt, circadian disruption and their impact on mission safety. I was able to accompany a crew throughout a planned 10 day support mission.

This report is based on my conversations with about 35 officers and enlisted transport crews. I was impressed with the similarity of the comments for the primary sources of frustration and fatigue in the conduct of C-5 missions. These are described more fully in the report but some deserve highlighting here. Many felt that once they did something demanding, like 20-hour plus missions or the then unprecedented three aerial refuelings needed in Somalia, it becomes expected, not the "one time only effort" they were told. There is a strong feeling that there are too many crews on BRAVO alerts, perhaps unnecessarily and BRAVO alerts were too long. Many were convinced that better collaboration with schedulers and the Wing would provide more realistic mission schedules.

Based on some objective data I collected, in-flight sleep on the C-5 may be less restful than previously thought. Analysis of the nutritional content available in the box meal were conducted and suggests good food is available but not often selected by crews. A decibel meter was used to sample sound frequencies at 6 locations on the C-5 and while louder than published in the C-5 operations manual, the Dash-1, is still within limits. Given that longer missions may be more frequent, noise limits may need to be reconsidered. It is my hope that this report will stimulate interest in a larger study of C-5 crew fatigue issues. Coping strategies for management of fatigue that are simple to follow and can be implemented in current operations are provided at the end of the report and were derived, in large part, from the experiences gained during this investigation.

Dr French has left the USAF and is now a fatigue consultant for shiftwork, jet lag and sustained duration operations.

INTRODUCTION

On 8 September 1994, I arrived at the 436th Airlift Wing at Dover AFB to discuss the issue of crew fatigue with members of the 3rd and 9th C-5 airlift squadrons. Our mission at the Sustained Operations Branch (CFTO) of the then Armstrong Laboratory at Brooks AFB is to evaluate the effects of sleep deprivation and circadian disruption in the lab and in operations and to develop counter-measures. We have experience in the past with monitoring the human issues associated with accelerated transport mission requirements like that, which occurred during the Gulf War (see References).

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Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

VALIDATION RESEARCH

September, like many other months before it, saw the mission requirement rates for the C-5 at close to maximum due to the operational demands of Haiti, supplying medicine and food to Rwanda and in support of many other trouble spots around the world. My intent was to accompany as many missions as possible during the 3 weeks I had available for the study. I had hoped to experience the tempo and to hear first hand from many crews where they felt the sources of fatigue were in these missions. Unfortunately, I quickly learned that C-5 mission schedules are often sporadic and longer than expected. After a few mission opportunities were delayed, the first mission I accompanied turned out to be my only one due to a C-5 breakdown. Our plane was a good bet to last the entire mission because it was the newest C-5, that is, the last C-5 built. However, it developed an oil leak in one of the engines that forced us to wait for parts in Fujairah (United Arab Emirates). I shared the frustration of the crew in getting stuck at the far end of the supply line when operational demands prevented much of an effort at our rescue.

The data that I compiled then are based on conversations with approximately 35 officers and enlisted at Dover AFB. I was able to collect additional data on nutrition, quality of sleep and noise levels associated with a typical C-5 mission. I believe the data reported herein are representative and a good snapshot of the lifestyle and unique demands placed on C-5 crews.

I contacted the 436th Aerospace Medicine Squadron to advise them of my mission and to ask for their suggestions and possibly obtain any background information on crew fatigue. We discussed the primary sources of fatigue for aircrews; mission schedules, nutrition and billeting. Maintenance personnel were also suffering from fatigue. Poor crew rest discipline, including excessive alcohol consumption and poor nutrition are still a problem for some air and ground crews.

I spent the day talking to crews in the 9th and 3rd Airlift Squadrons learning about ALPHA, BRAVO alerts and crew rest time requirements. A second mission opportunity was delayed by a few days. A mission to numerous US naval installations in Europe and the Near East was the third opportunity and would leave on the 11th. I requested permission to accompany them, expecting to be back in about 10 days to take advantage of the original stage mission to Frankfurt that would be departing about when we were scheduled to return. Prior to my arrival in Dover, a former C-5 crewman told me that the average mission length was about 5 days. Most of the Dover crews I met felt this to be a short estimate as missions of late were averaging about 10 days. The lack of clear definition of mission times and BRAVO's point to a very large part of the frustration in the life of a transport crew. Not only is it difficult to know when a mission is to go, it is almost impossible to determine when to plan for their return. Lately, multiple crews are kept on BRAVO alert to ensure one mission will go. This is very disruptive to scheduling family events and sleep/wake cycles since BRAVO extends the normal 6-8 hours that crews are legal for alert to as long as 48 hours. Most crews felt that they always seemed to get alerted on BRAVO's just as they were getting to sleep. This means that the mission started after the crews had been awake for most of their day already. The crews I met at all levels were quite candid and seemed eager to describe fatigue related issues.

A senior pilot told me that the pace of current C-5 operations was still the same as during the Gulf war. It had not slowed down for his wing. He and many of the Dover crews flew the multiple, long duration missions (24+ hours) of Operation Restore Hope II to Somalia and followed that with humanitarian aid to Rwanda. He had many comments that address well the frustration and fatigue the crews feel. He felt the primary statistic was airplane utilization rate but should be crew utilization rate. He also felt that most of the crews are young and did not have enough rank or experience to challenge their assignment to extremely fatiguing missions involving inadequate crew rest intervals. He thought this could lead to dangerous levels of fatigue. There were complaints about too many quick turn missions (crew rest intervals of 16 hours) many of which were perceived as unnecessary. Another officer suggested that their situation was best described as "management by BRAVO". That officer complained that preventative maintenance had not been done on C-5s for about 5 years due to the pace of missions. He speculated that perhaps the C-5 would be less likely to 'break' if such maintenance was accomplished. He felt that mission cancellations and BRAVO alerts left very little certainty to their lives and this was disconcerting.

The crews I met with seemed to feel that they were being misused in many instances. As an example, another pilot made the point that during Restore Hope II they were asked to do triple and quadruple aerial refueling and do 30 hour missions "just this once". He felt that since they had demonstrated they could do it, these were now considered within normal operations. He also cited the disruption and stress of too many BRAVO alerts. There was almost universal agreement amongst the crews I spoke with, that too often the schedulers were not familiar with C-5 operations and the demands placed on them by long duration missions. It was recommended that C-5 crews should rotate as schedulers for a few weeks out of the year. They would learn mission planning from the perspective of the scheduler and the schedulers might learn more about the unique demands placed on transport crews. I learned there was an excess of co-pilots at Dover. Since they need lots of training, this means that instructor pilots must spend many hours teaching as well as flying their own missions.

Dover AFB had just completed Phoenix Pace when I arrived. This allows the crews time to recover and not fly for 2 weeks. Although crews are supposed to be given relief from missions, one crewman told me that there was too much ground activity, simulator training and other exercises, to permit adequate recovery. When Restore Hope II was initiated, Travis AFB was engaged in it’s Phoenix Pace which required Dover to bear the brunt of those long missions. One of the schedulers at the 436th Wing showed me an interesting graph of utilization rate. Currently, the rate is about 40.5. The maximum rate is very close, 42. For comparison, a rate of about 28 is considered low and 35 is high. Three days after Phoenix Pace, the rate went from 4.0 to 40.0 and hovered around this "pain threshold" for many days.

Another senior officer told me that before the Gulf War, utilization rate was limited by airframes. Now that rate is limited by crew availability due to the drawdown from the war. However, schedulers still consider airframes in their utilization figures, as the other senior officer above had reported, not crews. Dover has 64 aircrews for 32 planes. He felt that surge type operations had become normal for today's operations. Everything had become urgent and that's why so many BRAVOs were scheduled. I discussed some alternatives with him and the best options seemed to be restoring routine maintenance on C-5s and reducing the time spent in BRAVO. For example, instead of 48 hours 'on the hook', the time might be reduced to 16 or 24 without too much of an impact.

The mission I could accompany arrived shortly thereafter. The crew seemed energetic and friendly. They seemed skeptical that we would make it back within 10 days so that I could pick up the Frankfurt stage mission. The crew were eager to point out things in the C-5 environment that were fatiguing. Sitting for long periods was an important fatigue factor but they realized that it came with the mission. The seats on the C-5 are very comfortable. The bunks are a great addition to the crew compartment. They can be sealed off and made dark and quiet. I learned the toilet fills quickly on the C-5 and since it would not get emptied normally until our return to Dover, they did not like to use the one in the flight deck. Instead, they go to the far end of the plane, the troop compartment, and use that one. The toilet in the flight deck area was in a large room and flushed well (maybe too well given the comment that it fills too quickly). The sink in the toilet room did not work. I'm told that the internal water supply for the C-5 has been turned off because of problems. Apparently, the water was foul tasting and discolored. Too often the water would freeze in the lines and create problems. The crew seemed content with the Igloo containers full of water. I wondered if that would be enough should we break down in countries where the local water was not safe.

One of the measures that we use in the field to determine length and quality of sleep is called an activity monitor or actigraph. These are wrist-watch sized devices that strap to the arm and count limb movements per minute for the duration of the battery life, about 2 weeks. I was very interested in using the activity monitor to compare sleep in the bunk with sleep in a bed during crew rest. I only had 3 activity monitors with me for the trip so I had to rotate them between crews to get an idea of sleep quality. Unfortunately, we had to wait in Fujairah a week for repair and crew rest became a non-issue. Also, data were lost due to battery failure on one of the actigraphs. From my records, I was able to go back and identify bunk time and sleep time on the record. No figures are available from the actigraphs for this manuscript since the data did not survive a computer crash in 1998. However, the results will be described albeit without reference to a figure. An advantage of the activity monitors is that it allows the sleep period counts to be evaluated; that is, to determine if the sleep period is restless compared to some baseline. Of course, inactivity in a pilots chair might also register scored

sleep inadvertently but not to the length of time that would be recognized as a sleep period. Since the activity monitors are very sensitive to motion, false sleep scores are not normal.

At Rota Spain, the Aircraft Commander (AC) went to sleep at about 2330 and awoke about 0700. At Sigonella Italy, the AC went to bed the next night at 0600 and slept until about 1230. The AC presents an interesting nap pattern. He will typically sleep for about 15 minutes and feel quite refreshed. Short naps, less than 40 minutes, are actually very sound lengths of time to nap without difficulty awakening (sleep inertia). I was careful to mark when he took a nap.

The engineer wore the monitor when we were awaiting repair parts for the airplane in Fujairah and I did not get a comparison sleep on the airplane. Since the times are Central Daylight Time, he was getting to bed about 1700 (CDT) or 0100 local time and awakening about 0100 (CDT) or 0900 local. A Loadmaster wore the actigraph when the AC did and also took a nap in-flight. Like the AC, his nap occurred around 2000 CDT. The activity associated with the C-5 bunk nap had the highest counts. The bunks on board the C-5 look very comfortable but the activity monitor suggests the crews are sleeping restlessly. This loadmaster stayed up upon landing in Rota until about 0500 (CDT) and then went to bed. He was remarkably consistent with his sleep time at Sigonella. Another crewman wore the activity monitor when the engineer did so no naps on board the aircraft were recorded. During the first crew rest in Fujairah (CR#1), an alarm clock in the engineer's room went off and kept him awake in bed trying to sleep for about an hour. He had also been drinking alcohol immediately prior to going to bed and I suspect this caused a typical alcohol induced insomnia on his rest. On the second night in crew rest (CR#2), he slept almost 12 hours, probably because of the poor sleep on CR#1. The activity monitor is a very useful way to objectively study the quantity and quality of sleep for crews.

The finding of restless sleep on the aircraft is important and needs to be supported by more data. If crews are not getting restorative sleep on the plane then techniques need to be explored that might help them to sleep more soundly. We have a simple handout describing how to maximize sleep that I have included in Appendix 1. It may be useful to expand on some of these principles in a two- three page guidance tailored to the transport crew.

The nutrition available to the crews is a very important aspect of the mission. Good food promotes good health, vitality and can influence morale. Accordingly, I paid careful attention to the meals the crew ate. I wrote down all the ingredients of the box meal and, with the help of the Armstrong Laboratory's research dietician, analyzed the contents for nutritional value. The crew said the choices were much better at Air Force bases but since this was a Navy support run we should expect limited food resources. Our food analysis however, favored the box meal from Sigonella in some ways over that from Frankfurt or Dover. Table 1 shows the Military Recommended Daily Allowance (MRDA) taken from AF Regulation 160-95 and the civilian Recommended Daily Allowance provided by the National Academy of Sciences (1989). Table 2 shows the summary for the 3 box meals as a percent of the MRDA recommendations in Table 1.

Table 1. Recommended Military and Cicilian Nutritional Components

	Military RDA	Civilian RDA
Kilocalories	3200 KC	2900 KC
Protein	100 g	63 g
Carbohydrates	440 g	446 g
Fat	124 g	96 g

Table II. Percent of MRDA provided by the box lunch at each location

	Kcalories	Protein	Carbohydrates	Fat
Dover	49%	51%	41%	64%
Signonella	37%	30%	36%	42%
Frankfurt	48%	78%	39%	51%

The box meal from Dover was quite good but slightly high in fat (64% of the MRDA) as shown in Table 2. The box meal is only supposed to supply 30% of the daily nutritional components and most of the meals supplied almost 50%.

Two of the crew seemed very interested in some of our work with the amino acid tyrosine. Since this essential dietary compound is the pre-cursor to catecholamines, a diet rich in tyrosine might enhance adrenergic activity. Colleagues at Army labs have shown an improved ability to withstand cold and altitude using tyrosine. We have not be able to show a similar result with sleep deprivation. Tryptophan is another dietary amino acid and is the pre-cursor to serotonin and ultimately, melatonin, the sleep promoting hormone. Therefore, foods rich in tryptophan may serve to promote sleep. We discussed the usefulness of meals 'ready for sleep' and meals 'ready for wakefulness' using these concepts and thought it a good idea.

Crew rest quarters at Rota were very comfortable and all the crew got single rooms. The crew however, managed to find lots of bad things to eat and drink. They were drinking lots of coffee and beer and most were eating a gastronomically difficult creation called a jumbo burger. We were given 24 hours in Rota which is not the best crew rest time. The AC and I discussed this issue and he felt the same way. Most of the time, it takes 2 hours to get the crew to billeting and to bed for a good 8 hours of sleep. Another 2 hours to get them back to the plane makes 12 hours a reasonable rest period. After that time, most people are ready for about 10 hours of activity before getting tired again. Scheduling a crew for 24 hours of crew rest gives them their 12 hour crew rest and their 10 hour activity phase but by the time they are tired enough to go to sleep, it is time to start the mission. A better amount of time would be either 16 hours or 36 hours of crew rest to avoid starting missions at the beginning of the normal sleep cycle. However, the crew seemed well-rested by their 24 hour rest period and ready for the next leg to Bahrain.

A few of the crew drank what I would consider to be more alcohol than would be conducive to a good sleep. Of course, the plane was broken and their frustration with getting it repaired might have been a factor. They were always careful to make the 12-hour alcohol free restriction but the crews need to know that alcohol can impair quality sleep and can produce insomnia. They should be advised to quit drinking a few hours before sleep as recommended in the suggestions in Appendix 1. I saw no one who seemed impaired the day following a night of drinking. However, the message about alcohol and sleep quality needs to be made even more forcefully, in my opinion.

The final measure I brought on board was a decibel meter. I was interested in finding the level of noise to which the crew were exposed. I used a Quest-Tech meter (510 Worthington St Oconomowoc, WI 53066 Phone 414 567-9157; calibrated on 14 July 1994). The meter came with a calibrator device that I was careful to use before every measurement since I am not skilled in collecting or interpreting sound. I took measures at each of 6 spots around the aircraft about 4 times and averaged the measures to reach a composite. I was careful to position the meter in the same spot each time and I only recorded when we had reached cruise altitude. I did get some additional decibel information during climb-out and during loading the aircraft. I also have data on altitude, ambient temperature and cabin pressure should the data need to be pursued. The AC showed me a table from his DASH-1 which concerned sound levels. In chapter 2A on page 97 were two tables that are relevant. One table showed the allowable exposure time to noise of varying magnitudes. I have reproduced it here in Table 3. The other showed similar measures taken around the C-5 which I have reproduced as Table 4. Figure 6 shows the decibel levels that I recorded from the 6 locations.

Table III. Partially Reproduced from DASH-1 to IC-5A-1

Decibel, dB	Required Ear Protection Allowable exposure (min) for 8 hour pd	
0 – 84	No Protection	
85 – 104	Headset/plugs	480 min
105 – 114	Headset/plugs	480 min
115 – 120	Headset/plugs	170 min
121 – 125	Headtset/plugs	71 min
126 – 130	Heatset/plugs	30 min

Table IV. Internal Noise on C-5

Flight Condition	Maximum location	Decibel
Ground/Take-off	Flight Sta	86
Normal Cruise	Flight Sta	84
Normal Cruise	Relief Crew	86
Normal Cruise	Courier	89
Normal Cruise	Troop	87
Normal Cruise	Cargo	94
Normal Cruise	Avionics	91

The meter I used was able to break the sound into component frequencies. There was also an all-pass feature that combined all frequencies. The legends in Figure 6 refer to the locations during cruise from which the measures were taken; FS=flight station, BN=bunk (door closed), RS=relief station, CG=cargo (mid-hold), TP=Troop and CO=courier. Inspection of Figure 6 shows that the all-pass levels I recorded are considerably higher than what was reported in Table 4. This could simply reflect a difference in which direction the meter was pointed during recording or some other methodological difference. It may be important to review the allowable exposure time in light of the longer missions the C-5 is flying (20+ hours) since that table is limited to 8 hours. It is interesting that the middle frequency (250 Hz) was associated with the highest dB levels. The higher frequencies showed the lowest dB values.

During climb-out, I recorded levels between 103 dB to 118 dB for about 5 minutes from the Relief Station. Only a few of the crew were wearing ear cushions in the relief stations. From the Flight station, the levels were between 100 and 108 dB on climb out, considerably higher than the levels reported in Table 4. Levels recorded during loading operations ranged between 70 and 90 dB in the mid-cargo bay area. Occasionally, a pallet related noise would get as high as 117 dB but infrequently. All the loadmasters had at least ear cushions in use during loading. I was interested in determining the level of noise attenuation by the ear cushions and the headset. However, I was unable to satisfactorily attach these protective devices to the decibel meter.

The crew complained that many who went to Mombassa had their plane re-assigned and we would likely be stuck there waiting for a new plane and mission. We communicated with another C-5 in-flight that had been in just that situation. They were long past their scheduled return time. Places like Mombassa or Rwanda easily became stages without being officially designated so.

Of all the crew, the engineer's task seemed to me to be the most fatiguing. They have to constantly monitor the panel for long shifts at all times. Research suggests that monitoring tasks are sensitive to long duration induced fatigue. I would guess the engineers would be the first to suffer microsleeps in-flight of all the crew. They did seem to regulate their own in-flight rests very well though. I did notice that most of the time, the crew slept in the chairs on the courier compartment or the relief station.

Our mission was supposed to take us 10 days. We ended up returning 15 days later. Due to the breakdown in Fujairah, we gave up our Navy mission to a functioning C-5 and picked up new missions as we returned. Our return mission took us to Frankfurt Germany, Keflavik Iceland and back to Dover. I was disappointed that there was no time to get on another mission since I had to return to Brooks AFB for 3 important meetings before the end of September. It is my hope that a broader study of the human problems associated with long endurance transport missions will be requested by AMC, particularly when the activity of transport crews is once again pushed beyond their usually high levels due to some national contingency.

CONCLUDING REMARKS

In our opinion, transport crews have the least predictable and often the longest missions in the Air Force flying community. Due to the extent of their transmeridian travels and the atypical sleep/wake patterns they must endure on missions, they likely suffer the most disruption of normal circadian cycles of all Air Force missions. The C-5 crews that I spoke with about fatigue felt that they could do the long missions if they had to. However, many perceived that limits were being escalated by their accomplishments and that gruelling missions were becoming the norm. Better collaboration with TACC by the Wing schedulers and pilots might promote less fatiguing mission schedules. The issue of fewer BRAVOs and shorter BRAVOs needs to be explored. In order to keep crews at peak readiness they need to have regularly scheduled sleep/ wake schedules as often as possible. BRAVO's disrupt these normal patterns and increase stress

The quality of food available to transport crews at their unusual hours of arrival and departure deserves more study. It would be useful to know what selections they have and what they are making throughout the missions. Since transport crews are relatively sedentary for long periods of time, the number and quality of calories consumed is very important and deserving of closer inspection. Crews should be better educated in what foods to bring with them and to select during a mission.

Some indications from the current study suggest that the quality of sleep in-flight and during crew rest could be improved. A careful study of in-flight naps is suggested to discover the timing and the conditions that make for the most restorative sleep.

An annual brief to transport crews about stress relaxation training, sleep hygiene, naps and circadian disruption among other topics, was proposed. This would additionally allow researchers to find out through discussion and surveys where the difficulties are from crews directly.

Finally, a careful study of the effects of mission demands on crews during a period when they are experiencing an increase in the number or duration of missions might provide important information for managing the human resource. Sleep/wake patterns, nutrition, exercise patterns and circadian disruption are among the fatigue related topics that should be further investigated in transport crews. It is likely that careful attention to fatigue management techniques (nutrition, sleep, schedules, exercise and others) like those proposed here might extend the ability of crews to make safer and longer missions. Coping strategies for managing fatigue that can be implemented in current operations were provided as part of the after-mission recommendations and are included in Appendix 1 of this report.

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Appendix 1

The Sleep/Wake Vigilance Strategies

FATIGUE MANAGEMENT TECHNIQUES FOR TRANSMERIDIAN TRAVEL AND SHIFT WORKERS

Your work may require you to be vigilant for long hours, especially during the early morning (0300-0700) circadian performance troughs. These are some techniques that may reduce your fatigue and improve your alertness. Before the extended duty period its very important to catch up on your existing sleep debt. Get as much uninterrupted sleep as you can for a few days before your mission.

Improve sleep. The quality of the sleep you get is vital to your performance when you are awake. You must do everything to increase the quality of your sleep.

1.) Make the bunks or beds you use as comfortable as possible. For example, use blankets for extra padding and take the time to protect your sleep environment from light, noise, vibration, temperature extremes wherever possible. If you get a chance to use a bunk on duty then you should because a chair is not nearly as conducive to a restful sleep. Otherwise, stretch out on the floor on padding. Get serious about your sleep. Take your shoes off if possible and loosen your clothing. Don't prop your head up too high when lying down because as you sleep your chin has a tendency to move towards your chest, preventing good respiration. Snoring and sleep disorders (insomnia at night, fatigue during day) can prevent a restful sleep and should receive medical attention. To help you doze off, practice muscle relaxation prior to sleep onset (ie. tense your hands and breathe deeply, tense your face and breathe deeply; continue this with all major muscle groups legs, stomach, shoulders, back, arms; imagine pleasant scenes).

2.) Schedule your sleep carefully. *Short naps* (no more than 30 minutes long) can be very helpful. *Bad naps*, naps between 1-2 hours long can be very difficult to awaken from since you will likely wake up in deep slow wave portion of sleep. *Long naps* however (about 3-4 hours long) are very restful. Neither short nor long naps should be too close to a long sleep opportunity (over 4 hour).

3.) Try very hard to get to bed at the same time each night and try to awaken at the same time. If a crew rest period starts in the morning (past 0600), compared to when you usually wake up (your body clock time), then you should get a long nap (3-4 hours) and get to bed as close to your usual time that night as possible. Don't allow yourself to sleep a full 8 hours at this unusual time or you will have trouble getting to bed later. In-flight or on duty share sleep availability with fellow crew equitably. Excessive alcohol disrupts normal sleep. It can cause insomnia and it can ruin a restorative sleep. Do not drink to excess and stop drinking about 2-3 hours before you go to bed. Try drinking water in the interim to hydrate yourself. Also, avoid caffeine 3-4 hours before naps or sleep.

Enhance vigilance. If you are required to remain awake and vigilant for long periods of time and especially during the circadian performance trough, you should take advantage of fitness, nutrition and the environment. Prior to long duty days or night work, you would do well to ensure that you have adequate sleep (7-9 hours) each night at least 2 nights prior.

1.) Periodically throughout a long duty day, try brief 10-15 minute exercise intervals to combat fatigue (stretch, aerobics, isometrics in seat). Stand and move about if possible and do so at regular intervals. Pushups, situps and other exercises in place can be invigorating. Exercise regularly. It helps to stay vigilant if you're in shape.

2. Use refrigerators and ovens on the aircraft or duty rooms and make an effort to eat healthier choices of food. Have your large meal for lunch rather than dinner. Generally, high protein foods upon awakening and complex carbohydrates before retiring. Stay hydrated. Caffeine and alcohol can dehydrate you. Brushing your teeth and washing your face, attending to your appearance periodically can refresh you.

3. Use as much light as available to illuminate crew environment (planning room, cafeteria, billeting, flight deck, office, break areas) especially at night to promote vigilance. Bright light at night can reduce fatigue. If you can't work in brightly lighted areas, take frequent 2 minute stretch breaks in light. Daylight (and fresh air) are best for these breaks.

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The Biochemical and Physiological Effects of 95 Days Endurance Exercise in Negative Energy Balance

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INTRODUCTION

In times of war, men push themselves to physical limits well beyond those that are conventionally thought possible, and hence it is extremely difficult to predict the limits of endurance capabilities using laboratory based studies. Of course, studies can be performed examining military exercises, although motivation levels may not be maximal and it is perhaps better to study rigorous military selection processes, where intense competition maximises the psychological drives. This approach has been utilised successfully by several groups. For shorter-term activities, it is also possible to study athletic performance which provides measures of sustainable levels of physical effort with some degree of military relevance, since athletes are very highly motivated. In the case of ultra-distance competitors, there is even the potential to assess physical performance and its decline over several days, and hence studies of these events should be encouraged in the context of furthering our understanding of exhaustive military Operations. However, when it comes to investigation of likely physical performance over very extended exhaustive activities, it becomes increasingly difficult to find potential study models which combine sustained physical work with the kind of motivation levels that would occur in potential life threatening wartime situations. It is in this context, that I believe that studies of prolonged Polar, or other expeditions can be of value.

Polar expeditions provide an opportunity to examine the effects of extreme sustained exercise combined with relative under nutrition in an adverse environment. The participants are pushed to their physiological limits and hence the data gathered can provide unique information regarding survival and function under the very worst conditions. This paper describes studies performed in relation to the first unsupported crossing of Antarctica - studies conducted by the Army Personnel Research Establishment, UK, which is now incorporated into the DRA Centre for Human Sciences.

THE EXPEDITION

In November 1992, two men (RF and MS) set off from the Atlantic coast of the Antarctic aiming to perform the first crossing of the continent unaided by other men, animals or machines. Each man pulled a sledge weighing 222 kg which contained 100 days of food, fuel and other essential survival equipment. They hauled the sledges for between 10 and 12 hours daily, initially for 20 days across the 350 km Filchner ice shelf (a region of glacial ice floating on the sea or grounded on the sea bed) before they met the Antarctic coast. There then followed a 340 km ascent to the Polar plateau at 3000m and 550 km across the plateau to reach the South Pole on day 68. Beyond the Pole, the men travelled a further 480 km on the plateau, before descending through the Trans-Antarctic mountains to reach the Pacific coast of Antarctica and the Ross ice-shelf on the 90th day of the journey. An attempt to cross this second ice-shelf, to reach the open Pacific ocean, was abandoned on day 95 when it became evident that the men were suffering from severe malnutrition. The expedition was the first to complete a crossing of the Antarctic mainland without the use of aircraft to ferry food and equipment, and at nearly 2300 km was the longest unsupported walk ever made. Still air temperatures ranged from –45^{o}C to –10^{o}C often accompanied by high winds (Stroud, 1993).

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

The choice of diet during the expedition was critical. Work performed on a previous expedition of similar nature showed that daily energy expenditure when manhauling in Antarctic conditions was likely to be around 25 $MJ.day^{-1}$ (Stroud, 1987). An intake that provided 23 $MJ.day^{-1}$ was therefore planned, with an acceptance that weight losses over the planned 100 day journey would be around 10 kg. Of course, the nature of our journey dictated that the food should weigh a minimum and hence should contain as high a fat content as possible. However, this requirement conflicted with that of achieving maximal glycogen resynthesis at the end of each day, for although the exertion was essentially of low intensity in physiological terms, it would still lead to marked glycogen depletion by the end of 10 to 12 hours of exertion. In the end, a compromise daily diet containing 23 MJ as 57% fat, 35% carbohydrate and 8% protein was selected, a decision supported by the successful use of a similar diet on the earlier expedition Stroud, 1987). There is also evidence to suggest that man can adapt to a high fat intake and improve muscle utilisation of free fatty acids (French et al, 1993; Phinney et al, 1983). Eventually, however, the planned diet was not adhered to since, after leaving the Pole, debilitation from a continued energy deficit became severe. The men therefore increased intakes to around 28 $MJ.day^{-1}$ until Day 84 when they commenced their descent from the plateau down the Beardmore glacier. They then reduced their intake to just 16$MJ.day^{-1}$ in the hope the excess food consumed could be recovered and the overall 100 day range regained.

AIMS OF RESEARCH

The expedition afforded an opportunity to examine energy balance over a prolonged period of heavy work in a cold environment and to study the associated changes in body composition, resting metabolism and metabolic responsiveness to food. Subject numbers were obviously limited to only 2 individuals and logistic considerations also limited the scope and detail of the work that could be carried out. However, the following studies were performed:

a) Body weight and composition changes.

b) Energy expenditure using dietary intake/weight losses and the isotope-labelled water technique.

c) Resting metabolic rates and metabolic and biochemical responses to a high fat test meal before and after the expedition.

d) Maximal aerobic capacity and isometric muscle strength before and after the expediton.

e) Skeletal muscle enzyme activity before and after the expedition.

f) Changes in biochemical parameters at during the course of the expedition.

METHODS

Subjects. The two men, RF and MS, were well trained. Their age, weight, height and maximal oxygen uptake (VO2max.) prior to the experiment were 48 and 37 yr, 95.6 and 74.8 kg, 188 and 169 cm, and 53.6 and 58.1 ml O2 kg-1.min-1, respectively. Informed written consent was obtained prior to the experiments.

a) *Body weight and composition changes*

Body mass was recorded in the UK 10 days prior to and 6 days after the expedition and, in addition, was recorded at the start and finish of the journey using a miniature load-cell based portable scale (Miniscale, Raviv-Aran, Israel). On each occasion, measurements were made after an overnight fast, defaecation and the voiding of urine. At the same time as the UK weighings, body composition was measured using underwater weighing (UWW), with measurements of residual lung volumes being made using helium dilution spirometry. No accurate food intake data were available for the periods between the UWWs and the expedition itself.

b) *Energy expenditure using dietary intake/weight losses and the isotope-labelled water*

During the experiment, the subjects ate a high-fat, energy dense (21.3 $MJ.day^{-1}$) diet consisting of freeze-dried meals supplemented with butter, chocolate bars, biscuits, soups and hot chocolate drinks. Dietary analysis was performed before departure with food values taken from tables (Paul and Southgate, 1978) and

from manufacturers' nutritional information. Since the food was weighed and pre-packaged into daily ration bags, and no food was left uneaten, accurate daily energy intakes could be calculated. Daily energy expenditure was then estimated by combining the daily intake figures with energy deficits calculated from the overall losses of lean tissues and fat. Lean tissue losses were assumed to be 73% water whereas fat losses were considered to be 100% fat. The calorific value of protein was taken as 18.39 $kJ.g^{-1}$ and fat was taken as 39.7 $kJ.g^{-1}$ (Brouwer, 1965).

Estimates of energy expenditure were made using the $^{2}H_{2}$ ^{18}O method (Prentice, 1990) modified to account for changes in likely background levels in the Polar snow water source (Stroud et al, 1993). Body isotope disappearance was followed by daily collection and later analysis of 2ml urine samples. Each subject had two determinations of energy expenditure, one between days 1 and 50 and the other between days 51 and 95.

c) *Resting metabolic rates and metabolic and biochemical responses to a high fat test meal before and after the expedition*

Following a standardised day's intake (12.0 MJ containing 35% fat, 53% carbohydrate, and 12% protein) and an overnight fast, indirect calorimetry was used to measure resting metabolic rate (RMR) 14 days prior to departure and 7 days after completion of the expedition. Following the measurements, subjects consumed a test meal of 4.8 MJ containing 55% fat, 10% protein and 35% carbohydrate. RMR measurements were then repeated at 15 min intervals for 120 min. Venous blood samples were taken prior to the meal and then at 15 min intervals for 60 min and 30 min intervals for the subsequent 300 min. The blood was analyzed for glucose, insulin, triglycerides and free-fatty acids.

d) *Maximum aerobic capacity and isometric muscle strength before and after the expedition*

Maximal oxygen uptake was measured from Douglas bag collections made during treadmill running using a continuous incremental exercise protocol. Dominant maximal voluntary contraction (MVC) force production was measured under isometric conditions in the muscle groups involved in elbow flexion, elbow extension, abdominal flexion, and leg extension using a Hermansen isometric rig (Hermansen et al, 1972), and for hand grip (Digimeter, MIE, UK) and an upright pull (Takei, Japan) using specialised dynamometers.

e) *Skeletal muscle enzyme activity before and after the expedition*

Ten days prior to the expedition and 6 days following its completion, muscle biopsy samples were taken from vastus lateralis using the percutaneous needle biopsy technique described by Bergström (1962). Samples were immediately frozen in liquid nitrogen and subsequently freeze-dried. The muscle samples weighing approx. 15 mg dry were dissected free from visible blood and connective tissue, and powdered before being homogenised and the enzyme activities determined for four different enzymes, chosen to be representative of different components of muscle energy metabolism (Wibom et al, 1992): Glyceraldehyde-3-phosphate dehydrogenase (Gly3PDH) from glycolysis; ß-hydroxyacyl-CoA dehydrogenase (HAD) from ß-oxidation of free fatty acids, citrate synthase (CS) from the citric acid cycle; and cytochrome-c oxidase (COX) from the electron transport chain.

f) *Changes in biochemical parameters at during the course of the expedition*

At 10 day intervals during the experiment, blood samples were taken to assess hormonal and biochemical responses. Venepuncture was performed approximately 30 min after the end of the daily 10 to 12 hours of exercise and at least 4 hours after the last food intake. The samples were collected into tubes containing fluoride oxalate which were then hung in the roof of the tent for 2 to 3 hours to allow partial red cell sedimentation. A small plasma sample of between 0.5 and 1.0 ml was then pipetted off and allowed to freeze. Following the experiment, the frozen samples were returned to the UK where standard enzymatic and radio-immuno assays were used to assess glucose, insulin, growth hormone (GH), cortisol, testosterone, luteinizing hormone (LH), thyroid function, cholesterol, triglycerides, total protein and albumin.

RESULTS

a) *Body weight and composition changes*

Following the adjustments to rations during the journey, the average the diet over the whole expedition provided 21.6 MJ.day-1 of which 56.7% came from fat, 35.5% from carbohydrate, and 7.8% from protein. Despite this intake, weight losses were severe (Table 1) and both men became severely debilitated.

Table 1. Body weight and composition pre and post expedition

Age		Body weight (kg)		% fat UWW		Weight	Fat	FFM
Subject	(yrs)	Pre	Post	Pre	Post	Loss	loss	loss
RF	48	95.6	71.0	19.0	1.9	24.6	16.8	7.8
MS	37	74.8	53.0	18.5	2.5	21.8	12.5	9.3

Table 1 also shows that body composition changes were marked and the post-expedition UWW measurements suggested body fat levels of around 2% compared to around 18.7% before departure. However, the technique assumes an unchanging density of lean tissues - an assumption that may be untrue under such extreme circumstances of weight loss.

b) *Energy expenditure using dietary intake/weight losses and the isotope-labelled water technique*

Estimates of energy expenditure from the dietary intakes and the UWW data gave mean values for the whole expedition of 29.0 $MJ.day^{-1}$ in RF and 27.3 $MJ.day^{-1}$ in MS. These were in good agreement with the overall estimates from the isotope-labelled water which gave mean values of 29.6 MJ.day-1 in RF and 24.1 MJ.day-1 in MS. However, although these figures are in themselves high, they actually masked exceptional values early in the journey. For the first 50 days, the energy balance data gave values of 32.8 MJ.day-1 in RF and 28.7 $MJ.day^{-1}$ in MS, which were very similar to those from the isotope technique of 35.5 and 29.1 $MJ.day^{-1}$ in RF and MS respectively. Furthermore, when estimates of energy expenditure from the isotope data were analyzed in 10 day periods, RF had an energy expenditure of 44.6 $MJ.day^{-1}$ and MS of 48.7 $MJ.day^{-1}$ between Day 20 and 30.

During the second part of the expedition, from Day 51 to Day 96, energy expenditures were much lower according to both the energy balance and isotope techniques giving values of 24.7 and 24.3 $MJ.day^{-1}$ respectively for RF and 23.6 and 18.8 $MJ.day^{-1}$ for MS. However, although lower values were expected at this stage, since sledges were lighter and the journey was partly downhill, the isotope estimates are difficult to trust since both men showed a surprising rise in urinary D and O18 levels at around Day 80. The isotope estimates for the entire second period were therefore based upon data for the limited period from 51 to 80 days. Since it is difficult to envisage why a change in enrichments of the natural background water source should have occurred beyond Day 80, the increases in D and O18 in both men can only be explained by the entry of isotopes from the breakdown of body tissues which do not usually exchange with body water at a significant rate. These must have then have either contained a "memory" of the higher background enrichments of the UK, or must have become "labelled" at the time of isotope dosing.

c) *Resting metabolic rates, and metabolic and biochemical responses to a high fat test meal before and after the expedition*

The measurements of resting metabolic rate before and after the expedition demonstrated unexpected changes with RMR kg^{-1} FFM increasing by 11.2% in RF and 8.8% in MS. The maximum metabolic responses to the test meal expressed as a percentage of RMR were also increased from 16.8% to 75.4% in RF and from 17.6% to 96.2% in MS.

The blood sampling following the test meal demonstrated some changes in the bodies fat and glucose handling with both men showing a more rapid appearance of circulating triglycerides after the expedition.

This suggests an improved fat absorptive capacity and post meal circulating gastro-intestinal peptide levels also rose to higher levels following the journey. RF showed evidence of increased insulin resistance with blood glucose levels rising to a maximum of 11.5 mmol.l^{-1} post-expedition, compared to 8.0 mmol.l^{-1} before.

d) *Maximum aerobic capacity and isometric muscle strength*

Following the expedition VO2 max. declined from 53.6 to 41.2 ml O_2 kg^{-1} min^{-1} in RF and from 58.1 to 46.0 ml O_2kg^{-1} min^{-1} in MS. The MVC force production measured in the different muscle groups had also declined by up to 19.9% in RF and 55.8% in MS (Table 2).

Table 2. Changes in maximal voluntary contraction kg isometric force production (dominant), pre- and post-expedition

	RF			MS		
	Pre	**Post**	**% decline**	**Pre**	**Post**	**% decline**
Elbow flextion	28.1	25.2	10.4	27.4	16.3	40.6
Elbow extension	23.2	18.6	19.9	20.2	14.7	27.2
Grip strength	46.1	45.4	1.5	64.9	40.9	27.0
Leg extension	121.1	113.8	6.0	192.1	85.0	55.8
Abdominal flextion	45.7	46.3	+1.3	52.5	42.7	19.7
Upright pull	144.7	131.1	9.3	183.2	113.0	38.3

e) *Skeletal muscle enzyme activity before and after the expedition*

The combination of relative undernutrition with the prolonged exercise produced some surprising changes in skeletal muscle biopsy samples taken from vastus lateralis pre- and post-expedition. These showed decreases in both cytoplasmic and mitochondrial enzyme activities of: 47% and 56% for glycerol-3-phosphate dehydrogenase (Gly3PDH); 49% and 18% for Hydoxy-acylCoA dehydrogenase (HAD); 35% and 13% for Citrate Synthase (CS); and 56% and 63% for Cytochrome oxidase (COX), in subjects RF and MS respectively (Table 3).

Table 3. Enzymes activities in vastus lateralis muscle biopsy samples taken pre- and post-expedition. Enzymes activities are pressed as nmol.min^{-1} kg^{-1} wet muscle at 25°C

	Glyc 3 P DH			HAD			CS			COX		
	Pre	**Post**	**%Δ**	**Pre**	**Post**	**%Δ**	**Pre**	**Post**	**%Δ**	**Pre**	**Post**	**%Δ**
RD 195	195	104	47	6.04	3.10	49	20.14	13.10	35	8.42	3.74	56
MS 216	126	96	56	5.86	4.91	16	23.33	20.22	13	7.48	2.80	63
Normal range	(146 - 370)			(4.6 - 8.7)			(10.6 - 37.6)			(7.00 - 25.00)		

Such decreases make an interesting contrast to increases of 20% to 80% that have been seen after 6 weeks of training with normal energy balance (Wibom et al, 1992) and together with the loss of muscle mass, probably caused the observed decline in isometric strength. However, it is not clear why MS should have suffered greater strength losses.

The decline in skeletal muscle mass and enzyme content also explain the decrease in maximal oxygen consumption since the capacity for peripheral utilisation would have been reduced. However, it is also likely that cardiac muscle was lost and that maximal cardiac output declined, changes that have been documented in man during less prolonged dietary restriction with a smaller deficit in energy intake vs. expenditure (Rahamadany et al, 1989).

f) *Changes in blood glucose, insulin, cortisol, growth hormone, protein and lipids at 10 day interval during the course of the expedition*

The blood samples taken during the expedition also yielded unusual results. During the expedition, end of day blood glucose levels were low with mean values of 3.0 $mmol.l^{-1}$ in RF and 2.8 $mmol.l^{-1}$ in MS, and on two occasions (days 70 and 95) both men were apparently grossly hypoglycaemic with values of around 0.3 $mmol.l^{-1}$. It is obviously tempting to assume that these very low values were artifactual, but on one occassion they were accompanied by a very raised growth hormone level which would be an appropriate response to hypoglycaemia. It would therefore appear that the prolonged exercise combined with the relatively low carbohydrate intake and the under-nutrition genuinely led to frank hypoglycaemia; and that the men had adapted to utilise ketones or other substrates in the CNS. Despite a daily intake of around 290g of mostly saturated fat, total cholesterol values remained esentially unchanged and HDL cholesterol rose in both men from pre-expedition levels of around 0.9 $mmol.l^{-1}$ to post expedition values of around 1.6 $mmol.l^{-1}$. It would therefore appear that very high levels of exercise can offset the adverse lipid effects of even the most abnormal of diets.

DISCUSSION

It is evident from the weight losses alone, that this expedition entailed extremely hard work, maintained for a period of over 3 months. This exceptionally hard work resulted once again in massive exercise induced weight losses despite the very high energy intake. The magnitude of the changes in body composition were extreme in both men, and even the Minnesota experiment of Keys et al. (1950) only described changes of 70% in fatness and 20% in muscle mass after 52 weeks of chronic undernutrition. The very high energy expenditures documented by both the energy balance and isotope techniques must therefore be most unusual and, as far as I am aware, the isotope figures for the period between days 20 and 30, of 44.6 $MJ.day^{-1}$ in RF and 48.7 $MJ.day^{-1}$ in MS, are the highest sustained levels ever documented.

However, although these energy expenditure figures are exceptional and probably close to what is physiologically possible, they do remain lower than a theoretical energy expenditure ceiling of 58.5 $MJ.day^{-1}$ that has been calculated as attainable by ultra-long distance runners (Davies and Thompson, 1979). They are also made more credible by their corresponding to the period when the heavy sledges were dragged uphill from the ice-shelf to the plateau. Later in the expedition, when the men were descending from the Polar plateau with lighter sledges, energy expenditures were nearer normal and the energy expenditure values for the overall journey do not look unreasonable considering the circumstances.

From the point of view of military relevance, it must be assumed that under extreme conditions, personnel could equal or even exceed these excessive values. They therefore raise some questions about the adequacy of military Operational Rations which rarely provide more than 23 $MJ.day^{-1}$ - the figure consumed by the men on this expedition. However, noticeable debility did not occur on this Polar journey for at least 50 days and a reasonable level of function was maintained for approaching 90 days. It would therefore seem likely that major debility would not occur within the likely span of a military operation without resupply. Of course, ration restriction in the context of a military operation may be more extreme due to the need to backpack heavy loads, and it is not unusual for personnel to restrict their rations to around 10 $MJ.day^{-1}$ for periods when carrying additional ammunition becomes the most critical consideration. However, even for this situation, the findings from the Polar expedition can be viewed as encouraging since between days 20 and 30 of the journey, deficits of the order of 20 $MJ.day^{-1}$ were sustained whilst the men were working hard for prolonged hours each day.

According to the UWW, losses of body fat were extreme and anecdotally these losses were accompanied by a markedly lower resistance to the cold, although the exercise induced hypoglycaemia may also have disturbed effective thermoregulation. This, at first sight, would appear to raise the possibility that military clothing adequate for short-term cold weather operations may be potentially inadequate for very extended periods. However, the finding is not really transferable since the men on the trans-Antarctic expedition chose to carry very little insulative clothing, relying instead upon continued work to maintain body heat. They were able to do this since there was never any reason to stop other than at the end of the day when the protection of the tent

and sleeping systems was available. Military operators, on the other hand, will always have to be prepared for periods of low-level activity outside, and therefore will be obliged to carry some highly insulative protection.

Perhaps not suprisingly, weight changes also comprised considerable lean as well as fat losses, and in addition to this loss of muscle bulk, there were marked declines in muscle enzyme activities. It is therefore not surprising that there were decreases in both isometric strength and aerobic capacity, and it should be borne in mind that these measurements were made 7 days after completion of the journey when some recovery would have already occured. With almost all of their fat stores consumed, it would seem likely that even a short further period of negative energy balance would have precipitated a catastrophic decline in physical performance. However, although these findings suggest that strength losses detrimental to operational effectiveness will occur if military activities are protracted enough, once again, it seems unlikely that military personnel will reach this near terminal state of decline before dietary resupply was available. However, the figures do give Operational analysts some "maximal" data which might be useful in the consideration of very extreme scenarios.

The blood samples taken during the expedition yielded some very unusual results. It is obviously tempting to assume that the very low blood glucose values were artifactual, and certainly they may have been exaggerated by the cold causing low peripheral blood flows and hence greater peripheral glucose extraction. However, samples were taken in the tent which was not generally cold, and the low values were accompanied on one occasion in MS by raised cortisol and growth hormone levels which would be appropriate responses to marked hypoglycaemia and there was a general trend for increasing cortisol and GH levels throughout the expedition. It would therefore appear that the prolonged exercise, combined with the relatively low carbohydrate intake, did genuinely lead to severe hypoglycaemia and it suggests that, since they were conscious, the men may have adapted to the use of other substrates in the central nervous system. However, in the military context, the time for such an adaptation could not be relied upon and hence it is probable that the combination of large energy deficits with hard work would lead to severe hypoglycaemia. This should therefore be borne in mind when formulating lightweight Operational ration packs when there is the temptation to maximise fat content in order to minimise weight. On the other hand, the results from the expedition demonstrating adaptation to the high fat diet and no adverse changes in lipid profiles would encourage the use of Operational diets with higher than normal fat contents.

The insulin levels of around 11 to 14 $mIU.l^{-1}$ seen over the first 70 days of the expedition were rather high considering that glucose levels were 4.0 $mmol.l^{-1}$ or less. This may be due to a loss of insulin sensitivity secondary to the very high fat diet, although the insulin levels of 15 and 30 $mIU.l^{-1}$ in RF and MS respectively on Day 95 would still seem totally inappropriate in the face of glucose levels of only 0.3 and 0.4 $m.mol.l^{-1}$. Indeed, it would seem likely that this inexplicable hyperinsulinaemia was causing the hypoglycaemia.

The increased RMR and thermogenic responses following the expedition were in marked contrast to the changes that would be expected if similar weight loss had been induced by dietary restriction, when both parameters would have been likely to decline (Morgan, 1984). However, it has been shown that if "dieting" is accompanied by exercise, such reductions are limited and that if weight loss is achieved through increases in exercise whilst on a normal intake there are probably no declines at all (Mole et al. 1989). These data would therefore support a spectrum of change with RMR and DIT actually increasing if weight loss is induced by high levels of exercise during a period of high dietary energy intake.

The striking decline in testosterone in both men was similar, although more marked, to that reported by Aakvaag et al. (1978) in soldiers undergoing a combination of physical stress and near total sleep deprivation for 5 days. However, LH also declined during the Antarctic expedition, while Aakvaag reported little change or small increases in LH levels.

Overall, although there were only two subjects in these studies, the findings demonstrate that marked biochemical and physiological changes occur when men work hard in negative energy balance for a prolonged period. They therefore provide unique information regarding the body's adaptations under circumstances of extreme exercise and, since the men appeared to be close to likely physiological limits, they also give

indications regarding the period that well motivated men could sustain military effectiveness on inadequate rations. Certainly, it would be of great interest to obtain similar measurements on any future expedition of such duration and severity.

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The Effects of Exhaustive Military Activities in Man. The Performance of Small Isolated Military Units in Extreme Environmental Conditions

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MAN IN ISOLATED AREAS

ABSTRACT

Denmark has since 1951 operated its dog-sledge patrol SIRIUS along the North-eastern shores of Greenland. On each patrol 2 men and 10 dogs covers between 3000 to 4000 km. In this service they may not have any encounter with other humans for a period of 4-5 months. For this duty predeployment screening, selection, and training is of outmost importance. The decisive factor in succeeding in this military task is that each man gain confidence in himself and his colleagues. The role of experience is vast, but as each man is only assigned to this duty for 2 consecutive years it means that the "memory" knowledge stored within the members of the SIRIUS patrol only amounts to two years. Each individual thus has to master all the details of Arctic life necessary to live and accomplish his tasks. One of the operational principles is to regard the service in the patrol not as one of survival, but one of doing a regular job. The term survival technique is only attributed to those situations, where life is really at stake.

The aim of this article is to focus on selection criteria for duty in isolated areas, on factors relevant for such a duty, the importance and content of training, the role and duties of the back-up organisation, and finally how this is accomplished in the operations of the Danish dog-sledge patrol in the Northernmost Arctic.

Operating small isolated military units especially under extreme climatic conditions present a series of problems. Most nations do have such units dedicated to these tasks. The problems will change from mission to mission, but there are certain general aspects. One way to define these is to look at specific groups and from these try to come to a conclusion and determination of the common problems. Many of these may present themselves as very different, but analysed it may turn out, that the difference between operating in the cold of the high Arctic or the heat of the desert may less than expected. In Denmark the service of the Danish Sledge Patrol SIRES in the isolated parts of Northern Greenland may serve as the model for describing the problems in operating isolated military units.

Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

BACKGROUND

The Danish dog-sledge patrol SIRIUS was founded in 1951 as a Naval Force in the time of the Cold War. It had its predecessor in the sledge patrol formed during WW II in cooperation Beethoven the Danish Government in Greenland and the U.S. Coast Guard. This earlier patrol consisted of fur-trappers isolated in North Eastern Greenland. During the war, the sledge patrol located and destroyed several German weather bases along the coast. The weather of Greenland being the cradle for the meteorological conditions of the North Atlantic and Northern Europe. It has been stated, that it was weather reports from Greenland that made General Eisenhower decide to go on with OPERATION TORCH, the landings in Normandy in 1944 in spite of the local bad weather. The role of the sledge patrol was and still is to exercise the Danish / Greenlandic souverainity in this otherwise completely uninhabited part of Greenland, stretching from 70°N to 81°N. The patrolling is carried out in summer by airplane and boats (in the southern parts), but the main task is to patrol the coastline and its deep fjords by dogsledges. The patrolled coastline thus expands to about 40.000 km. The weather during winter is a harsh Arctic climate with frequent storms up to around 60 knots, blowing snow, and air temperatures down to - 50°C. Each dog team consists of 2 men and 10 dogs, using a travelling technique that has been adopted but modified from the original Eskimo dog sledge technique. In summertime depots of provisions and necessary reserve equipment have been placed along the coast by air or by boat as far north as the ice permits. In the area smaller huts have been erected not so much for the benefice of the men, but to protect the depots from the frequent visits by the polar bears, who does not recognise and respect the logo of the Danish Government.

THE JOB

The SIRIUS patrol consist of volunteers that has signed for this service for a period of 2 years. Before their deployment to Greenland they are trained for a period of 6 months in those skills and techniques that can be taught in Denmark (elementary ski-training is carried out in Norway with the Norwegian Forces). When arriving in Greenland they are assigned to a specific dog-team, and they will for the next two years always identify themselves with that dog team and its 10-12 individual dogs. The relation man / dog is one of the most important factors in their duty. This relationship will develop into a nearly personal relationship. Each team will feed its own dogs, take care of them and thus develop a "friendship" that will carry them together through the long journeys of the winter. Each individual dog-sledge team will consist of an "old hand" who is the leader and a newcomer. The old man is not only the leader, he is before anything else the trainer. Next year he will leave all the responsibilities to the new "old man". This principle of apprenticeship is a tradition in Greenland, and it is the basis for the operations of the patrol. At any time traditions and knowledge and memory is only as old as the oldest man in the patrol. To ensure the continuation of operations a former patrol-man is in charge of the administration and command of the patrol from his desk in the Danish Naval Command in Denmark. Operational control is exercised by the Island Commander Greenland (ISCOM GREENLAND) in Southern Greenland, but all provisions and logistics are provided from Denmark.

THE WINTER JOURNEYS

The winter journeys are the main objective of the SIRIUS patrol. Each team will have a route of between 3-4.000 km to cover. Dog sledging is hard work. The sledge has to be pushed, dragged, lifted, kicked forward sometimes on smooth fjord ice, where occasionally men and dogs feel like flying along, and at other times through loose deep snow, where every meter is a hard won victory, where the daily distance covered may be down to 4-5 km, and where distant mountains seem unchanged in appearance and distance for days. The daily accomplishment for a team is its covered distance, the reward is the tent, a pipe (if so inclined), and to relax with a hot meal - radio communication with main base - and sleep. Temperatures are far below zero. High wind may be prevailing. Wind-chill index is only a theoretical consideration without any practical relevance. You must learn to live in the nature as it present itself, as the Arctic animals, dependant completely on yourself and your techniques. Not surviving, but cunning is the principle. The margin between success and failure, between life and death may be narrow, but not narrower than you always have time to think and analyse - but in many cases not to err. Everybody realises, that he has only himself and his buddy to depend upon. Nobody can bring in any help within days. If the team drives through the ice, they are faced with a hard

job to free themselves, get out of the water, continue to a safe place where they with icing in the clothing, frozen fingers must untie the tent, rise it and lit the stove to dry out the wet clothing and material. This may take place at the height of the winter, where the sun has not been over the horizon for weeks, and where the only light is from the stars and the moon - if present. In the northern parts of the area it stays under the horizon like the sun and does not rise for a week or more.

The daily routine during the sledge voyages follows a fixed pattern, each sledge team make s its own adjustments, but these are seldom changed when first established. The ringing of the alarm clock wakes the one responsible for lighting the stove for melting snow. Breakfast consisting normally of oat meal with milk and tea or coffee. Melting of extra water for filling the thermos bottles with fluid for the whole day. Then breaking camp. Loading the sledge (each sledge carries around 400 kg of equipment, inclusive of extra (emergency) rations for men and dogs for 10 days). During the travel stops are made when necessary, either for rest or for fighting or rearranging the dogs who have their own appreciation of how and with whom they want to spend their day. During the day small meals will be taken, often consisting of raisins, chocolate etc. When the goal has been reached, the dogs are freed from their harnesses and for a short time are allowed to wander freely about - they know they will not be fed before they are back at their specific place in the chain. The tent is risen, snow is melted for the daily heavy meal. Radio communication with base is established, but not many unnecessary words are spoken, batteries should last the whole journey, and everybody knows that to o long correspondence s may mean , that power for transmission has to been produced by cranking the dynamo, which is no fun. This is the time for talk, at an outdoor temperature of -45° it is difficult to talk Sleep comes quickly. Personal hygiene is carried out in the evening. It hurts to meet the cold with fresh washed skin.

The darkness of the Arctic winter is often represented as a period, where man becomes depressed and moody. This can only be overcome by demanding work and routines, never slackness where the individual may "sink into himself'. On the sledge journeys the hard work is the medicine, at base winter time is the time, where preparations for the next journey takes place. Each team is responsible for its sledge and its dogs. Each team makes its own sledge from the raw materials. They must know every part of it and be able to repair any damage. The sledges themselves is the pride of its team. The basic construction is standard to facilitate the use of spare parts left in the depots along the route, but each team will find some way or other to distinguish their "ship". Discussions on sledge construction and sledging practices are always sure to bring everybody together.

Discussions are a measure of the psychological temperature of a group. This so much more in the small isolated group. It is typical of the small group, that discussion may be agitated. Everybody knows the other person so well, that his way of arguing, and his attitudes can be foreseen - if he not for the sake of the argument - chooses to take the opposite point of view. From an outstander the argument may be heated, and he may feel uneasy about the vigour and the hard points made - but in a well functioning group - and basically I know not of others in the Arctic - there are certain rules which nearly always are obeyed. Even the most frightful discussion may suddenly stop, everybody calms down and a cup of coffee or tea is produced - everything forgotten. This is the sign of the well-functioning group. Everybody knows the other, he may even dislike him or be opposed to him, but the society is too small, the group - and the individual - has to live together also tomorrow and the next day, week, month, perhaps year. When your whole company may be smaller than ten persons you cannot afford to loose any of them. As an old hand on a weather station at one of my first visits to the Arctic once answered when I asked him if he did not at times feel lonesome: "See Doc, here I know 7 other persons, I know, really know everything about them, and they me just as well - how many do you know, I mean, really know" - I was abashed and had learnt one of my first lessons of life in the Arctic.

Within the s ledge- team, where two men has to work closely together, share the same experiences, when not working living in a tent 2 times 3 meter. To be completely dependant upon each other and not having the possibilities of speaking to anybody else is a heavy demand on a person in his twenties. There may be periods, even days, where the communication may be non-verbal, where only the most necessary words are exchanged. This may not be a negative sign, but rather a sign of the complete awareness and knowledge of the other. The talkative person has a long and perhaps not too pleasant way to go before he learns that much can be said without words.

To the Arctic mammal living is not survival. It has been said about the Arctic peoples the Eskimos, that their culture was a culture of survival. But no culture can live on the brink of survival for any extended period. A culture must exhibit a surplus to exist. Nobody can live and prosper on survival terms. One of the secrets of living in any isolated and harsh environment is to make the daily life a well established routine. It has to be based upon fixed and well established procedures. Expedition life is often described as a kind of scouting, where the success depends upon improvisations and will to overcome deprivations. This is the unprofessional way of the small group. If one wishes to succeed in and accomplish the task given one of the most important principles is to "be prepared", not as a boy scout for everything, but for any conceivable contingency that may arise. To be a member of the sledge patrol it is necessary to be well trained for this particular work. But like any other military task it is just a job perhaps unlike any other, but it is the job you are trained to do.

The principle behind the way the Danish sledge patrol rests on well established procedures. The individual is trained theoretically and during his first year "on the job" in living as a "mammal" in its environment. He should have full protection from his clothes, and his equipment is designed for the task. In the Arctic the most dominant feature is the cold. But if the energy balance of a sledge team on journey is made up, the only real heat input is that provided by the food and the fuel used is mainly to melt the necessary snow to cover the unavoidable water loss.

Water is one of the main enemies of the Arctic traveller. Water in excess of what is needed for consumption is threatening man's well-being and perhaps even his existence. Water, frozen to ice will destroy the insulation of his clothes, his sleeping bag. It will accumulate and make the equipment heavier day by day. In certain military exercises water accumulation has been measured amounting to more than one litre per day per person.

The water removal from clothing and equipment is managed by the tent system. As mentioned the tent is not heated. It consists of a single layer of cotton. It is shaped like half a barrel as this shape has been found to be best in avoiding drifting snow to cover the tent. A tent like this could not tolerate rain, but its permeability, where the dry Arctic air is allowed to penetrate dries out the clothing during the night. The increase in weight of the equipment (clothing, sleeping bag etc.) amount to a few kilograms during a sledge journey of 3-4 months.

All equipment has to function after four months of heavy duty as well as on the first day or night. This leads to the preparations necessary for any longer isolated stay. All equipment must be thoroughly well prepared and tested. Even with the rightly chosen personnel any mission may have to be aborted due to faulty materials. Confidence in and knowledge of your equipment, its functions and limitations is of paramount importance. Amundsen knew this when he dashed to the South Pole in 1911, Scott the brilliant and courageous amateur succumbed. Preparedness is not only a state of mind it is as well a logistic and engineering hard accomplished condition. Too many parties (military, sportive or scientific) has endured unreasonable hardships if not complete failure due to foreseeable but not met requirements.

Food is one of the soldier's best rewards. This has always be recognised by the military leaders. Bad or lacking food has been the cause of most mutinies in the navies. The military history is full of examples of the deleterious effect of faulty supplies. The provision of good and plenty food is one of the most important parts of the planning for any isolated group that has to carry out a task in an isolated environment. It should be recognised that every individual has his favourite tastes. These can of course not all be met, but the food should be thus composed that it gives amble possibilities for individual variation. A package of different spices may be the factor that changes that for caloric reasons necessary pemmican into a endurable and even palatable and varied meal. Food is not only calories (joules or whatever). For a person that endures fatigue, cold, uncertainty, fright or perhaps even pain food is the daily reward. It should be sufficient in energy content and at the same time varied. Whenever possible the men should themselves be encouraged to participate in the selection of their food under the supervision of someone who can make the necessary corrections to ensure its sufficiency.

The recommended composition of U.S. military food is a caloric distribution of: 60-65% as Carbohydrates, 12-15 as proteins and 20-25 % as fat (+ vitamins, minerals etc.)

The caloric distribution in the Danish rations for the sledge patrol is: carbohydrates 17%, protein 28% and fat 55%. This is the basic composition when operating at temperatures around -40 to -50° C. At higher temperatures the fat content is diminished and replaced by carbohydrates.

In the cold experience has shown that the body besides an increased caloric input demands a very high fat intake (2). Some may speak of a craving for fat. Butter and ingredients with a very high fat content is used on everything. The traditional arctic food ration of Pemmican has a very high fat content, and the regard for this non-tasting high caloric foodstuff increases with the length of the journey - but even Pemmican needs to be accompanied by some other kind of food to relieve from the dietary boredom that is the complaint of so many expeditions.

Besides it should not be forgotten, that the caloric value of fat is twice that of protein and carbohydrates and thus provides the highest caloric value for a given weight.

Food composition and especially enhancement of physical performance by dietary means has had a high research priority (6). From the Danish experiences some of the debates on the optimal caloric composition of food seem to more to reflect the public debate on the influence of diet on general health than the basic needs for getting the soldier sufficient nourishment. For patrols operating for prolonged periods in extreme cold the caloric demand is very high. For a patrol of months duration food must be adequate if the soldier shall meet the demands. Dog sledging is hard work. When the environmental temperature drops below -40° C the experience is that food requirements changes. At lower temperatures the caloric intake is high, corresponding to the hard work and is around 7.000 Cals per day. Weight determinations before and after a sledge journey show that most individuals keep their weight constant.

But when it gets colder only fat can meet the demands, and food intake rises toward 10.000 Cals per day. As a patrol man said: "at normal temperatures (i.e. around -30 °C) we are hungry, and eat accordingly - below 40 ° we guzzle our food. I can eat butter directly out of the tin. We eat everything with a lot of sugar".

The same experiences are known from many of the earlier Arctic expeditions, where one had to live on hunting. Protein might even be abundant, but the lack of fat was the determining factor. On the 2.Thule expedition one of the members even committed suicide and in his last letter to his family gave the reason that he could not live on only on rabbit meat -they were only four days from the end of their journey.

The renowned Arctic Explorer Wilhjalmur Stefansson(3) wrote a whole book on the role of fat (pemmican) in the Arctic food. Eskimos regard the traditional fatty food not only as a delicatessen, but as a basic requirement.

The composition of the Danish Pemmican (LØVE-PEMMICAN) is given in Annex A.

Body functions

Regular body functions are of importance for anybody in an extreme climate. Constipation is very often a problem at the beginning of any special mission it might be favoured by dehydration and change in diet. Very often this is difficult to accomplish, especially the first days on a mission. The food should be composed accordingly with an amble content of fibres or a high content of fat and in the training the issue should be rised as an important problem.

BASE ACTIVITIES

The SIRIUS sledge-patrol has its base at 74° N. To the unprepared visitor the base does not live up to the expectations for an isolated outpost hundreds of miles from any other human habitation. The base has all modern facilities. Satellite TVs etc. The rationale is again, that living even in the high Arctic does not mean to live in a primitive way. On base life is "normal". The reason is the same as seen at military airbases. On base everything is as normal as possible. The military duties in the air, or out in the Greenland wilderness should be

in a contrast to routines at base. To live in a primitive environment does not increase efficiency - it only makes life more difficult. All efforts should be concentrated on the real objective, which is the patrolling.

The human relationships at base adheres to the principle, that everybody takes his share in the daily work. This means that although some jobs are dealt out to those with the appropriate training most jobs are performed on a rotational basis. Everybody - even visitors have to take their share in doing the dishing, cleaning and other trivial tasks. This is regarded as very essential to avoid a semi- social stratification. The commander participates on equal terms with his men

TAYLOR ( ) dealt in detail with the psychological problems of smaller isolated groups and the psychological stresses that frequently arose. His aim was to find selection criteria for selecting people for duty in isolated areas. But one of the main problems were associated with the boredom, if the group settled down to daily routines. Even in the best selected groups problems may arise. One of the remedies is to let everybody do his share. If the total work load is low, any workload how small it is may be a burden, and the cause for discussion. It should be a principle, that every work - and especially the mean routine tasks should be shared equally. If a social stratification might occur even within a little group, and this might eventually split the group. With soldiers on a specific mission these problems unless less pronounced, but the leader should always bear his responsibilities for the whole group in mind. He should be the first man to show that success depends on the work of every single member of the group. Cohesion within the group is a must, one of the non visible enemies is boredom. He might benefit from the old Danish saying: Idleness is the root of all evil.

From the Antarctic studies (TAYLOR, RIVOLIER et al.) there are reports on lack of group cohesion and other parameters, which influence the daily life and functions of personnel. These studies may have their relevance on isolated Arctic or Antarctic scientific stations, where the scientific tasks may result in different selections criteria for the group. The scientists are chosen because of their scientific qualifications, and it may be difficult to match these with the groups necessary for supporting the scientific studies. It has been argued, that scientific bases for instance in the Arctic may be a useful model for the manning of space stations. As I do not find similar problems in the small military community, I attribute the better social and psychological performance of the military groups to the uniformity of the group, and especially to the specific training of the group, a training which have many similarities to that of ie astronauts. One important factor for conserving group cohesion is that all tasks, even and perhaps especially the most humble tasks are equally shared by everybody.

During the sledge season (September to December and February to July) only a radio team (one of the sledge teams) is at the base. They keep the daily radio contact with the sledge teams. They are also the only contact with the outer world. At a fixed hour they will broadcast to the teams a survey of the overall weather situation, special news from Denmark and the world. They may have personal messages to individual members. These will often be open to everybody although crypto may be used. But everybody accepts, that news are common news and accepts that their colleagues in other teams are part of the same family and thus shares joys and sorrows. Doctoring is also accomplished from the base. In emergencies the base coordinates eventual search and rescue missions as well as other operation al orders like change in routes and missions etc.

SELECTION CRITERIA

The SIRIUS Sledge Patrol consist of volunteers. Application criteria are: normal health, full eyesight, normal colour vision, single - officers and NCOs. If these requirements are met there are no specific physical criteria - they will be trained physically during their service. Psychological screening was introduced 15 years ago. In the beginning the psychological screening did not contribute much to the selection. This was due to a lack of background knowledge. The criteria for selection for SIRIUS did not follow the criteria normally valued high in the military establishment. High scores in leadership abilities for instance showed to be a negative quality. Good co-operative abilities when working in a group is more essential. Stamina and self-confidence is positive as long as it does not lead to the domination of others.

As more applicants are accepted than actually needed the last selection is made by peer judgement after the initial training period of 6 months. The result of this last screening may not be according to the expectations gained by the initial screening, but within the small group itself small not detectable but very important aspects of personality are uncovered. This peer selection is done on the basis of each individual writing the names of whom he would like mostly to work with and whom he finds he would like the least. The outcome of this final "peer selection" gives nearly each year a very clear selection criteria. The same procedure is used in the patrol, where the "old hand" and the newcomers have to form next years teams. It is very seldom that a solution has to be pressed through.

PREDEPLOYMENT TRAINING

The aim of predeployment training is naturally to train the coming member of the SIRIUS Patrol in all the different aspects of the service that can be done in Denmark before he arrives in Greenland. This is training in his weapons, demolition, radio equipment and its use, boating, skiing and medicine. The medical training corresponds to that given to a shipmaster. He is trained in medical communication in order to enable him to act as the physicians eyes, ears and hands (Radiomedical procedures). He should be able to carry out simple life saving intervention, perform simple emergency operations (minor surgery) like suturing, removal of foreign bodies, plastering of broken limbs etc. The aim of the medical training is to give him confidence that he and his colleagues in an emergency can carry out these procedures and is part of that training in self-confidence and self-reliability which is regarded as essential.

During his more formal training he becomes acquainted with those in Denmark he might contact in emergencies. It is regarded as very essential that he has a good knowledge of those, who in an emergency will take over and direct him. This is regarded as one of the foundations of the safety organisation that it is based upon a personal and intimate knowledge of the persons involved. This consideration goes both ways.

CLOTHING AND PERSONAL EQUIPMENT

In any harsh climates efficiency and profound knowledge is essential to success. The clothing should ensure that the body temperature remains high and normal for the given activity, but body temperature is only one side to cold protection. Optimal functioning is even more important. Optimal functioning is dependant upon the persons ability to maintain high local temperatures in hands and feet ( ). Even in the high Arctic animals maintain very high peripheral temperatures in the muscles of the extremities. A sledge dog sleeping at – 40 °C will have a temperature between the footpads of about + 35 °C. The reason is obvious. Optimal function is temperature dependant. To catch the prey or evade the predator demands optimal physiological functioning. Man fells as good as his feet does is an old saying of people in cold climates. When cold, when threatened by central cooling (hypothermia), the function hands and feet are sold out to preserve life as long as possible. But local cold injury and frostbite is only minutes away in a blizzard at low temperatures. One of the most important lessons for man operating in extreme cold is to know the danger signs of impending local cold injury. Numbness due to cold occurs at a local tissue temperature of 6-7 °C. From that local temperature and lower there is no sensation left. He should know, that the last he may feel of his feet- is that he feels nothing. I have treated serious cases of frostbite, where the patient did not know anything of his condition before he came home and took of boots and socks.

In cold the clothing system should offer correct protection. As stated earlier, the concept is, that man must be able to live in the Arctic as part of it. His natural protection lies within the clothing system. In the later years there has been many new clothing materials. This development has been spurred by the affluent market within the civilian (sporting activities) sector. Much of this has also found its way into military clothing. Some materials are good and even better than the older ones. But any new material or item has to be thoroughly tested before introduction into the service. It seems astounding, but is a fact, that the clothing system used in the cold Arctic winter does in principle not differ much from that used by soldiers operating in Denmark. When working heavily even at very low temperatures, the insulating demand is very diminished. But to the clothing additional protection must be available in order to cover the insulating needs at times where the physical activity is low. The thermal demand on a clothing for the extreme cold is more dependant upon its

ability to change the overall insulation (by ventilating) than the absolute thermal insulation. Water in the clothing, due to sweating and melting snow and ice is the constant danger, as the water creates "cold bridges" between body and environment, and as water displaces the insulating airlayer, that is the basis of all thermal insulation. The clothing system adopted for use in extreme cold has changed very little during the last century. Eskimo clothing although extremely efficient is not acceptable for use as it needs constant repair, and as it will be difficult to obtain in the needed quantities. An arctic explorer from the beginning of the century would marvel over the modern zipper, but the rest would as well in design as in materials be familiar to him. The system is that of the "layer principle", where increased protection against weather and cold is obtained by adding new layers to the system.

The clothing system used for extreme cold consist of an inner "hygienic layer". As there is no way of washing clothes to any sufficient extent for 3-4 months it is essential to teach the modern youth, that many of the rules of basic hygiene he has observed in his normal life has to be disregarded and that this does not to any larger degree create a health hazard. He himself will rapidly observe, that the senses and especially the sense of smell adapts very quickly.

Over the hygienic inner layer the insulating layers are added. These may change according to the thermal load, where it should not be forgotten, that even at very low environmental temperatures, heavy work produces so much heat, that the demands on insulation are very diminished 8). To this decreasing demand, the energy cost of wearing a heavy Arctic clothing has to be added. Modern man-made materials are changing the principles of clothing. But old fashioned wool and cotton are still in very high regard. The modern thermopile fibres have an advantage in being lighter for a given in sulation, but their role in water accumulation and water transmission has yet to be evaluated. One disadvantage is that most of these materials are inflammable and thus when used in the small space of the tent may present the most dreaded hazard of the Arctic - fire.

CONCLUSIONS

The Danish Armed Forces have for more than 25 years maintained small isolated military groups in the Arctic. It is the Danish experience, that in maintaining these groups on a high level of efficiency the following principles weights very heavy:

- the members of the group should be selected not on basis of their individual cognitive skills, but more for their ability to work subordinate to the common demand of their peers.
- in the small isolated group the need for good traditional military leadership is of less importance and little relevance, as the group exists on the basis of every members ability to work subordinate to the common goal.
- training together for all the common necessary skills is important to avoid "social stratification within the small group.
- in the very small group everybody is important, and within the group there will be a very sensitive psychological mechanism, which tends to eliminate the risk of anyone "dropping out".
- premission training in all necessary job skills is necessary. In a harsh environment you have to "be prepared" for all conceivable emergencies. All experiences should be analysed and find their way into the future training . This is especially important in groups that only have a "life-span" of only two years, as that means that the collective memory is very short.
- the group should always have a consultant at base 7 home, that personally has earlier experience in the functions of the group, and who "at home" can follow the group. He should know all the individuals. He will eventually be the consultant in emergencies.
- before mission all parts of the equipment (clothing, weapons, machinery etc.) should be well known to everybody. In a harsh environment, there will be very little time for mistakes.

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ANNEX A

LØVEN PEMMIKAN

Contents of Pemmican used by the Danish Dog-sledge Patrol in Northeastern Greenland.

12 % soyaprotein
10 % milkprotein
30 % pea flour
3+ % vegetable oil

2-3 % vitamins
mikrominerals
antioxydant
15 % water

Energy distribution: 28 % protein
55 % fat
17% carbohydrates

Energy content per 100 g: 2266 KJ = 536 Kal.

The same Pemmican is used by dogs and man. The transportation costs are so high, that it would not be logistically relevant to have two different Pemmicans.

In extreme cold man requires 7.000 to 10.000 Kal per day.

The sledge dog requires and gets beween 3.500 to 4.500 Kal per day.

Special Rations for Long Range Reconnaissance Troops

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INTRODUCTION

In 1987 the Federal Armed Forces decided to develop or search for a light-weight **high calorie** field ration intended for the supply of soldiers on extended special missions outside of the supply area and solely dependent on themselves. Since they have to be provided with all provisions required for the duration of their mission, the field ration is reduced in volume and weight and.comprises various food items of high calorie density specially adapted to the operational conditions of long range reconnaisance troops, frogmen, arnoured reconnaissance units and paratroopers.

The Federal Agency for Defence Technology and Procurement composed two different lightweight rations of high calorie density consisting of ready to eat "energy bars" and **dehydrated** food to which hot water has to be added before consuming, which was specially adapted to the operational conditions of long range reconnaissance troops, armoured reconnaisance troops, and SEALS.

The Division for Exercise Physiology was asked to assist in evaluating possible medical or physiological risks during a planned 20 day field exercise during which only light-weight rations were to be eaten.

INTRODUCTION

The exercise took place from the end of april to the beginning of may at Putlos **shooting range** at the border of the Baltic Sea.

The participants (=subjects(Ss)) of the study were recruited on a semi volunteering basis from three different LRRT companies. The total of 36 men were divided into two groups: 24 test persons (age 22.8 ± 3.6 y and body height 179 ± 6 cm) who consumed the special light-weight ration (SLR) and 12 controls (age 21.8 ± 2. y and body height 180 ± 5 cm) who got the German field rations (GFR). The exercise consisted of typical LRRT-tasks *id est* long distance marching, observing and reporting, shooting and a parachute jump. The observers made sure that no additional food whatsoever was available for either group except tap water ad libitum.

However, they had to protocol the number of water bottles consumed per day. Additionally, the Ss filled in all nutrients not consumed into a nutrition diary so that eating habits and preferences of different parts of the field rations could be deducted together with the whole caloric intake. The medical/physiological tests were obtained on days 1 and 2, 9 and 10, **and 17** and 18 of the field exercise. On the morning of the first laboratory day the Ss **reported fasting** after voiding for measurements of weight (in shorts), body fat according to **Durning and** Womersley and blood withdrawel for the analysis of hemoglobin, hematocrit, glucose, uric acid, triglycerides, total cholesterin, total protein and sodium, potassium and calcium. The second laboratory day was spent in the nearby Institute of Naval Medicine, where cycle ergometry was performed using four ergometers in parallel applying a 2 min 25 Watt step protocol starting at I Watt/kg BW. Ergometry was stopped when HR 170/min was reached or the Ss felt exhausted. The Ss were retested on the same cycle ergometer at the same **time of** the day to reduce intraindividual circadian influences. From the HR response to

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Paper presented at the RTO HFM Workshop on "The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation", held in Oslo, Norway, 3-5 April 1995, and published in RTO MP-042.

the cycle ergometry the Watt load was calculated for which the S needed a HR of 170 min '; this is called Physical Working Capacity at heart rate 170 min^{-1} (PWC_{170}). Division by body weight results inthe relative PWC_{170} [Watt/kg].

RESULTS

By introducing the new special light-weight rations the total weight to be carried by the LRRT for a 20 day patrol was reduced by 30 % or 22 kg. Thus the first aim was reached. The psychological effects of the food were interesting. Prior to the experiment, the SLR was looked upon as "space food" or something very special and the control group was pittied to have to eat the unloved SFR. After seven days the opinion had changed completely: the test group envied the control group for their food and the control group teased the test group for getting "soups only".

The physiological results were as follows: (Table I).

Table I. Anthropometry

Measure	Group	Date		
		18 April	26 April	4 May
Body Weight (kg)	Army Test Group Army Control Group	75,5 78,2	72,7 76,7	71,6 75,9
Rel Fat Content (%)	Army Test Group Army Control Group	16,2 15,7	14,6 15,2	13,7 13,9
Fat Free Mass (kg)	Army Test Group Army Control Group	63,1 65,9	62 65,1	61,7 65,4

Both groups were identical with respect to age, body weight, body height and body mass **index** (BMI), although the control group consisted of only 12 Ss while the test group comprised 24 Ss.

Body weight in both groups decreased during either week, the drop during the first week, however, was more pronounced. In total the test group lost 3.9 kg while the control group lost 2.3 kg which is 5.2 and 2.9%, respectively, of the initial body weight. In the control group this weight loss was not caused by a hypocaloric supply but by a combination of boredom by the repetitions of GFR and an intended weight reduction during a period of high energy **output.** The latter effect has been observed with US military as well.

Relative body fat was also reduced in both groups in either week. The test group lost 2.6% points while the control group lost 1.8% points. This is 16% and 11.5%, respectively, of the initial value.

Fat free body mass decreased -surprisingly enough- too. The amounts, however were **small** although in the test group statistically significant: the test group lost 2.2% FFM and the control group gave up 0.8% of their FFM.

From the weight losses and from the food consumed we calculated an average daily energy turnover of 3700 kcal which, according to German definition of work physiology, is strenuous work.

The data of the blood analyses are summaried in table II. The blood analysis showed a paralled drop in hemoglobin and hematocrit of approximately 6% in both groups. All values, however, remained well with in normal physiological limits. The cause for this finding is **uncertain and** may be explained by the experts present at this meeting.

Table II. Data of blood analyses

Measure	Group	Date		
		18 April	**26 April**	**4 May**
Serum Sodium (mmol/l)	Army Test Group Army Control Group	140,7 142	140,3 140,9	140,6 140,4
Serum Potassium (mmol/l)	Army Test Group Army Control Group	4,2 4,2	4,4 4,2	4,3 4,2
Serum Calcium (mmol/l)	Army Test Group Army Control Group	2,4 2,4	2,4 2,4	2,4 2,3
Total Serium Protein (g/l)	Army Test Group Army Control Group	68,7 68,4	71,5 67,5	73,5 71,5
Serum Clucose (mg/dl)	Army Test Group Army Control Group	77,1 80	78,5 78,7	70,9 73,9
Serum Triglycerides (mg/dl)	Army Test Group Army Control Group	113,4 167,5	49,1 61,1	66 88,1
Total Serum Cholesterin (mg/dl)	Army Test Group Army Control Group	200,5 207,5	162,7 175,7	152,9 163,9

The changes in serum electrolytes were minute and in either direction. From this we state that neither food preparation would cause an electrolyte deficit.

Total serum protein increased during either week in the test group while in the control group it rose quite pronouncedly during the second period after an initial drop during the first period. All increases are statistically highly significant and totalled in +7.1 and +4.5%, respectively. This finding was surprising as the drop in hematocrit could have been interpreted as **hinting to** a hemodilution while the increased total serum protein would point towards a hypohydration due to the strenuous exercises and the hypocaloric diet. This idea is supported by the finding that the control group showed only an insignificant drop in total serum protein in the first period, when they consumed almost all of the GFR, while during the second week, **during which the** consumed less (because they were fed up), the incresase was almost as **great as the** total augmentation over the whole period in the test group.

Serum glucose concentrations showed an overall drop of roughly 8%, which is statistically significant. As the changes observed in the first week were bidirectional and not statistically significant and as the decreases measured in the second period were statistically significant this may be explained as an effect of the hypocaloric nutrition which, in the second period, was pronounced in both groups.

This is supported by the changes observed in serum triglycerides: They dropped by **more than** 40% in either group over the whole period. During the first part of the exercise this drop was even more pronounced amounting to more than 50%. This is a well-known effect of periods of high-energy turnover and intended by those soldiers who know about it. Total serum cholesterol depicted si mi l ar behavi our : the total d rop was about 2 5% with a greater reduction in the first week as compared to the second week. All changes except the drop in the second period in the control group were highly significant. This is to a certain extent surprising as the initial values of 200+35 mg/dl were indeed not very high. Unfortunately the laboratory was not able to differentiate into HDL and LDL sub groups. However, changes in these subpopulations have already been documented by others.

After the biochemistery of blood showed no alterations that could not be tolerated from a standpoint of nutrition physiology it seems interesting to look into the data **of cycle ergometry,** the data of wich are summarized in table III.

Table III. Results of the cycle ergometry

Measure	Group	Date		
		18 April	26 April	4 May
PWC_{170} per kg actual BW	Army Test Group Army Control Group	3,5 3,2	3,7 3,5	3,9 3,8
PWC_{170} per kg actual FFM	Army Test Group Army Control Group	4,2 3,9	4,3 4,1	4,6 4,4

Both groups showed a rather high value of the relative PWCl70 i.e. 3.57 and 3.29 Watt/kg. These data, related to fat-free body mass, will amount to 4.27 and 3.90 Watt/kg FFM. **During** the observation period, this measure of physical fitness increased to 3.95 Watt/kg BW and 4.65 Watt/kg FFM in the test group and to 3.83 Watt/kg BW and 4.49 Watt/kg FFM in the control group. The latter increase was more pronounced. However, the control group having a lower value to start with, it could be argued that the identical training stimmulus led to a higher training effect in the (slightly) less trained group.

One of the problems with dry food is the water supply. The control group consumed almost 2 I/day while the test group used 0.5 I/day more. This is on one hand surprising as **the water** deficit of the LSR is more than 500 ml, on the other hand one could assume that the control group had a higher water output, which we were not able to control. Anyhow, a water turnover of 2 or 2.5 l/day is adequate for strenuous exercise conditions during spring time on the borders of the Baltic Sea. The control of early morning urine for ketone bodies, which was done by the Ss using test-sticks gave no hints for keton uresis.

The results show that dehydrated food with a caloric content of 2100 koal/day is well tolerated by LRRT in spite of the fact that a decrease in body fat content was observed.

Ergonomic improvements resulted in food containers into which the hot water could be poured without the need to use the mess-tins and additional water for "dish washing".

In the meantime the variety of dishes has been augmented. It comprises 27 different food items as well as multipurpose paper, matches, plastics bags, and water purification tablets wich are combined to 5 types of ration sets (type I through V).

REPORT DOCUMENTATION PAGE

1. Recipient's Reference	2. Originator's References	3. Further Reference	4. Security Classification of Document
	RTO-MP-042 AC/323(HFM)TP/23	ISBN 92-837-1054-1	UNCLASSIFIED/ UNLIMITED

5. Originator Research and Technology Organization
North Atlantic Treaty Organization
BP 25, 7 rue Ancelle, F-92201 Neuilly-sur-Seine Cedex, France

6. Title

The Effect of Prolonged Military Activities in Man. Physiological and Biochemical Changes. Possible Means of Rapid Recuperation

7. Presented at/sponsored by

the former DRG Workshop held in Oslo, Norway, 3-5 April 1995 (the publication was sponsored by the Human Factors and Medicine Panel - HFM - of RTO).

8. Author(s)/Editor(s)	9. Date
Multiple	March 2001
10. Author's/Editor's Address	**11. Pages**
Multiple	134

12. Distribution Statement There are no restrictions on the distribution of this document. Information about the availability of this and other RTO unclassified publications is given on the back cover.

13. Keywords/Descriptors

Military operations	Survival training	Hematology
Continuous operations	Military training	Endocrinology
Human factors engineering	Sleep deprivation	Cold stress
Biomedicine	Performance evaluation	Starvation
Stress (physiology)	Cognition	Recovery
Biochemistry	Circadian rhythms	Fluids
Fatigue (biology)	Hormones	Erythropoietin
Exercise (physiology)	Immunology	

14. Abstract

New technology and operational concepts have provided new challenges to military personnel's health, safety and performance. These proceedings include ten papers, dealing with different aspects of the biomedical consequences of continuous military operations. They were presented at a workshop organised by the former DRG of NATO in Soria Moria, Oslo, Norway, 3-5 April 1995, and this publication has been sponsored by the Human Factors and Medicine Panel (HFM) of RTO.

The papers show large alterations in hormones, metabolites, red and white blood cells, as well as mental performance. There was an adrenergic desensitization, decrease in thyroid function due to energy deficiency, and decrease in androgens due to physical exercise. Unspecific immune function (granulocytes and monocytes) were stimulated, while specific immune function (lymphocytes) were inhibited. Acute sleep deprivation mainly affects mental functions and have limited influence on endocrine, metabolic and immune functions.

The key questions addressed by the workshop were how to select and train personnel for continuous operations, and how to intervene to enhance physical and mental performance by such means as nutrition, sleep, work-rest schedules and drugs. The optimal treatment for soldiers' recovery and reentry to the battlefield following high intensity direct action missions was also discussed.

The participants agreed that there is a need for more realistic field studies with systematic interventions with different stress factors to reveal their relative significance and to find counter measures.

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(Membre de la Corporation St-Joseph)
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Printed by St. Joseph Ottawa/Hull
(A St. Joseph Corporation Company)
45 Sacré-Cœur Blvd., Hull (Québec), Canada J8X 1C6

ISBN 92-837-1054-1