Immune, endocrine and metabolic changes related to exhaustive and repeated exercise sessions

Doctoral thesis

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To Jorunn, Sebastian and Adrian

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1. Immuno-endocrine and metabolic changes during a World Cup cross-country ski race in elite male and female athletes. *Scand J Med Sci Sports*, *Accepted for publication May 2003*. Ola Ronsen, Elisabet Børsheim, Roald Bahr, Bente Klarlund Pedersen, Egil Haug, Jens Kjeldsen-Kragh, and Arne T. Høstmark.

2. No effect of seasonal variations in training load on immuno-endocrine responses to acute exhaustive exercise. *Scand J Med Sci Sports, 2001, Vol 11, pp 141-148*. Ola Ronsen, Kristian Holm, Hanne Staff, Per Kristian Opstad, Bente Klarlund Pedersen, and Roald Bahr.

3. Increased neuroendocrine response to a repeated bout of endurance exercise. *Med Sci Sports Exerc, 2001, Vol.33, No 4, pp 568-575*. Ola Ronsen, Egil Haug, Bente Klarlund Pedersen, and Roald Bahr.

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6. Residual effects of prior exercise and recovery on subsequent exercise-induced metabolic responses. *Eur J Appl Physiol,* submitted December 2002. Ola Ronsen, Øystein Haugen, Jostein Hallén, and Roald Bahr.

7. Recovery time affects immuno-endocrine responses to a second bout of endurance exercise. *Am J Physiol Cell, 2002, Vol 283, No 6, pp 1612-1620,* Ola Ronsen, Jens Kjeldsen-Kragh, Roald Bahr, Egil Haug, and Bente Klarlund Pedersen.

Summary of the thesis

In study I we examined the magnitude of change in immune, hormonal, substrate and metabolic variables during a long distance ski race among 10 male and 6 female international cross-country skiers. From before to immediately after the race, we found increases in the concentrations of granulocytes, natural killer (NK) cells, epinephrine (EPI), norepinephrine (NE), growth hormone (GH), cortisol, glucose, free fatty acid, creatine kinase, uric acid, non-organic phosphate, and a decrease in insulin concentration.

In study II we studied the impact of variations in seasonal training and competition load (TC-score) on the acute response to exhaustive exercise in 10 male, international cross-country skiers. We tested the hypothesis that increased training and competition load would result in more pronounced stress responses in connection with an exhaustive exercise test. We found the TC-score to be twice as high during the in-season compared with the off-season period. However, during and after a standardized exercise test, there was no difference in the magnitude of change in concentrations of neutrophils, lymphocytes, EPI, ACTH, or cortisol, between the in-season HI and off-season LO tests; and only minor differences between norepinephrine and the IL-6 concentrations.

Study III examined the impact of a previous bout of strenuous endurance exercise on the responses to a subsequent exercise bout the same day, and tested the hypothesis that immune, endocrine, and metabolic responses would be more pronounced during and after a second bout compared with a single bout of exercise. Compared with the single bout of exercise, the second bout resulted in higher plasma concentrations in EPI, NE, ACTH, cortisol, and GH; similar concentrations in insulin, FSH, LH, TSH, F-T4, IGF-1, and glucose; higher concentrations of total leukocytes, neutrophils, lymphocytes, CD4+, CD8+, and CD56+ cells; reduced NK-cell activation, higher levels of plasma IL-6 and IL-1ra; higher mean O_2 uptake, heart rate (HR), rectal temperature (T_R), excess post-exercise oxygen consumption (EPOC) and lower respiratory exchange ratio (RER). For the most part, our results confirmed the hypothesis.

Study IV examined the impact of different recovery periods between two daily exercise sessions on the responses to a second bout of exercise, and tested the hypothesis that changes in neuroendocrine, immune, and metabolic variables elicited by the second bout of exercise would

be more pronounced when preceded by a short compared with a long rest. Compared with the long rest between the exercise bouts, the short rest resulted in higher plasma concentrations EPI, NE, ACTH, cortisol and insulin; similar concentrations in GH, FSH, LH, TSH, F-T4, IGF-1, and glucose; higher concentrations of neutrophils; similar concentrations of CD4, CD8, CD56 cells, IL-6, and IL-1ra; similar lymphocyte activation; lower post-exercise lymphocyte concentrations; higher O_2 uptake, HR, T_R, and lower RER. For the most part, our results confirmed the hypothesis.

List of abbreviations

ACTH: adrenocorticotropic hormone
ANOVA: analysis of variance
ADP: adenosine di-phosphate
AMP: adenosine mono-phosphate
ANS: autonomic nervous system
ATP: adenosine tri-phosphate

CD: cluster designation
CD3+ cell: T-lymphocyte
CD4+ cell: T-helper lymphocyte
CD8+ cell: T-cytotoxic lymphocyte
CD16+ cell: large granular (NK) lymphocyte
CD56+ cell: large granular (NK) lymphocyte
CD69+ cell: activated lymphocyte
CNS: central nervous system
CHO: carbohydrate
CRF: corticotropin releasing factor
CRP: C-reactive protein
CV: coefficient of variance

ECG: electrocardiogram
ELISA: enzyme-linked immunosorbent assay
EPI: epinephrine
EPOC: excess post-exercise oxygen consumption
Ex-M: exercise bout in the morning
Ex-A: exercise bout in the afternoon

FFA: free fatty acids
FIA: fluoro-immunometric assay
FIR: fluorescence intensity ratio
FSH: follicle stimulating hormone
FT-4: free fraction of thyroxin

GH: growth hormone
GLUT-4: glucose transport molecule-4

HPA: hypothalamic pituitary adrenal axis
HPLC: high performance liquid chromatography
HR: heart rate
HSL: hormone sensitive lipase

IgA: immune globulin A
ICAM: inter cellular adhesion molecule
IGF-1: insulin-like growth factor-1
IL-1: interleukin-1
IL-1ra: interleukin-1 receptor antagonist
IL-6: interleukin-6

IRMA: immuno-radiometric assay
ILA: immuno-luminometric assay

LH: luteinizing hormone
LONG: experimental trial with long rest between two bouts of exercise

NE: norepinephrine
NK-cell: Natural Killer cell

ONE: experimental trial with one bout of exercise

R: correlation factor
RER: respiratory exchange ratio
REST: experimental trial with 25 h of rest
RIA: radio-immuno assay

SD: standard deviation
SEM: standard error of the mean
SHBG: sex hormone binding globulin
SHORT: experimental trial with short rest between two bouts of exercise
SNS: sympathetic nervous system
SPSS: statistical package for social sciences

TCS: training and competition score
TG: triglyceride
T_R: rectal temperature
TSH: thyroid stimulating hormone
TWO: experimental trial with two bouts of exercise

VCAM: vascular (inter) cellular adhesion molecule

1. Introduction

1.1. Exercise in a stress research model

Exercise elicits a multitude of changes in biological functions in the body and could thus be regarded as a stressful stimulus that may disrupt a well-regulated state of homeostasis. In a stress research model, a stressful stimulus is labeled the stressor and the following response is described as the stress reaction (160;193;194). The stressor-induced reactions are observed in the context of a pre-set and meticulously regulated balance, often referred to as homeostasis in the biological science. As early as 1929, W.B. Cannon described homeostasis as a ”coordinated physiological process, which maintains most of the steady states in the organism”. Thus, stress may be defined as a disruption of homeostasis (29), and the subsequent reactions are programmed to counterbalance the perturbations of the stressor and to re-establish the homeostatic balance after the stressful stimulus is terminated. This gives the following sequence of events:

Stressor → Stress Reaction → Re-establishment of homeostasis

In a setting of physical activity, exercise becomes the stressor and exercise responses the associated stress reactions. In keeping with this stress model, recovery may be viewed as the re-establishment of homeostasis after exercise is terminated. Thus, with regard to exercise stress we have the following sequence of events:

Exercise → Exercise Response → Recovery

The magnitude and character of the exercise-induced responses are dependent on several factors related to the exercise stimulus itself (intensity, duration, mode, etc.), environmental factors (temperature, humidity, altitude, etc.) as well as conditions related to the internal milieu of the body (hydration, feeding state, time of day, etc). In the field, environmental conditions may represent additional impact on the exercise response. However, in a laboratory setting intensity and duration of the exercise become the most important determinants for the magnitude as well as the type of such responses.

Traditionally, exercise science has focused mainly on the changes occurring during exercise. However, a number of studies have also characterized post-exercise changes in various physiological systems and functions (2;8;120;125;128;165;172), while others have used interventions during recovery known to influence the normalization of biological processes (16;19;20;149;150;169;234). Still, several investigations have failed to follow up during a sufficiently long period after exercise to observe complete normalization of the variables studied. It can also be questioned whether normalization of exercise-induced changes during the recovery period actually represents a valid expression of complete homeostasis. One way of addressing this question could be to introduce a second identical bout of exercise as soon as possible after normalization of the responses from the first exercise bout, and compare the changes related to the first bout with those resulting from the second bout of exercise.

However, before giving a more detailed description of the ideas and aims of the four studies included in this thesis, a short introduction to some components of the immune system, neuroendocrine-immune system interactions, and endocrine regulation of muscle metabolism during exercise is warranted. The selection is based on the indices of immune, endocrine and metabolic function measured in the four studies of this thesis.

1.2. Components of the immune system and exercise

1.2.1. Leukocytes and cytokines

The immune system is an interactive network of organs, tissues, cells and specific molecules with the overall purpose of providing protection against foreign substances that are intolerable to the internal milieu of the body (121;218). Immune cells have many different characteristics and functions and a systematic nomenclature for all leukocytes (Cluster Designation) refers to groups (clusters) of specific antigens found on the cell surface for each sub-population of leukocytes. These CD antigens may be identified by the binding of a fluorescence-marked monoclonal antibody to the specific surface antigen of a leukocyte. Thus, all thymus-derived lymphocytes (T-cells) are identified as a population of lymphocytes by the presence of a common surface antigen called CD3, i.e. CD3+ (positive) cells. However, sub-populations of these CD3+ T-cells may also be identified by the co-expression of another surface antigen called CD4 (T-helper cells) or CD8 (T-cytotoxic cells). Moreover, there is also a certain degree of overlap in these antigen markers between the different leukocytes. Some T-cytotoxic cells can express both the CD8 and CD4 marker, and may therefore be identified as a T-helper cell as well (116). To further complicate the

matter, in most instances no single surface marker may be able to identify all functionally related leukocytes. For example, a group of large granular lymphocytes --often referred to as Natural Killer (NK)-cells-- may express either the CD16 or CD56 antigen, or both. However, the overlap between these two antigens is quite extensive, thus by using monoclonal antibodies towards one of the antigens, usually > 90% of the NK cells will be identified (36).

Interleukin-6 is a cytokine with several biological effects in different tissues, mediating increased osteoclast activity in bone, regulating inflammatory activity in immune cells and liver, and possibly metabolic activity in muscle and fat tissue (48;168;217). The increase in several cytokines during strenuous exercise has to a large extent been attributed to activation of the neuroendocrine and immune system (164), but recently it has been demonstrated that exercise-induced changes in peripheral organs and tissue can lead to increased cytokine production locally (103). IL-6 has traditionally been categorized as a pro-inflammatory cytokine, because it is secreted from immune cells together with IL-1 and other cytokines in connection with local tissue damage (90). However, IL-6 also seems to mediate anti-inflammatory responses by up-regulating the production of interleukin-1 receptor antagonist (IL-ra), thus blocking the biological effects of IL-1 (223). Moreover, IL-6 has also been suggested to mediate endocrine and metabolic effects in several tissues (217). During prolonged exercise IL-6 is produced in the working muscle and large amounts is secreted into circulation as exercise exceeds 2 h (168). This muscle-produced IL-6 is suggested to have hormone-like action on liver and fat tissue and mediate a change in mobilization of substrates for the working muscle (214).

Several components of the immune system interact closely with the endocrine and central nervous systems (CNS) (163;241). Therefore, in addition to being activated by microorganisms and foreign substances, the immune system may also be triggered by neuroendocrine factors released from the hypothalamic-pituitary-adrenal (HPA)-axis, pituitary hormones and neuronal pathways of the autonomic nervous system (ANS) (48;140;206). This is mediated by the binding of neuroendocrine signal molecules to specific receptors on immune cells, and via the distribution of sympathetic nerve endings into lymphatic tissues. Thus, most types of stressful stimuli that activate the neuroendocrine system --including physical exercise-- will also exert a significant impact on several functions of the immune system (229).

1.2.2. Effects of acute exercise on components of the immune system

Many exercise-induced changes in circulating components of the immune system show a near-linear relationship to the intensity of short term exercise, i.e. the higher the intensity of exercise, the stronger the perturbations in the immune system (78)(134;134;151;201;201). Prolonged strenuous exercise (>1 h) may lead to additional increases in some leukocytes concentrations and soluble components of the immune system (163). However, after more than 2 h of exercise, the duration more than the intensity of the exercise may have a stronger impact on concentrations and functions in leukocytes (184).

Table 1.2.1 Overview of changes in components of the immune system in connection with strenuous exercise. Arrow up means increase, arrow down means decrease in concentrations. Arrow sideways means no change. Two arrows imply that the magnitude of change is large.

See text for references.

	During exercise	**After exercise**
Neutrophil count	↑↑	↑↑
Monocyte count	↔	↑
Lymphocyte count	↑	↓
CD4+ T-cell count	↑	↓
CD8+ T-cell count	↑	↓
CD19+ B-cell count	↑↑	↓
CD56+ NK-cell count	↑↑	↓↓
NK-cell activity	↑	↓
Lymph. proliferation response	↓	↓
Antibody response in vitro	↓	↓
Plasma Immunoglobulins	↔↓	↔↓
Salivary IgA	↓	↓
C-reactive protein	↔	↑
Plasma Interleukin-1	↑	↑
Plasma Interleukin-1ra	↔↑	↑↑
Plasma Interleukin-6	↑↑	↔↓
Plasma Interleukin-10	↑	↑

The direction of change in various components of the immune system during and after strenuous exercise listed in table 1.2.1, are based on consistently reported findings in numerous exercise studies and summarized in several reviews (22;73;102;153;163;197;201;250). A more detailed description of perturbations associated with various protocols of strenuous endurance exercise will be offered in chapters 3, 4, 5, and 6 of this thesis.

1.2.3. Leukocyte trafficking

One of the most studied effects of exercise on the immune system is the change in leukocyte concentrations in the blood, also called leukocyte trafficking (62;134;163). Alterations in circulating leukocyte concentrations have also been examined to understand potential neuroendocrine effects on the immune system (10). However, there are several limitations to the usefulness of examining exercise-induced changes in concentrations of circulating leukocytes. One limitation concerns the issue of compartmentalization of the observed changes in the blood, and thus the relevance of these changes to the immune competence in other parts of the body. This may be exemplified by the fact that the circulating pool of blood leukocyte makes up only about 1% of the total number of leukocytes in the body. Another concern regarding such measurements is the biological significance of the exercise-induced changes.

However, exercise-induced changes in leukocyte concentrations are closely linked to the intensity of exercise and show consistent patterns of change between subjects performing the same exercise (163). Thus, the relative workload and degree of stress that a subjected experience during exercise may be reflected in the alterations of circulating leukocytes (151). The most prominent changes in leukocyte trafficking are granulocytosis (mainly neutrophils) and lymphocytosis (mostly CD8+ and CD56+ cells) observed *during* exercise, and lymphocytopenia along with a second neutrophilia *after* exercise (table 1.2.1). The main sources of additional circulatory neutrophils and lymphocytes appearing during exercise are primary (mostly bone marrow) and secondary (spleen, lymph nodes, gut, etc) lymphoid tissues, as well as and recruitment of neutrophils from the endothelial wall of peripheral veins (129;139;146;233) . Increased sympathetic activity in the lymphoid tissues and elevated plasma levels of EPI and cortisol may facilitate the mobilization of these cells into circulation during exercise (10;57).

Catecholamines and cortisol have been suggested to induce the increase in lymphocytes, but to a lesser degree the neutrophilia observed during exercise (102;134;163). Neutrophilia during exercise seems to be more closely linked to an increase in GH (100;176), while post-exercise neutrophilia is related to the increase in cortisol and may in part be a result of reduced diapedesis of neutrophils across the endothelium (40;100;224). In contrast to its effect on neutrophils, cortisol contributes strongly to the efflux of lymphocytes out of the circulation after exercise and a subsequent lymphocytopenia if exercise has been both strenuous and prolonged, i.e. resulted in high cortisol levels (73;148;199).

One of the explanations for this trafficking of cells in and out of circulation could be altered expression of adhesion molecules on the endothelium of blood vessels, in lymphoid organs, lungs and on the surface of circulatory leukocytes (66;196). Adhesion molecules from the immunoglobuline superfamily (mainly ICAM, VCAM), selectins and integrins, are responsible for the adherence between the endothelium and leukocytes. Neuroendocrine factors and exercise have been shown to alter this adherence and consequently a change in the concentrations of circulating leukocytes will occur (66;137;178;233). Thus, altered leukocyte trafficking during exercise seems at least in part to be a reflection of the degree of neuroendocrine activation and stress exposure. Hemodynamic changes in the form of increased shear stress in the vascular bed is also considered to affect leukocyte trafficking substantially during exercise (129;134;139). The mechanism behind this effect on several leukocytes may be linked to hemodynamically-induced changes in adhesion molecules on endothelial and circulating white blood cells (4;94;200). This is discussed further in chapter 3 of this thesis.

1.3. Interactions between the neuroendocrine and immune system during exercise

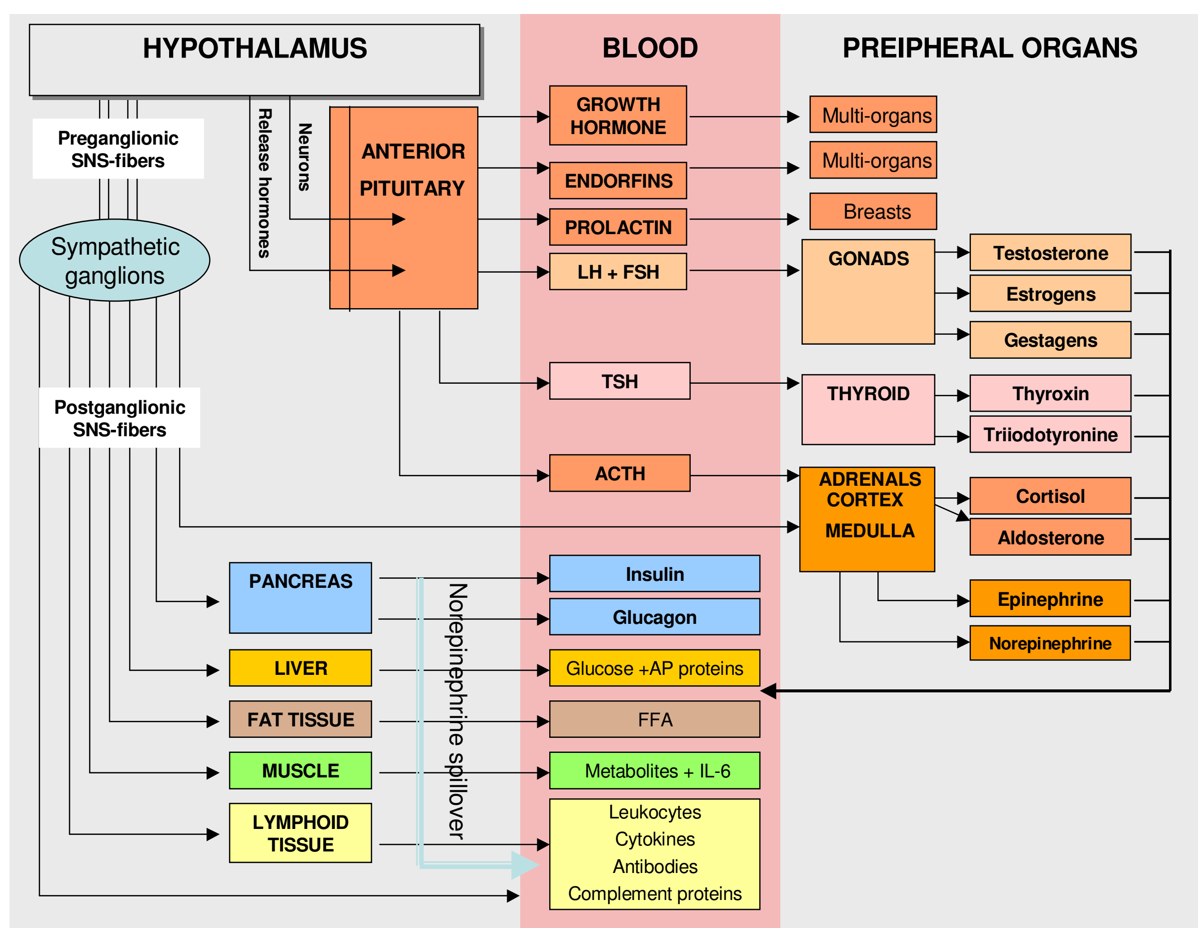
1.3.1. Links between the neuroendocrine and immune system

Some of the early information about a possible link between the neuroendocrine and immune system was gained in the 1940's through the first treatments with glucocorticoids and subsequent clinical observations of changes in inflammatory diseases (241). Since then, extensive research has established a network of connections involving anatomical (neuronal) and humeral (mainly hormones and cytokines) pathways between these systems. Neuroendocrine and immune interaction is based on reciprocal and bi-directional communication (1;14;241). A schematic overview of the neuroendocrine system and its connection to major organs and tissues, including tissues and components of the immune system, is given in figure 1.3.1.

Input from the CNS to single cells, tissues and organs of the immune system follows two main pathways: 1) An endocrine pathway based on hormones secreted by the hypothalamus, the anterior pituitary gland and the adrenal cortex. The various hormones exert their effect by coupling to specific receptors in the plasma membrane of an immune cell (non-steroid hormones) or in the cytosol (steroid hormones). The HPA axis with its secretion of glucocorticoids from the adrenal cortex is the major contributor to immune system regulation in this pathway (67;187;229). 2) The neuronal pathway consisting of pre-ganglionic autonomic

nervous system (ANS) fibers which originate in the CNS (hypothalamus) and connect to post-ganglionic sympathetic (SNS) fibers that innervate lymphoid tissues directly, or stimulate the adrenal medulla to secrete epinephrine (EPI) and norepinephrine (NE) (118;241). EPI is responsible for about 80% and NE about 20% of total catecholamine output. Spillover of NE from peripheral SNS activity in many organs and tissues will also end up in the circulation, and thus plasma NE has two origins. Yet, of the two adrenergic pathways, it is EPI that mediates the strongest influence on the immune system during acute stress and exercise (118).

Figure 1.3.1. Overview of the major neuronal and neuroendocrine pathways, which connect the CNS with tissues and organs and tissues with endocrine, immune and metabolic functions.



Multiple neuronal, hormonal and cytokine links between the neuroendocrine and immune system have been established during the last decades through numerous investigations using different methodological approaches (1). Documentation of a neuroendocrine and direct neuronal impact

on various aspects of immune function has dominated this research, but evidence for a reciprocal effect where alterations in the immune system affect CNS activity is accumulating. A summary of the evidence for anatomical links and functional interactions between the two systems is presented in tables 1.3.1 and 1.3.2, and based on the scientific methods and/or observations reported in the following review articles (1;10;67;118;163;175;187;241) .

Table 1.3.1 Evidence for neuroendocrine → immune communication

- Discrete lesions in the brain (i.e. hypothalamus or pituitary) affect immune function
- Surgical/chemical lesions of neuronal pathways (sympathectomi) alter immune responses
- Presence of post-ganglionic SNS-fibers and NE release in lymphoid tissues
- Stimulation of SNS-fibers elicits changes in the immune system homeostasis
- Presence of receptors for neuroendocrine factors on immune cells in circulation and tissues
- Clinical deficiency in neuroendocrine factors is associated with impaired immune function
- Infusion of neuroendocrine factors elicits changes in the immune system homeostasis
- Neuroendocrine receptor blockers attenuate/blunt neuroendocrine-induced immune responses
- Psychological stress alters innate and adaptive immune responses by activating the CNS
- Acute exercise stress alters immune responses by activating the neuroendocrine system

Table 1.3.2 Evidence for immune system → CNS communication

- Presence of cytokine receptors in the hypothalamus and other parts of the brain
- Infusion of cytokines induces altered neuronal activity
- Cytokine deficient/knock-out animals display altered CNS activity
- Cytokines regulate local norepinephrine release from SNS-fibers
- Inflammatory cytokines can increase vagus nerve signaling (afferents) to the CNS
- Mitogen induced activation of T-cells causes increased expression of adrenergic receptors

Plasma concentrations of most hormones linked to the neuroendocrine system increase in connection with acute stress, and strenuous exercise consistently elicits substantial elevation in

EPI, NE, GH, ACTH and cortisol. An overview of the changes during and after strenuous exercise in the plasma concentrations of these and other hormones is given in table 1.3.3 and based on the following review articles (70;71;108;163;180;204).

Presently, there is a general consensus in the literature that neuroendocrine factors are involved in many of the changes in circulating components of the immune system during strenuous exercise of approximately 1 h or more (table.1.2.1) (163;241). Part of the evidence for this view has been based on correlation analysis between concentration changes in hormones and several variables of the immune system. However, since this does not imply a causal relationship, infusion studies with neuroendocrine hormones have been performed. By and large, most of the exercise-induced changes have been mimicked by a single hormone or a combination of several hormones (52;100;118;191;212;224;225;227). Yet, the association is by no means perfect and a causal link between neuroendocrine activation and perturbations in the immune system in connection with exercise may not exist.

Table1.3.3 Overview of the changes in plasma concentrations of hormones during and after strenuous exercise. Arrow up means increase, arrow down means decrease in concentrations. Arrow sideways means no change. Two arrows imply that the magnitude of change is large.

	During exercise	**After exercise**
Epinephrine	↑↑	↓↓
Norepinephrine	↑	↓
ACTH	↑	↓
Cortisol	↑	↓
Growth hormone	↑↑	↓↓
Prolactin	↑	↓
Endorphins	↑	↓
TSH	↔↑	↔↓
FreeThyroxin	↓	↑
LH	↔	↔
FSH	↔	↔
Testosterone	↑	↓
Insulin	↓	↑
IGF-1	↔↑	↔↓
Glucagon	↔↑	↔↓

1.4. Hormones affecting substrate metabolism in the working muscle

This overview is based on the following publications if not otherwise specified (50;71;79;107;108;132;133;180;204;228). Skeletal muscles represents 40-45% of the total body mass in an adult and is the largest organ in the body. At rest, skeletal muscles receive approximately 20% of the arterial blood provided by the heart (1 L· min^{-1} of a cardiac output of 5 L· min^{-1}), but during high intensity exercise this fraction of cardiac output increases to >80% (i.e. >20 L· min^{-1} of a cardiac output of 25 L· min^{-1}). This increased muscle blood flow reflects a large increase (20-25-fold) in whole body oxygen consumption --which at rest may be about 0.3 L· min^{-1}-- but at maximal aerobic exercise capacity in an elite endurance athlete may be 6-7 L· min^{-1}. A parallel change in muscle metabolism requires sufficient energy substrate availability, either from glycogen and triglyceride stores within the muscle cell or through circulatory supply of glucose and free fatty acids (FFA). In short, strenuous endurance exercise will result in increased glycogenolysis, glycolysis and lipolysis in the muscle, as well as increased glycogenolysis and gluconeogenesis in the liver, and lipolysis in fat tissue. These exercise-induced changes in muscle substrate turnover, together with the cardiovascular adjustments needed to accommodate for such a large increase in metabolism, require a fine-tuned regulation within the muscle itself as well as between muscle and other tissues/organs of the body.

Muscle metabolism is influenced by a variety of factors, such as neuronal input, membrane potential, pH inside the cell, and several other variables. Moreover, substrate oxidation (mainly from fat and carbohydrate) is largely dependent on the energy state (i.e. the balance between ATP, ADP and AMP levels) inside the cell. Therefore, any intra-cellular substance or process that affects the enzymes regulating the flux of these phosphates will also strongly influence the substrate turnover. Contractile activity also has a major impact on substrate metabolism, and this is largely mediated through changes in the phosphorylation state of the muscle cell (182). However, of the extra-cellular factors with influence on substrate metabolism in the muscle, endocrine factors exert the strongest control. The following section will give a brief presentation of some of the hormones with a significant impact on muscle metabolism and substrate turnover during exercise; i.e. catecholamines, insulin, GH, adrenocorticotropic hormone (ACTH), and cortisol, all of which were measured in the studies included in this thesis.

1.4.1. Catecholamines

The biological effects of EPI and NE are executed through binding of these hormones to adrenergic receptors in the plasma membrane of a cell, resulting in the activation of the intra-cellular enzyme adenylate cyclase and subsequent increased production of cyclic AMP, acting as a second messenger inside the cell. NE has its strongest influence on the contractility, vascular tone and other cardiovascular adaptations during exercise, while EPI has a dominant role in the regulation of substrate metabolism in the working muscle as well as liver and fat tissue. This difference is for the most part due to the distribution of α- and β-adrenergic receptors in different tissues, but also to the unequal affinity between EPI and NE for these receptors. The fact that muscle tissue contains almost exclusively β-2 adrenergic receptors, and that EPI has the strongest affinity for this receptor, makes EPI much more influential than NE in the regulation of muscle metabolism.

During exercise, plasma concentrations of EPI and NE increase exponentially in proportion with workload and near-linearly to duration of exercise at exercise intensities above 50-60% of maximal O_2 uptake. Endurance training results in decreased catecholamine secretion at the same absolute workload, but increases the capacity to secrete catecholamines at the same relative workload. Both hormones have a very short half-life in blood (2-3 min), and metabolic clearance rate (speed of elimination from the plasma) of EPI and NE is slightly reduced (20%) during low intensity exercise and similarly increased during high intensity exercise. In contrast to NE, secretion of EPI seems to be sensitive to the general level of sympathetic activity in the body and in particular to hypoglycemia.

Epinephrine enhances glycogenolysis via a β-adrenergic effect on both muscle and liver cells. This is accomplished through an increase in cyclic AMP, which promotes the conversion of the regulating enzyme phosphorylase from an inactive form (b) to an active form (a). Increased levels of phosphorylase-a then enhance the oxidation of glycogen to glucose-6 phosphate, which is the prime substrate for further glycolysis. Simultaneously, EPI also inhibits the reverse reaction (glycogen synthesis) through inhibition of the enzyme glycogen synthase, resulting in increased availability of glucose-6 phosphate for energy (ATP) generation through glycolysis. The activity of major glycolytic enzymes like hexokinase and phoshofructokinase are also up-regulated by EPI. The net result of increased catecholamine levels and sympathetic activity in the liver is enhanced glucose release to the circulation. Interestingly, EPI also seems to inhibit glucose

uptake in the working muscle, perhaps to avoid severe hypoglycemia during strenuous exercise. This discrepancy between catecholamine-stimulated hepatic glucose output and reduced uptake by the working muscle may result in increased plasma glucose levels even when muscles may be glycogen-depleted towards the end of prolonged exercise. Recently, it has been shown that low pre-exercise glycogen levels enhances the plasma EPI and NE responses after about 90 min of a subsequent exercise session (213). Thus, the working muscle seems to be able to activate the neuroendocrine system and thereby mobilize energy reserves (mostly fat) in cases of intramuscular substrate shortage. Interleukin-6 has been proposed as a candidate for mediating this signal of metabolic crisis in the muscle (168).

Adrenergically stimulated glycolysis may turn into an aerobic process where glucose-6 phosphate is completely oxidized through the glycolytic pathway, citric acid cycle and subsequent oxidative phosphorylation. In contrast, under anaerobic conditions, glucose-6 phosphate will be "diverted" in the glycolytic pathway and incompletely oxidized from pyruvate to lactate, which cannot be further oxidized. However, lactate along with glycerol (released during fat oxidation) and some other metabolites, may become useful as substrates in the gluconeogenic pathway, where glucose is synthesized from these precursors. Gluconeogenesis can only take place in the liver, but may represent an additional energy source during strenuous exercise through increased hepatic output of glucose. Epinephrine has been suggested to facilitate gluconeogenesis by way of promoting increased amounts of precursors such as lactate and glycerol.

Lipolysis in muscle and fat tissue is also strongly regulated by the plasma level of EPI, and to some degree NE. This is mediated through increased levels of the second messenger cyclic AMP, which activates the rate-limiting enzyme hormone sensitive lipase (HSL). The phosphorylation state of HSL regulates the speed of fat oxidation, mainly from triglyceride (TG) depots, and thus the subsequent release of FFA and glycerol. FFA may be further metabolized in the mitochondria of the muscle cells through β-oxidation and enter the citric acid cycle as acetyl-CoA in parallel to aerobic oxidation of glucose. Recently, β-adrenergic desensitization in fat tissue has been demonstrated both after exercise and catecholamine infusion (19;123;208), which indicate that there is a catecholamine-mediated effect of exercise on post-exercise lipolysis.

1.4.2. Insulin

Insulin is secreted by the β cells of the islets in the pancreas in response to increasing levels of glucose in the blood and subsequently inside the β cells after meals. In the resting muscle, insulin facilitates glucose uptake over the plasma membrane through translocation of the transport protein GLUT-4 from inside to outside of the sarcolemma. However, during exercise, insulin becomes less important for this mobilization of GLUT-4 proteins to the surface of the plasma membrane and thus for glucose transport into the muscle during exercise, because increased contractile activity seems to stimulate this GLUT-4 translocation in the working muscle. This is why plasma insulin levels can be substantially reduced during exercise without a significant influence on glucose uptake in muscle. An exercise-induced decrease in plasma insulin is for the most part a result of α-adrenergic inhibition of insulin secretion from the pancreas. The magnitude of this decrease is dependent on the duration and intensity of exercise at workloads above 50-60% of maximal O_2 uptake.

Besides the task of facilitating glucose uptake in the muscle --mostly during rest-- insulin exhibits two important inhibitory actions that ultimately influence on muscle substrate turnover: 1) insulin inhibits hepatic glucose output, and 2) insulin inhibits lipolysis by reducing the phosphorylated form of HSL. Thus, insulin and epinephrine have opposing actions on the mobilization of glycogen and fat stores as well as glucose output from the liver. Consequently, it is advantageous that the plasma levels of these two hormones change in opposite directions during strenuous exercise when substrate availability in the working muscle becomes critical. At rest, when catecholamine levels are minimal, insulin exerts considerable anabolic effects on skeletal muscles by stimulating glycogen synthesis, inhibiting proteolysis, as well as mediating fat storage. Insulin sensitivity is increased significantly with training, which probably enables the body to support and sustain these important metabolic effects at lower plasma insulin levels during exercise.

1.4.3. ACTH and cortisol

ACTH is released by the pituitary as a result of increased production of corticotropin releasing factor (CRF) from the hypothalamus and stimulates the production of cortisol and other adrenal cortex hormones such as aldosterone and androstenedione. ACTH is under considerable negative feedback control through the inhibitory effect of cortisol on CRF, and both ACTH and cortisol show substantial diurnal variations. Peak plasma levels are seen early in the morning and the lowest levels early at night ACTH and cortisol increase during strenuous exercise, but substantial

elevation of cortisol is observed only after 50-60 min of such exercise. Exercise-induced elevations in plasma ACTH and cortisol are related to the relative intensity of the workload, and the metabolic clearance rate of cortisol is increased at high intensities of exercise.

Despite consistent increases in plasma levels of ACTH and cortisol during strenuous exercise, these hormones do not seem to have the same degree of influence on substrate metabolism in the working muscle, as is the case with catecholamines and insulin. However, some evidence for a role of cortisol in metabolic regulation has been shown since both prior meals and increased plasma glucose availability attenuate the normal cortisol response during strenuous exercise. Furthermore, cortisol has been suggested to contribute to increased hepatic glucose production through gluconeogenesis. Recently, cortisol has also been shown to increase regional lipolysis (47), which may imply a role in stimulating fat oxidation during prolonged exercise that elicits significant elevations of cortisol. Additionally, cortisol may exhibit some indirect metabolic effects in the muscle, but in sum the contribution of cortisol and ACTH in muscle substrate turnover has so far been considered minor.

1.4.4. Growth hormone

The anterior pituitary secretes GH in a pulsatile manner with short bursts at approximately 2 h intervals, mostly during sleep at night. A nocturnal peak is observed about 1 h after the onset of sleep. Growth hormone secretion is stimulated by a hypothalamic releasing hormone (GH-RH) and inhibited by somatostatin. The half-life of GH in the blood is 15-20 min, therefore plasma concentration of this hormone outside the individual bursts is very low. GH receptors are found on all cells of the body and the biological effects of GH are multiple, mainly promoting cell division and growth in a wide variety of tissues. However, most of the metabolic effects of GH are mediated through the action of insulin-like growth factors of which IGF-1 and IGF-2 exert the strongest effects. These growth factors are produced locally, but mediate both autocrine and paracrine actions.

Exercise is a strong stimulus for GH secretion and may increase plasma GH concentrations by 20- to 40-fold. The increase is strongly linked to the intensity of exercise, but also with duration of exercise above 30% of maximal O_2 uptake. Peak GH concentrations are not substantially changed with training, but the GH response to exercise at the same absolute intensity is reduced with training. Exercise-induced GH release is further enhanced by a decrease in glucose

availability, hypo-insulinemia, and by increased body temperature, suggesting a significant role for GH in exercise metabolism. In the liver, GH promotes gluconeogenesis, but seems to have even stronger metabolic influence in skeletal muscle. Here, GH stimulates protein synthesis, inhibits glucose uptake and mobilizes FFA from triglycerides, thus mediating both anabolic and catabolic effects on skeletal muscles. Some of these metabolic effects are also observed in other types of tissues like bone, cartilage, fat, etc. In short, GH mediates many of the same metabolic changes as EPI in the working muscle, and has opposing effects to insulin on several metabolic functions.

1.5. Background and purpose for the studies

1.5.1. Study I

A large number of studies have characterized the biological changes related to various intensities and durations of exercise, as well as other factors influencing exercise responses, such as training status, nutrition, hydration, age, gender, heat, cold, hypoxia, etc. Regarding the impact of training status on the response to exercise, several factors like aerobic capacity, muscle strength, technical skills etc., may be of importance. Having developed extraordinary capacity and skills within a certain sport, athletes have been useful subjects in a variety of exercise studies, both for the purpose of investigating the effects of single bouts of exercise, as well as the effect of long-term exercise training. During exercise athletes and sedentary people display several physiological differences (35;84;85;109). Since trained athletes may reveal particular physiological characteristics during strenuous exercise, we became interested in examining a group of extremely well-trained endurance athletes during a prolonged exhaustive effort. Thus, we chose to study world-class cross-country skiers during a long distance World-Cup ski race with regard to changes in immune, endocrine and metabolic variables.

The issue of possible health risks involved in long distance ski racing was brought to public attention after the total collapse at the finish line of the Norwegian gold medallist in the 1998 Olympic 50 km race. Additional anecdotal reports of other skiers having collapsed during and after long distance ski racing on earlier occasions, prompted a need to investigate possible signs of health risks associated with such racing. Moreover, in contrast to for example marathon running, information on the physiological changes during cross-country ski racing among international level cross-country skiers is limited (49;106;158;226).

Therefore, the main purpose of this field study was to describe alterations in immune, endocrine and metabolic variables in connection with a long distance cross-country ski-race among extremely well-trained endurance athletes. Additionally, this would enable us to compare the results with investigations among marathon runners with similar race time and intensity, as well as the changes observed with a different exhaustive exercise protocol used in our own laboratory.

1.5.2. Study II

Elite endurance athletes experience periods of heavy training loads often combined with frequent international competitions. The total physical and psycho-social stress that these athletes experience during such periods may affect their performance (104;114;136). Consequently, monitoring the ability to adapt to large stress loads has received increased attention in professional sports (12;86). In contrast to several studies that have purposely overtrained their subjects (87;113;114;232;236), the present study wanted to examine the effect of normal, yet substantial seasonal variations in training and competition load on typical acute stress responses to a standardized exhaustive exercise test in the laboratory. We wanted to examine if any of the stress-sensitive variables measured during and after a standardized treadmill test would show a different pattern of change in a period of high compared with low training load. If so, such variables could be further examined as possible indicators of overload training (overreaching). Since most investigations that so far had examined the impact of chronic exercise on the acute exercise response did not systematically observe the subjects in the post-exercise period, we chose to obtain most of our measurements during the first hours of recovery. This was based on the idea that potential effects of changes in training load could appear in the period *after*, rather than during the acute exercise test.

Thus, the main purpose of this study was to examine the effect of normal seasonal variations in training and competition load among elite endurance athletes on the typical pattern of change in variables linked to the immune- and endocrine systems, which could be sensitive to both acute and chronic exercise stress.

1.5.3. Study III

In most studies examining a single bout of exercise, great care has been taken to control for or eliminate the influence of prior exercise sessions, because persisting effects of a previous bout of

exercise could represent a confounding variable. Yet, the daily training schedules of elite athletes often include two, and sometimes even three exercise sessions on the same day. Consequently, there is limited amount of time for recovery between sessions. When using the term recovery it is essential to keep in mind that this includes a large number of processes involved in restoring the balance in several physiological systems. Furthermore, the time frame of these recovery processes may be vastly different. Some aspects of recovery may be completed within the first hour after exercise, like the normalization of plasma catecholamine levels, while other perturbations such as increased cortisol levels, leukocyte concentrations, oxygen consumption, or decreased muscle glycogen levels may need from 4 to 24 h to reach baseline values after strenuous exercise (6;120;147). For the elite athlete, this means that a second exercise session on the same day may be started long before complete recovery from the previous exercise is achieved.

The idea of studying the potential effect of previous exercise on a subsequent exercise session materialized from observing national team cross-country skiers and speed skaters performing two prolonged and often intense exercise sessions with only 3-4 h of rest in between. With such a compact training and recovery schedule it seemed likely that the physiological stress from the morning exercise session could have a "carry-over" effect on the next exercise session. Consequently, a larger degree of stress and possibly increased metabolic cost connected to a second exercise session on the same day would be expected. Moreover, if residual effects from the first exercise session result in an increased relative workload during the second bout of exercise, this may in turn affect recovery after the second exercise session.

At the time this study was planned, few other investigations had applied a design with repeated exercise sessions in one day and only one had used a protocol with prolonged exercise (189). Table 1.6.1 summarizes all the studies that so far have examined repeated, separate exercise sessions in one day (23;24;56;68;97;98;126;135;144;186;189;195;216;244). Therefore, the purpose of this investigation was to examine possible residual effects of prior exercise on changes in endocrine, immune and metabolic variables in connection with a second exercise session on the same day.

Table 1.6.1 Studies with a repeated bouts of exercise design

Study	n	Peak VO_2 ml/min/kg	Ex Int $\%VO_2$	Ex.Dur min	Rest min	# Rep	Feeding/Water
Galasetti 2001	8	31	48	90	180	2	Glucose after Ex-1
Stich 2000	7	46	50	60	60	2	No
Weltman 1998	6	53	70	30	60 / 210	3	No
Kanaley 1997	6	53	70	30	60 / 210	3	No
Rhode 1997	8	66	73	60+45+30	120	3	Food + Water
Nielsen 1996	8	65	Max	6	220	3	Food + Water
Severs 1996	11	47	50	30	45	2	No
Brenner 1996/97	11	47	50	30	45	2	No
McCarthy 1992	8	37	70	30	180	2	Food + Water
Kaciuba-Uschilco 1992	10	?	50	30	30	4	W
Field 1991	12	44	100	13	60	2	No
Marliss 1991	12	44	100	13	60	2	No
Sawka 1979	7	65	70	80	90	2	Food after Ex-1

1.5.4. Study IV

In many sports, 4-6 h of exercise per day during most of the year is necessary to perform at a top international level. This is accomplished by splitting the daily exercise load into two or more sessions per day, resulting in limited periods for recovery between exercise sessions. Therefore, an increased focus on recovery routines in connection with each training session has appeared, including liquid and caloric intake, various forms of local muscle treatment, relaxation techniques, etc. Probably, the quantity and quality of rest in itself may also be an important determinant for the speed of recovery after exercise. As previously mentioned, several aspects of recovery may take longer time than the athlete is able to spend between exercise sessions. Naturally, an essential question is how many hours of rest is necessary between exercise sessions in order to ensure a positive effect of performing a second bout of exercise on the same day.

Some of the major findings from studies on rehydration and food intake after exercise have successfully been integrated in the practical recovery routines of most athletes. Unfortunately, few studies have provided relevant information on how various recovery regimes may affect physiological responses to a second exercise session on the same day. In fact, we have found only one study that has attempted to describe the effect of different duration of resting periods between two separate exercise sessions (98;244). Therefore, in study IV we wanted to pursue the issue of recovery further and examine the effect of two different recovery regimes between the first and second exercise sessions on the same variables as in study III.

Thus, we designed a study that compared neuroendocrine, immune and metabolic responses during days with two equal bouts of high intensity endurance exercise, but allowing different periods of rest between the first and second bout.

1.6. Study aims and hypotheses

1. To examine the magnitude of change in variables linked to immune, neuroendocrine and metabolic function during long distance ski racing among extremely well-trained male and female endurance athletes (study I).

2. To examine the impact of variations in seasonal training and competition load on the acute response to exhaustive exercise. Specifically, we wanted to test the hypothesis that increased training and competition load would result in more pronounced stress responses during and after an exhaustive exercise test (study II).

3. To examine the impact of a previous bout of strenuous endurance exercise on the responses to a subsequent exercise bout the same day. Specifically, we wanted to test the hypothesis that a previous bout of strenuous endurance exercise would result in augmented immune, endocrine and metabolic responses to a subsequent exercise bout the same day (study III).

4. To examine the impact of different recovery periods between two daily exercise sessions on the responses to the second bout of exercise. We hypothesized that the changes elicited by the second bout of exercise would be more pronounced when the rest period between the exercise sessions was 3 h compared with 6 h (study IV).

2. Materials and methods for study I, II, III, and IV

2.1. Subjects

Elite endurance athletes recruited from the Norwegian national teams in cross-country skiing, triathlon and speed skating took part in all four investigations included in this thesis. Thirty out of the 32 athletes were at an international level in their sport and eleven athletes have won Olympic gold medals. In study I, ten male cross-country skiers (age range of 23-32 years, and a maximal O_2 uptake between 82-92 mL · kg^{-1}· min^{-1}) and six female skiers (age range 22-32 years and maximal O_2 uptake between 65-74 mL · kg^{-1}· min^{-1}) from the Norwegian national cross-country ski team participated. In study II, eleven male athletes, age 21-29 years and a maximal O_2 uptake between 70-82 mL · kg^{-1} · min^{-1} were recruited from the national teams in cross-country skiing (n=6) and Nordic combined (n=5).

Subject characteristics of the speed skaters and tri-athletes in study III and IV are shown in table 2.1. One member of the national team in speed skating and two from the triathlon team declined to participate, and one subject chose to leave after the first trial because of discomfort during the blood collection procedure.

Table 2.1 Subject characteristics of the national (Nat) and international (Int) level athletes participating in study III and IV.

Subject	Age	Height	Weight	Maximal O_2-uptake		Workload	Sport	Level
				pre-test	post-test			
	(yrs)	(cm)	(kg)	($ml·kg^{-1}·min^{-1}$)		(W)		
A	21	180	70	69,7	67,3	219	triathlon	Nat.
B	25	178	74	70,6	no	270	skating	Int.
C	23	172	71	65,1	66,1	213	skating	Int.
D	23	183	83	67,1	67,5	260	skating	Int.
E	22	176	70	71,7	64,4	246	skating	Nat.
F	23	175	73	76,8	69,1	272	triathlon	Nat.
G	27	200	83	66,2	67,2	280	triathlon	Nat.
H	22	189	70	65,8	63,6	215	triathlon	Nat.
I	23	185	78	68,8	67,7	254	skating	Nat.
MEAN	23	182	75	69,1	66,6	248		
SD	1,8	8,6	5,4	3,7	1,8	26		

2.2. Design and procedures

2.2.1. Study I

The ski race investigation was performed during a World Cup competition with the purpose of examining changes in blood constituents from before to after a long distance ski race under normal preparations and race procedures (paper 1). The females performed a 30 km race (mean finish time 104 min) and the males a 50 km race (mean finish time 142 min); both in the classical skiing discipline. The pre-race blood sampling was done immediately after the athletes had arrived at the arena and the post-race sampling was performed within 1 min after completing the race. The indices of immune, endocrine and metabolic functions that were analyzed are given in table 3.1 in chapter 3.

2.2.2. Study II

To examine the effect of seasonal variations in training and competition load on the acute stress responses to an exercise test, the subjects were scheduled to test on four occasions; twice during the competitive season and twice during the off-season (paper 2). The athletes kept records of their daily training and all competitions were logged during the season. A training and competition score (TCS) was calculated for a three-week period prior to each of the four test days based on this information. The number of hours spent on different types of training was recorded and this value was multiplied with an intensity factor and added up as a total training and competition score on a weekly basis (table 1, paper 2).

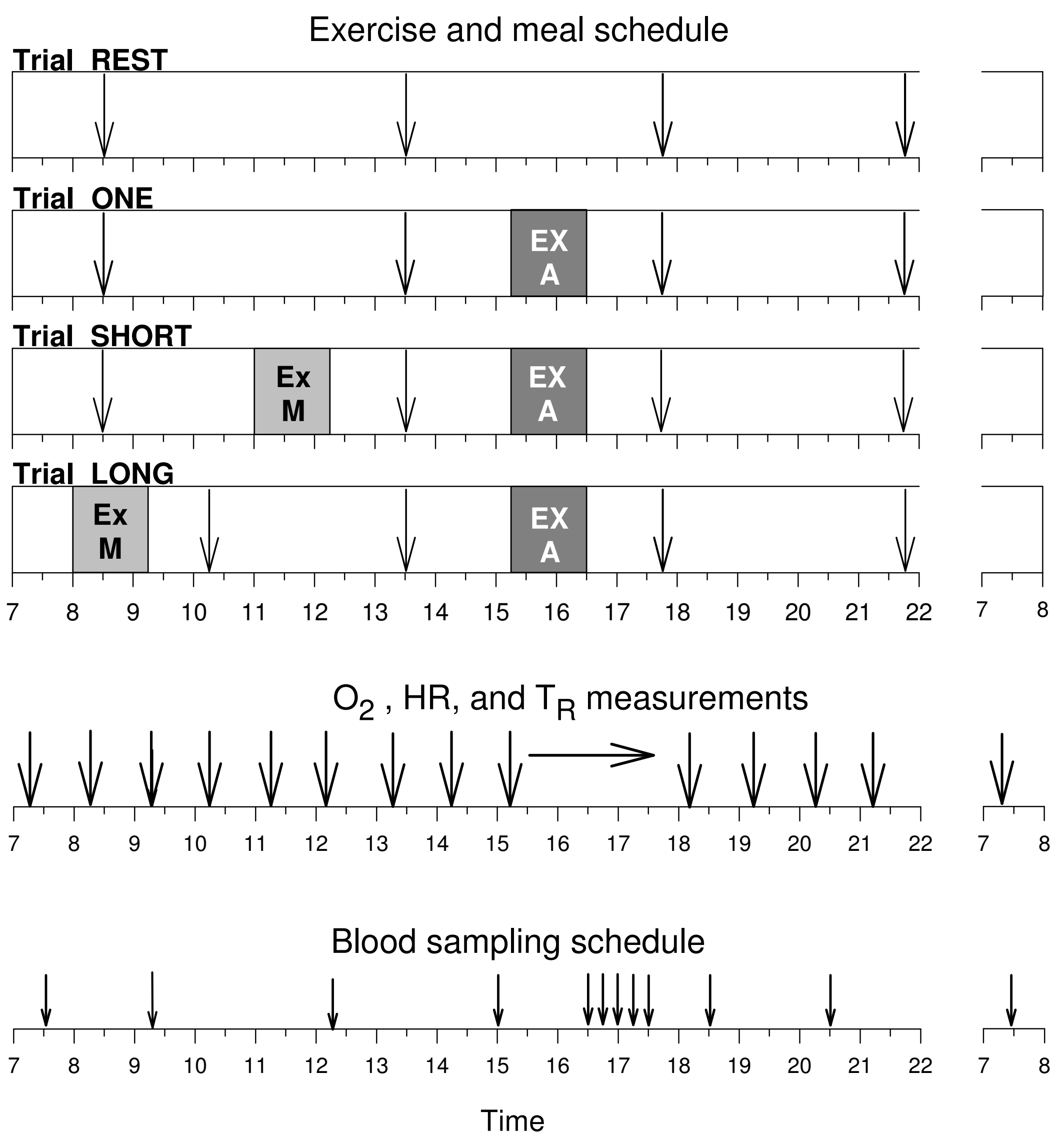
Each exercise session included an incremental treadmill test where the speed was increased every five minutes until volitional exhaustion, at which point the athlete could not keep at pace with the treadmill speed. A 30 s pause for blood sampling and speed adjustment intercepted each increment and the tests lasted from 45 to 50 min. Subsequently, the subjects where observed during the first four hours of recovery. Blood samples for hemoglobin, hematocrit, total leukocytes, neutrophils, lymphocytes, IL-6, catecholamines, ACTH and cortisol analyses were collected 15 min prior to exercise and 0, 15, 30, 60, 120 and 240 min following exercise.

2.2.3. Studies III and IV

In the repeated bouts of exercise studies each subject participated in four trials (Fig. 2.2), each lasting from 07.00 to 08.00 the following day: 1) complete bed rest (REST), 2) one bout of exercise from 15.15-16.30 (ONE), 3) two bouts of exercise (11.00-12.15 and 15.15-16.30) with 3h

of rest in-between (SHORT), and 4) two bouts of exercise (08.00-09.15 and 15.15-16.30) with 6 h of rest (LONG). In study III, trial SHORT served as the two bouts of exercise trial (trial TWO) and was compared with trial ONE and trial REST. In study IV, trial SHORT was compared with trial LONG and trial REST.

Figure 2.1 Experimental design and protocol study III and IV



In contrast to previous studies on repeated bouts of exercise (table 1.4.1), a separate trial day (ONE) with a single bout of exercise at the same time of the day as the second bout in the two exercise trial was added when designing the study (figure 2.1). This was done to eliminate the

influence of diurnal variations and allowed us to make valid comparisons of the genuine exercise response with and without a previous exercise session. Additionally, we added a separate trial with 24 h complete bed rest in order not only to control for the diurnal changes, but also to measure them. To our knowledge this has not been done systematically over a 24 h period in elite athletes, and the observations add information on the magnitude of the diurnal changes in selected immune, endocrine and metabolic variables.

All trials were separated by 12-17 days in order to ensure complete recovery between trials, and randomized in a counterbalanced order with each subject serving as his own control. Except for the last two days before each trial, when exercise was regulated by the study protocol, the subjects followed their regular training program without any interruption during the study period. From the pre-trial testing a workload corresponding to 70% of maximal O_2 uptake was calculated and used for the three trials (papers 3-7).

High intensity exercise for more than 60 min duration was chosen because previous studies have demonstrated that significant changes in certain immune and endocrine variables do not occur with moderate intensity exercise of less than 60 min. Secondly, most endurance athletes perform repeated strenuous exercise sessions lasting more than 1 h. Another concern when designing the protocol in study IV was to give the subjects a "reasonable" short and long recovery period between the two exercise sessions performed on the same day. A minimum "worst case" 3 h period and a "maximum" 6 h period of rest was chosen, including one or two meals respectively, in trials SHORT and LONG.

The trial procedures were almost identical in the four trials (for details see papers 3-7) and may be summarized as follows: The subjects arrived in the laboratory at 07.00, emptied their bladder, had their body weight measured and thereafter laid down on a bed. A flexible temperature probe was inserted 10 cm into the rectum, and the subjects were connected to a temperature, ECG and heart rate monitor. A catheter was inserted into a cubital vein and kept there for the whole trial. All exercise bouts were equal in duration and intensity and consisted of a 10 min warm-up period at 50% of maximal O_2 uptake, immediately followed by 65 min at their predetermined workload. All subjects completed all the exercise sessions, but the workload had to be reduced temporarily in five cases in order to avoid premature exhaustion (table 2, paper 6). The subjects rested in bed at all hours except when exercising and slept in the lab the following night until 07.00 the next

morning. During each trial, the subjects were served four standardized meals of sandwiches at 08.30 (10.30 in trial LONG), 13.30, 17.40 and 21.45 (Fig. 2.2), each consisting of 1000 kcals. Water was consumed ad libitum during exercise and recovery except for the first 60 min post-exercise, when O_2 uptake was measured continuously.

The sampling protocol may be summarized as follows (for details see papers 3-7): O_2 uptake and HR were measured for 60 s after 15, 30, 45, 60 and 70 min of exercise, as well as continuously during the first hour post-exercise and for 10 min every hour during the subsequent recovery period. Blood for hormone, glucose and leukocyte measurements was collected at 07.30, 12.15, 15.15, 16.30, 16.45, 17.00, 17.15, 17.30, 18.30, 19.30, 20.30 and 07.00 next morning in all trials except in trial LONG where the post Ex-M sampling took place at 09.15 instead of 12.15 (Figure2.2). Blood for cytokine measurements was collected at 07.30, 15.15, 16.30, 17.30, 18.30, 19.30, 20.30, and 07.00 next morning in all trials. Blood for lymphocyte subset counts and responsiveness was collected at 07.30, 15.15, 16.30, 20.30, and 07.00 next morning. After the subjects had emptied their bladder at 07.10, all urine was collected for the next 24 hours. The urine was collected from each of the following diurnal periods: 07.10 –15.05; 15.10-21.40 and 21.40-07.10 next morning.

2.3. Measurements

We chose a relatively broad specter of variables in all of our studies for the following reasons: 1) we wanted to characterize as widely as possible the responses to an exhaustive ski race in a special population of athletes (study I); 2) we did not know what type of measurements during our exhaustive laboratory test that may be affected by seasonal changes in training and competition load (study II); and 3) we wanted to characterize the exercise-induced changes to a new exercise and recovery protocol (study III and IV).

2.3.1. Leukocytes and cytokines

An in-house Sysmex K 1000 automatic cell counter was used for the standard hematological measurements including hemoglobin, hematocrit, neutrophils and lymphocytes. All lymphocyte sub-population measurements were performed at the Department of Immunology and Transfusion Medicine, Ullevaal University Hospital. A standard flow-cytometric method for the measurements of lymphocyte concentrations was used (65), except for the estimation of lymphocyte activation (responsiveness) where a relative new method was applied (58;111;221).

Traditionally, lymphocyte function has been assessed in bioassays using mononuclear cells that have been separated from the plasma and all other blood cells prior to testing for functional capabilities like proliferation and cytotoxicity. It is conceivable that such a removal of cells from their natural milieu in the circulation may have a significant impact on the viability of the cells. Recently, by using whole blood and the flow cytometric method of identifying populations of cells that share a common antigen on the cell surface, lymphocyte responsiveness to mitogens has been assessed by measuring the expression of the CD69 antigen (58;111). Since the CD69 molecules appear on the surface of lymphocytes after only a few hours of stimulation (130;253), this is a rapid method which provides an accurate measurement of lymphocyte responsiveness when compared with the traditional 3H-thymidine assays (122;246).

The percentage of CD4+, CD8+ and CD56+ lymphocytes as well as the absolute number of the cells that expressed detectable levels of CD69, were calculated as the main outcome measures of lymphocyte responsiveness. An estimate of the amount/density of CD69 molecules on each lymphocyte (fluorescence intensity ratio; FIR) was calculated as the ratio of the geometric mean of the CD69 fluorescence of stimulated cells divided by non-stimulated cells. This was done in order to examine quantitative changes within each cell and not only changes in the number and percentage of cells that were activated. Natural Killer (NK) cells is a heterogeneous population of lymphocytes that lack the CD3 surface antigen shared by T-lymphocytes, but are identified by expression of the CD56 and/or the CD16 antigen. Thus, a pure population of NK cells is impossible to select on the basis of one surface antigen. The CD56 marker was used to identify NK cells in this investigation, but 10-15% of the CD56+ cells may be non-NK cells. As explained in the introduction, the degree of heterogeneity in CD56+ cells may give an uneven CD69 expression before and after exercise when stimulated with the CD2 mitogen, because exercise may recruit sub-types of CD56+ cells that have different sensitivity to the CD2 mitogen. The cytokines were analyzed by commercially available high sensitivity enzyme-linked immunosorbent-assay (ELISA) at the Institute of Immunology, National University Hospital, Oslo, and all measurements were performed in duplicate.

2.3.2. Hormone measurements

Plasma cortisol and ACTH measurements were performed using radio-immunoassay (RIA) methods. Plasma and urine catecholamines were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection as described by Hallman et al (76) and

Peaston (161) with a inter-assay CV of 19% for E and 10% for NE. Urinary excretion was expressed as a product of the concentration of EPI or NE and urinary volume measured over the observed time period.

Serum growth hormone (GH) and insulin levels were measured by in-house RIA methods established in the Hormone Laboratory, Aker University Hospital, Oslo. The intra- and inter-assay CV's are between 7-10 % and 11-13%, respectively. Plasma ACTH levels were measured by an immunoluminometric assay (ILA), with intra- and inter-assay CV of 4-7% and 6-8%, respectively. Serum LH, FSH, TSH, and FT4 levels were measured by fluoroimmunometric assays (FIA), with intra- and inter-assay CV between 2-4% and 2-9%, respectively. Serum cortisol and testosterone levels were measured by RIA with both intra- and inter assay CV between 3-8%. Serum levels of insulin like growth factor one (IGF-1) was measured by immunoradiometric assay (IRMA) with intra-and inter assay CV of 5% and 10%, respectively.

Immunoglobulin A, G and M and CRP levels were measured in study I only, and determined in immunometric assays where the specific antibodies react with antigen in the sample to form an antigen/antibody complex and the concentration is measured by light absorption. In the same study, sodium, potassium, and chloride were measured by an indirect method using an ion-selective electrode. Magnesium, inorganic phosphorus acid and uric acid were measured by an enzymatic colorimetric method. Urea and CK were measured using a kinetic UV assay and UV test, respectively. Plasma glucose and albumin were measured by the Roche Glucose HK liquid enzymatic assay on a Roche/Hitachi 917 analyzer and on an automatic Technicon Chem I analyzer. Total plasma FFA concentration was determined enzymatically (95). The distribution of albumin-bound (non-esterified) fatty acids in plasma was estimated as described by Lepage and Roy (115). Lactate concentration was analyzed on an automatic enzymatic lactate analyzer using a 50-μl fingertip capillary blood sample.

2.3.3. Metabolic measurements

During the pre-trial test for study III and IV, the O_2 uptake was measured during the last 90 s at each level of speed at the treadmill using an automated Oxycon Champion System, and gas exchange was recorded for every expiration. In the four study trials, the O_2 uptake was measured by collection of expired air in Douglas bags. The CO_2 and O_2 content were measured on separate O_2 and CO_2 analyzers. Additional measurements of air pressure and temperature were performed

on an EOS-sprint system. Calculations of respiratory exchange ratio (RER) were based on the ratio of CO_2 production to O_2 uptake. Assuming a non-protein RER, an estimate for whole body RER, carbohydrate (CHO) and fat oxidation were calculated by indirect calorimetry, using standard equations published previously (5). Heart rate and rectal temperature were measured using a Siemens 6000 monitor.

2.4. Statistical analyses

The sample size in the four studies was in the range typically used for exercise physiology experiments (6-12) where the subjects serve as their own control and the inter- and intra-subject variance of the test variable is known to be relatively small. Parametric tests were used in all the statistical analyses, mainly analyses of variance (ANOVA) and Student t-tests, thus assuming a normal distribution of our data. In the few instances where the distribution of our data was skewed, additional non-parametric tests was performed, but did not yield a different outcome with regard to the significance level of $p < 0.05$.

All leukocyte, hormone and cytokine concentrations in study II, III, and IV were corrected for plasma volume changes relative to the values from the first morning sample, according to the method of Dill and Costill (46). In study I, Student's paired t-test was used to test for effects of exercise on concentrations of all the blood constituents. Since Hgb concentration did not change from pre- to post-race, a correction for plasma volume change was not done in this investigation. In study II, comparisons were made between the test after the period with highest TCS (in-season HI) and the test after the period with the lowest TCS (off-season LO) for leukocytes, IL-6, ACTH, cortisol, and catecholamines using a two-factorial ANOVA model for repeated measures. The factors were test (in-season HI, off-season LO) and time (pre, 0, 15, 30, 60, 120 and 240 min post-exercise). The ANOVA procedure was performed to check for both main and interaction effects, followed by separate t-tests using a Bonferroni correction where multiple comparisons were performed. T-tests were also used in the analysis of TCS changes (delta-values) from the in-season HI test to the off-season LO test.

In study III and IV, the analysis of changes in all hormones, total leukocyte, neutrophil and lymphocyte counts was done with an ANOVA procedure for repeated measures to estimate main effects (trial or time) and interaction effect (trial x time) including all four trials (REST, ONE, SHORT and LONG). Nine measurements from 15:00 till 07:30 next morning were included and

the Huynh-Feldt method for adjustment of degrees of freedom for the F-tests was applied. Where significant effects were found, separate tests were performed for effects of exercise (trials ONE, SHORT, and LONG, respectively vs. REST), for the effect of a previous bout (trial SHORT/TWO vs. trial ONE), and for the effect of extended recovery time between exercise sessions (trial LONG vs. SHORT). Since changes in the CD4+, CD8+, and CD56+ cells and their respective CD69 expression occurred mostly during exercise, the effect of previous exercise on the lymphocyte subset concentrations was analyzed with Student's t-test. Additionally, Student's t-test with Bonferroni corrections for multiple comparisons was used for pre- and post-trial comparisons and for comparisons at the same time point in the different trials.

The correlations made in chapter 6 are based on the changes observed during and after Ex-A in trial ONE from study III and in trials SHORT and LONG from study IV (i.e. nine subjects in three exercise trials, resulting in 27 observations). The degree of inter-trial dependency for these observations was corrected for by estimating a correction factor σ using the following formula: $\sigma = (MSb-MSw) / MSb+ (n-1) \times MSw$; where MSb is the mean sum of squares between subjects and MSw is the mean sum of squares within subjects.The corrected t-value was then calculated using the r-value from a Pearson's correlation analysis in the following formula: $t = r / \sqrt{} (1-r2) \times \sqrt{} (n-2) \times 1 / \sqrt{} (1+2 \times \sigma)$.

All statistical calculations were performed using the software package SPSS. Results are presented as means ± SEM unless otherwise noted. Exact p-values are generally given, and p-values < 0.05 were considered significant.

3. Study I: Effects of an exhaustive long distance ski racing on aspects of immune, endocrine and metabolic functions in extremely well trained endurance male and female athletes (paper1)

3.1. Results

Table 3.1 Immune, endocrine and metabolic changes during long distance ski racing in males and females

	MALES 50 Km ski race				**FEMALES 30 Km ski race**			
	PRE ±SD	POST ±SD	% change	P-value	PRE ±SD	POST ±SD	% change	P-value
Hemoglobin (g · dL^{-1})	16,4 ±0,8	16,0 ±1,0	-2	NS	14,5±0,5	14,4 ±1,0	-1	NS
Leukocytes (10^{3} ·uL^{-1})	5,5 ±0,9	21,4 ±2,9	289	<0,001	5,3 ±0,9	17,2 ±2,5	225	<0,001
Granulocytes (10^{3} ·uL^{-1})	3,3 ±1,0	18,0 ±3,7	445	<0,001	2,8 ±0,5	14,1 ±3,3	404	<0,001
Lymphocytes (10^{3} ·uL^{-1})	1,9 ±0,4	3,1 ±1,4	63	<0,05	2,1 ±0,7	2,8 ±1,2	33	(0,86
Monocytes (10^{3} ·uL^{-1})	0,29 ±0,1	0,30 ±0,1	3	NS	0,35 ±0,12	0,29 ±0,18	-17	NS
B-cells (% of lymphoc)	9,8 ±3	10,5 ±3	7	NS	9,1 ±2	10,1 ±3	11	NS
T-cells (% of lymphoc)	62,6 ±9	45,3 ±10	-28	<0,001	69,5 ±9	52,1 ±10	-25	<0,01
NK cells (% of lymphoc)	21,8 ±8	32,4±8	49	<0,01	14,3 ±3	26,8 ±6	87	<0,05
Epinephrine (nmol · L^{-1})	0,4 ±0,2	4,6 ±2,6	1050	<0,001	0,4 ±0,1	5,2 ±2,5	1200	<0,005
Norepinephrine (nmol · L^{-1})	3,7 ±1,3	19,7±6,0	432	<0,001	3,1 ±1,3	21,5 ±5,5	594	<0,001
Cortisol (nmol · L^{-1})	462±114	957±154	107	<0,001	736 ±374	1023 ±491	39	<0,01
Testosterone (nmol · L^{-1})	16,0±3,9	15,3±5,4	-4	NS	1,2 ±0,2	2,7 ±0,4	125	<0,05
GH (mIE · L^{-1})	1,2 ±2,2	27,1±13	2158	<0,001	1,7 ±1,0	51,1 ±29,9	2906	<0,001
Insulin (nmol · L^{-1})	299 ±168	59 ± 20	-80	<0,001	224 ±42	109 ±47	-51	0,005
SHBG (nmol · L^{-1})	24 ±6	25 ±6	4	NS	84 ±55	85 ±58	1	NS
S-Glucose (mmol · L^{-1})	5,4 ±1,0	7,2 ±1,8	33	<0,05	4,3 ±0,6	8,0 ±2,3	86	<0,05
S-FFA (mmol · L^{-1})	0,18 ±0,06	0,69 ±0,22	283	<0,005	0,26 ±0,17	0,65 ±0,31	150	<0,03
S-Albumin (g · L^{-1})	50 ±3,0	51 ±2,4	2	NS	47 ±2,4	50 ±1,9	6	<0,05
S-IgA (g · L^{-1})	2,1 ±1,2	1,9 ±1,1	-10	0,007	1,5 ±0,5	1,5 ±0,6	0	NS
S-IgG (g · L^{-1})	9,1 ±1,7	8,5 ±1,5	-7	0,002	10,0 ±2,1	10,3 ±1,5	3	NS
S-IgM (g · L^{-1})	1,1 ±0,4	0,9 ±0,4	-18	0,004	1,4 ±0,5	1,3 ±0,4	-7	NS
S-Creatine Kinase U · L^{-1})	154 ±57	286 ±86	86	<0,001	81 ±38	185 ±41	128	0,001
S-Urea (mmol · L^{-1})	5,3 ±0,7	6,8 ±0,8	28	<0,001	5,2 ±0,7	6,1 ±0,3	17	0,007
S-Uric acid (umol · L^{-1})	360 ±40	454 ±43	26	<0,001	267 ±47	372 ±60	39	<0,001
S-Phosfate (mmol · L^{-1})	0,87 ±0,16	1,69 ±0,22	94	<0,001	0,9 ±0,1	1,7 ±0,3	89	<0,001
S-Sodium (mmol · L^{-1})	142 ±1,0	144 ±2,0	1	0,012	141 ±1,2	143 ±0,5	1	0,025
S-Potassium (mmol · L^{-1})	4,6 ±0,2	4,4 ±0,2	-4	NS	4,2 ±0,3	4,3 ±0,4	2	NS
S-Cloride (mmol · L^{-1})	104,7 ±1,8	106,7 ±3,1	2	NS	105 ±1,2	107 ±2,7	2	NS
S-Magnesium (mmol · L^{-1})	0,73 ±0,04	0,66 ±0,07	-10	0,05	0,81 ±0,09	0,66 ±0,04	-19	0,006
S-Haptoglobin	0,6 ±0,4	0,5 ±0,4	-17	NS	0,7 ±0,2	0,6 ±0,2	-14,29	NS
S-CRP (mg · L^{-1})	<10	<10	0	NS	<10	<10	0	NS

The changes from pre- to post-race in all measured variables from the female 30 km and the males 50 km competition are listed in table 3.1.

3.2. Discussion

Both the female 30 km and the male 50 km ski race resulted in substantial changes in concentrations of granulocytes, catecholamines, GH, cortisol, insulin, glucose, FFA and

metabolites like CK, urea, non-organic phosphates and uric acid (table 3.1). In general, the perturbations in biochemical and cellular variables described in the present investigation were somewhat larger than what has been reported after exhaustive endurance exercise in the field (64) (219), in laboratory studies (145;154), but by and large within the ranges observed in our own observations in papers 2, 3, and 4. Furthermore, the increased blood glucose concentration at the end of the race did not indicate any substrate shortage to the CNS or other vital organs. Plasma levels of FFA also increase, but not to levels assumed to be toxic (>4 mmol · L^{-1}) (89;245). Based on the pre- and post-race hemoglobin concentrations, only minor changes in plasma volume were observed during the races and serum electrolytes remained almost unchanged. Thus, from the analyses of more than thirty cellular and biochemical variables in this field study, we did not observe changes that indicate potential health risks in well-trained endurance athletes participating in a long distance ski-race where carbohydrate-electrolyte drinks are consumed regularly and no athletes collapsed.

3.2.1. Comparisons with marathon racing

The rationale for making a comparison between two races with similar duration and intensity, but a considerable difference in the amount of muscle involved, comes from observations that muscle mass may affect the degree of change in catecholamine and glucose uptake in the muscle during exercise (110;181;188). According to this hypothesis, cross-country skiing, which requires use of almost all skeletal muscles in the body, should result in larger changes in catecholamines and possibly substrate and immune system changes under hormonal control, as opposed to marathon running with the same intensity and duration where mostly leg muscles are at work.

Table 3.2 Comparison of percentage change in immuno-endocrine variables during a 50 km cross-country ski race (n=10) and data collected from studies on marathon running detailed in the appendix.

	50 km ski race % change	**Marathon race** range of % change
Total leukocytes	291%	154-224 %
Granulocytes	448 %	236-372 %
Lymphocytes	63 %	-22 to +19 %
NK cells	153 %	-65 to +72 %
S-Epinephrine	1063 %	62-250 %
S-Norepinephrine	472 %	69-463 %
S-Cortisol	107 %	65-312 %
S-Growth hormone	2158 %	233-1468 %
S-Insulin	-80 %	-30-70 %
S-Glucose	33 %	0-15 %

Changes in some variables of the immune and endocrine system, as well as blood glucose, from the following marathon race investigations (127;128;148;156;185;190;192;219) are listed in table 3.2, along with the data from the present study. The studies on marathon racing reported in table 3.2 all used experienced runners with finish times in the range of 135-180 min, which is roughly comparable to the 135-146 min in the ski race. Clearly, there are several limitations to such a comparison, including differences in subject populations, timing for the blood sampling, environmental conditions, food and liquid intake before and during the race, and perhaps differences in racing intensity. Nevertheless, it is interesting to see that the magnitude of change in almost all the variables listed in table 3.2 is larger during the 50 km ski race compared to what has been found in the marathon studies. Obviously, it is not possible to ascribe these differences to one particular factor, and most likely there are several mechanisms involved behind the observed differences. However, based on the suggested link between activated muscle mass and changes in catecholamines and glucose uptake during exercise, it is not unlikely that the involvement of larger muscle mass in cross-country skiing as opposed to running could in part be responsible for some of the observed differences.

3.2.2. Relation between changes in hormones and leukocytes

Several studies have proposed links between exercise-induced changes in concentrations of circulatory leukocytes and changes in epinephrine, cortisol, GH, and possibly others stress hormones (101;102;134;151;162;163). However, even though some of the exercise changes in leukocyte counts have been partly mimicked by infusion of these hormones under resting conditions (40;100;224;225;227), the endocrine changes cannot fully explain the changes in neutrophil and lymphocyte counts during intense exercise. In study I we measured perturbations in both leukocyte populations and stress hormones before and after exhaustive endurance exercise. This enabled us to make comparisons between changes in leukocytes and hormones such as epinephrine, norepinephrine, cortisol and GH. We found no correlation between the pre- to post-race changes in granulocytes and epinephrine ($r=0.32$), norepinephrine ($r=0.34$), cortisol ($r=0.31$), or GH ($r=0.13$). Likewise, there was no correlation between the pre- to post-race changes in lymphocytes and epinephrine ($r=0.47$), norepinephrine ($r=0.37$), cortisol ($r=0.28$), or GH ($r=0.18$). Thus, the increases in granulocytes and lymphocytes during a long distance race in elite skiers do not seem to be associated with changes in plasma concentrations in the above-mentioned hormones.

This lack of correlation does not necessarily exclude a role of stress hormones in the regulation of leukocyte trafficking during strenuous endurance exercise. There is a well-documented relationship between changes in concentrations of catecholamines, beta-adrenergic antagonists as well as cortisol, and altered expression of adhesion molecules on endothelial cells and circulatory leukocytes (10;11;30;138;157). Hormone-induced changes in the expression of adhesion molecules on these cells may therefore represent a potential link between endocrine changes and leukocyte trafficking in the circulatory compartment. Additionally, several exercise studies have shown that exercise-induced changes in concentrations of adhesion molecules on leukocyte and endothelial cells may explain a substantial part of the alterations in leukocyte trafficking during strenuous exercise (66;137;178;233). Thus, exercise-induced increases in hormones could still exert their impact on the circulatory pool of leukocyte indirectly through changes in adhesion molecules (196).

Nevertheless, how can the lack of correlation between changes in stress hormones and leukocyte counts in the present study be reconciled with previous findings? One explanation may be an antagonistic effect of EPI and cortisol on circulatory lymphocyte concentrations; EPI causing an influx and cortisol an efflux of lymphocytes to the peripheral circulation (52;159;225;227). Possibly, there was a catecholamine-induced influx of lymphocytes early in the race, but then after about an hour increases in cortisol could have caused a redistribution of lymphocytes out of the circulation (163). This dual, counter-regulatory effect of EPI and cortisol on circulating lymphocytes during prolonged strenuous exercise is supported by the observation of only minor elevation in the concentration of these cells at the end of the race compared with granulocytes in the present study as well as in others (184).

Since there is an apparent discrepancy between the findings from infusion studies during rest and exercise studies regarding the relationship between stress hormones and circulating leukocytes, it is likely that non-endocrine factors also play a significant role in the redistribution of leukocytes during exercise (134). Perhaps the most obvious difference between the exercise and rest settings is the generation of a substantial shear stress against the vascular endothelium as a result of increased cardiac output and peripheral blood flow during exercise (42). Since granulocytes represent the largest pool of marginated leukocytes loosely attached to the endothelium at rest, increased shear stress during exercise may mobilize a proportionally larger number of granulocytes than lymphocytes into the peripheral circulation (134). The large recruitment of

granulocytes compared with lymphocytes observed at the end of the ski race may be interpreted as support for this hemodynamic explanation (57;129;139;222). Interestingly, recent studies propose that the mechanism by which increased shear stress contributes to leukocytosis is in fact through modulation of adhesion molecules on the endothelium and leukocytes (4;94;200). Thus, both endocrine and hemodynamic changes in the blood compartment may alter leukocyte concentrations during exercise by means of modifying the expression of adhesion molecules on endothelial cells and leukocytes.

3.2.3. Relation between changes in hormones and energy substrates

Significant correlations were found between increases in glucose and GH ($r=0.66$, $p<0.01$), and between glucose and insulin ($r=0.54$, $p<0.05$), but no correlation was found between changes in hormones and FFA. We did observe significant elevations in blood glucose at the end of both races, a finding that has been reported previously in connection with prolonged strenuous exercise (33;205). A higher rate of glucose production from the liver (rate of appearance) than glucose utilization by the active muscle (rate of disappearance) has been found during strenuous exercise (109), resulting in an elevated blood glucose level towards the end of such exercise. As indicated earlier, the activation of a large percentage of total muscle mass during the cross-country ski race along with a substantial increase in catecholamines may also have contributed to this imbalance (110).

Substantial increases in catecholamines, with subsequent inhibition of insulin secretion, may have triggered such poor regulation of blood glucose level (207). GH is also known to inhibit glucose uptake in the muscle and enhanced gluconegenesis in the liver. Thus, the large increase in GH could have contributed to the elevated glucose levels towards the end of the race. It is worth noticing that the highest levels of blood glucose were observed among the females who also had a larger increase in GH concentrations. Interestingly, a gender difference has been found in both GH secretion (171;248) and glucoregulatory responses during intense exercise (69;124), demonstrating a greater imbalance between hepatic output and muscle uptake of glucose in females than males. This could at least in part explain the larger hyperglycemia among the females than males in the present study.

3.2.4. Comparisons between continuous and split exhaustive exercise (race vs. laboratory)

Table 3.3 gives the absolute values at the end of the race as well as the percentage change in immuno-endocrine variables examined in the 50 km male ski race and the investigation on repeated bouts of endurance exercise, reported in study III. Certainly, the 140-150 min continuous work performed during the ski race was different from the two bouts of 75 min high intensity exercise, separated by 3 h of rest, in our laboratory study. However, the subjects were exhausted after completion of both the race and the second exercise session. Thus, we used the opportunity of having tested elite athletes in two types of exhaustive exercise protocols (i.e. continuous vs. split exercise), and compared the perturbations in the immuno-endocrine variables observed in the ski race with those found in the laboratory study.

Table 3.3 Comparisons of changes during a 50 km ski race (n=10) and a split exhaustive exercise protocol in the laboratory (n=9) in elite endurance athletes. Percentage change and the post race/exercise concentrations are given for each variable.

	50 km ski race	**2 x 75 min lab.exerc.**
Total leukocytes	+291 % (21,4 $\cdot 10^3 \cdot ul^{-1}$)	+118 % (12,9 $\cdot 10^3 \cdot ul^{-1}$)
Neutrophils	+448 % (18,0 $\cdot 10^3 \cdot ul^{-1}$)	+141 % (7,6 $\cdot 10^3 \cdot ul^{-1}$)
Lymphocytes	+63 % (3,1 $\cdot 10^3 \cdot ul^{-1}$)	+ 94 % (4,1 $\cdot 10^3 \cdot ul^{-1}$)
CD56+/NK cells	+153 % (1,1 $\cdot 10^3 \cdot ul^{-1}$)	+200 % (1,0 $\cdot 10^3 \cdot ul^{-1}$)
S- Epinephrine	+1063 % (4,7 $nmol \cdot L^{-1}$)	+4450 % (9,1 $nmol \cdot L^{-1}$)
S- Norepinephrine	+472 % (19,7 $nmol \cdot L^{-1}$)	+1558 % (31,5 $nmol \cdot L^{-1}$)
S- Cortisol	+107 % (957 $nmol \cdot L^{-1}$)	+ 188 % (721 $nmol \cdot L^{-1}$)
S- Growth hormone	+2158 % (27,1 $mE \cdot L^{-1}$)	+10660 % (53,8 $mE \cdot L^{-1}$)
S- Insulin	-80 % (59 $pmol \cdot L^{-1}$)	-82 % (44 $pmol \cdot L^{-1}$)
S- Glucose	+33 % (7,2 $mmol \cdot L^{-1}$)	-25 % (4,3 $mmol \cdot L^{-1}$)

Table 3.3 shows that changes in EPI, NE, cortisol, and GH generally were larger at the end of the second bout of exercise compared with the ski race. The percentage change in insulin was almost the same in the two studies, but the pre- and post-concentrations were slightly lower in the laboratory study compared with the ski race. Blood glucose increased during the ski race, but showed a slight decrease during the second exercise session in the lab.

We suggest that the differences in insulin and glucose levels at the end of the ski race vs. the second bout of exercise in the laboratory may for the most part be due to the carbohydrate intake. Carbohydrate liquid was consumed both before and during the ski race, while only water

was allowed during the exercise sessions in the laboratory. The 3 h rest and one meal between the two lab-exercise sessions could only have resulted in a partial repletion of the glycogen stores before starting the second exercise session (16). Thus, most likely the subjects were severely glycogen depleted towards the end of both the 145 min ski race and the second lab exercise. Assuming near-equal state of glycogen depletion at the end of the ski race and second exercise session, the most obvious difference between the protocols is the lack of exogenous carbohydrate supply during the laboratory exercise. The subsequent decline in blood glucose availability may have been an important trigger for the more pronounced increase in catecholamines, cortisol and GH observed in the laboratory study (147), because these hormones are essential for increased fat oxidation in a glycogen depleted state (25).

By maximizing energy and liquid intake before the ski race, the athletes probably increased the pre-race concentrations of insulin, glucose, and FFA. It is also likely that the post-race level of blood glucose was influenced by the CHO consumption during the races. Additionally, the immediate blood sampling after the race (within 1 min) has to be considered when comparing our data with other field studies from marathon racing where the post race sampling may have taken place as late as 10-15 min after the finish.

Regarding the comparison of changes in leukocytes and lymphocyte subsets between the ski race and laboratory study, we observed a substantially larger change in granulocyte concentrations (> 90% are neutrophils), but a slightly smaller change in lymphocyte concentrations during the ski race compared with the second bout of exercise. If plasma levels of hormones regulate leukocyte trafficking, a more pronounced increase in GH would be expected in association with the larger increase in granulocytes in the laboratory study. However, that was not the case, which supports the lack of correlation between changes in plasma levels of stress hormones and changes in circulating leukocytes found in this study.

3.2.5. Conclusion and implications

Long distance ski racing resulted in substantial changes in several immuno-endocrine and metabolic variables, but no sign of energy substrate shortage in the blood in world-class cross-country skiers. There was no correlation between the exercise-induced changes in leukocytes and stress hormones. The magnitude of these perturbations may be linked to the large amount of

muscle mass activated during cross-country skiing, but further studies are needed to test this hypothesis.

Since this study showed that long distance ski racing results in major disturbances in several physiological functions, it may be reasonable to suggest that a correspondingly extended recovery time would be needed to attain complete homeostasis. For practical reasons we were not able to follow up with a proper protocol of measurements during the first hours of recovery after the race in these athletes. Therefore, the present study cannot substantiate such a suggestion. A new study that includes extended measurements during the post-race period could give valuable information about the duration of various recovery processes after a long distance ski race in elite athletes.

4. Study II: Variations in seasonal training and competition load on immunoendocrine responses to acute exhaustive exercise (paper 2).

4.1. Results

4.1.1. Training and competition score and exercise responses

The average training and competition score was more than twice as high during a three week period prior to the in-season HI (16.0 ± 3.9) compared to the off-season LO test (7.0 ± 4.4; t-test, $p<0.001$, figure 4.1a). In the in-season HI test, time to exhaustion was longer compared with the off-season LO test (35.3 ± 2.1 min, and 32.9 ± 3.1 min; respectively, p=0.04). However, at the end of the exercise test there was no difference in heart rate (HI: 190 ± 7; LO: 193 ± 6) or lactate concentration (HI: 7.8 ± 1.2 mmol/l; LO: 7.2 ± 1.2 mmol/l; see Fig.2, paper II).

4.1.2. Hormonal, leukocyte and interleukin-6 responses

A small difference was found in NE, but not in EPI response between the in-season HI and off-season LO tests ($F_{1,9}=8.7$, p=0.018 and $F_{1,9}=1.27$, p=0.29; respectively, figure 4.1b). There was no difference between the in-season HI and off-season LO tests in ACTH or cortisol levels ($F_{1,9}=0.80$, p=0.55, and $F_{1,9}=0.21$, p=0.66; respectively; figure 4.1c) during exercise and recovery. Furthermore, there was no difference between the in-season HI and off-season LO tests with regard to the neutrophil and lymphocyte responses during exercise and recovery ($F_{1,9}=0.01$, p=0.937; $F_{1,9}=3.62$, p=0.090; respectively, figure 4.2). Regarding the IL-6 response, a trial by time interaction effect between the in-season HI and off-season LO tests ($F_{1,9}=6.36$, p=0.007) was found. However, there was no significant difference between the IL-6 levels in the HI and LO tests at any single time point.

Figure 4.1a) Training and competition score (TCS) 3 weeks prior to the in-season HI test (hatched bars) and the off-season LO test (open bars). 4.1b) Epinephrine, norepinephrine and 4.1c) ACTH, cortisol concentrations before and after an incremental treadmill run to exhaustion at the in-season HI test and the off-season LO test (mean ± SD, n=9 for catecholamines, n=10 for ACTH and cortisol)

Figure 4.1

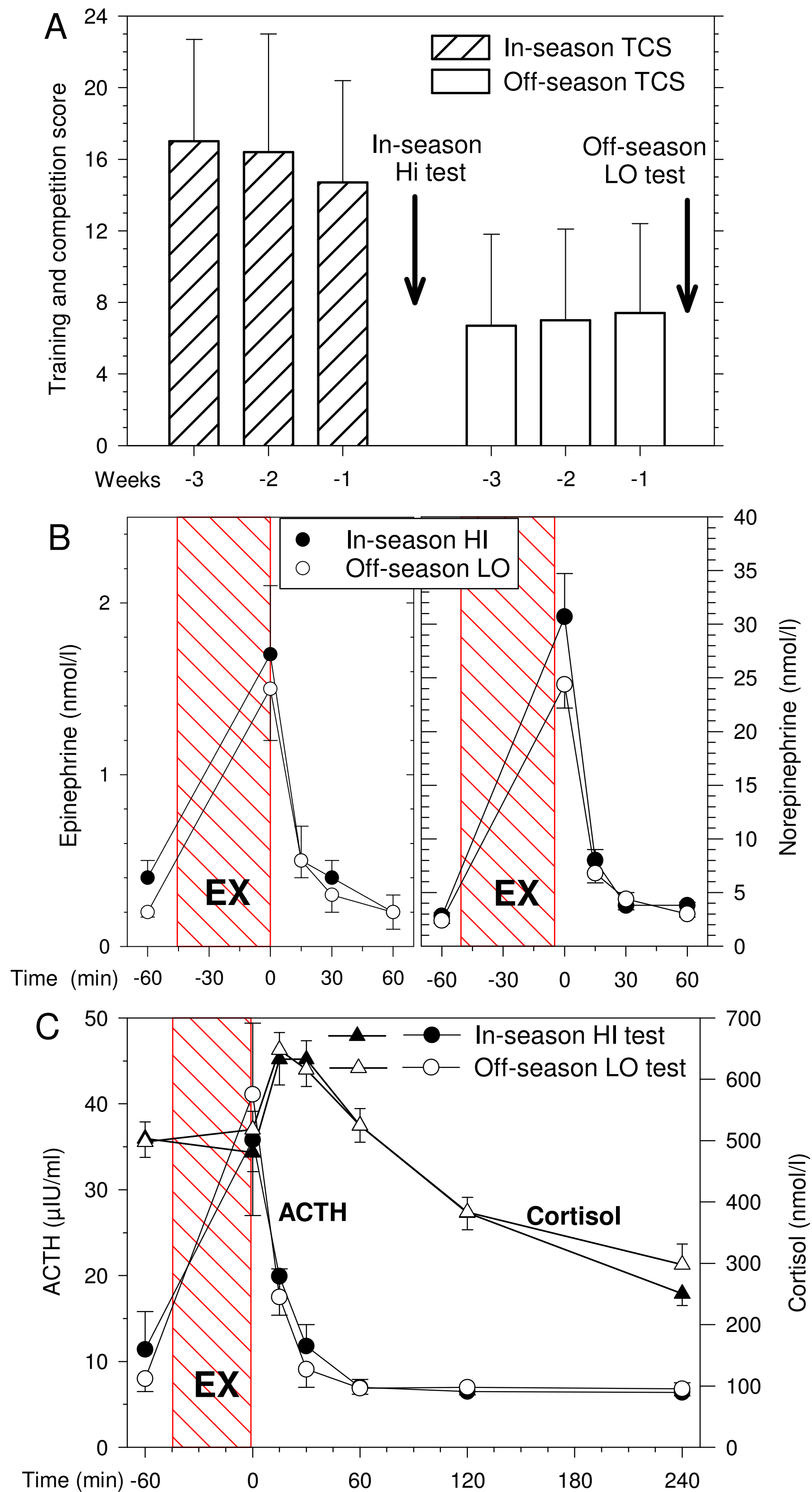


Figure 4.2

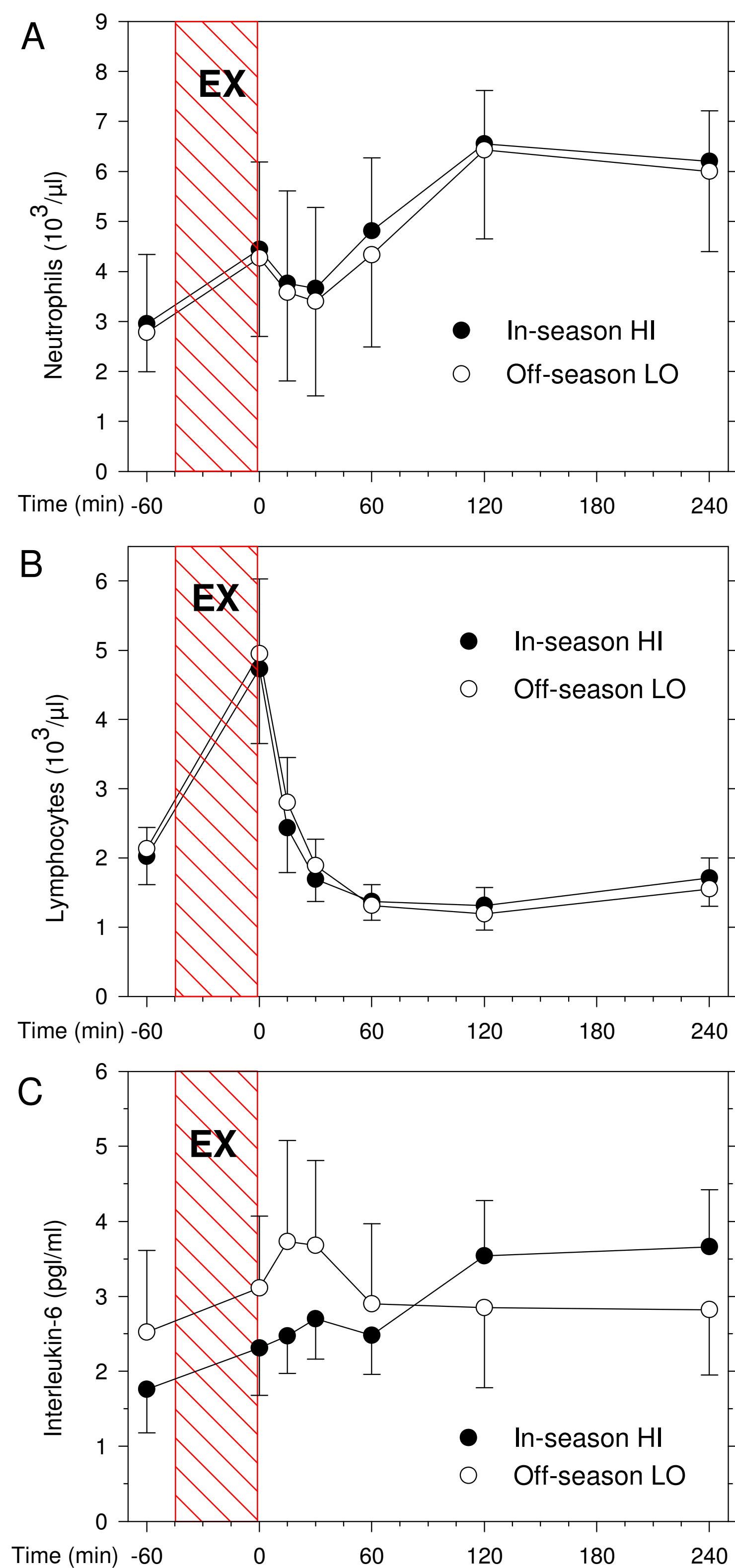


Figure 4.2 a) Neutrophil, 4.2b) lymphocyte and 4.2c) IL-6 concentrations before and after an incremental treadmill run to exhaustion at the in-season HI test and the off-season LO test (n=10).

4.2. Discussion

Longitudinal studies have demonstrated that variables linked to immune and endocrine function can be altered by chronic exercise (9;74;142;230;238). Therefore, it is conceivable that immuno-endocrine responses to acute exercise could reflect seasonal changes in training and competition loads, and thus be used to monitor the impact of altered training regimens throughout a sport season. However, there is no consensus on how variations in training load over a given period of time affect the hormonal and immune responses to a single bout of exercise (55;74;75;114;142;231;232;237;238;247). There are several plausible reasons for this discrepancy, which has been briefly discussed in paper 2. One problem when comparing the findings of the above studies is the poor or total lack of registration of changes in exercise load during the study period. Therefore, we carefully designed a sports-specific training and competition score (TC-score) to compute physical effort during days of training and competition (further details are given in paper 2).

The present study did not find a difference in the exercise-induced changes in neutrophils, lymphocytes, EPI, ACTH, and cortisol concentrations between the in-season HI test and the off-season LO test, despite the fact that the training and competition load was more than twice as high during the competitive season compared with the off-season. Thus, in these athletes long term variations in exercise stress do not seem to affect their reactions to an acute bout of exhaustive exercise. This is in accordance with the findings in some studies (142;235), but in disagreement with others (55;74;236). However, in the studies where an effect of variation in chronic training stress was observed, the changes were small and transient.

Explanations for the disparity between the findings of the cited studies may be found among the following circumstances: 1) Some investigations examined the effect of chronic exercise on variations in circulatory cells, hormones and biochemical substances in the resting state, while others have examined the changes during an exercise test. 2) Protocols for the chronic training component have varied from deliberate overtraining to normal seasonal variations, and the protocol for the acute exercise bout has also been different. 3) The training status of the athletes entering these investigations has varied.

A possible explanation for our findings may be found in the athletes' ability to adapt to large loads of training and competitions during the competitive season. This adaptability could in turn

be linked to a genetic predisposition and an acquired capability to tolerate large loads of physical stress through many years of systematic training (32;77;82;85;105;170;220). Also these were experienced athletes with ditto skills in balancing large volume of training with optimal recovery (27;104;165;198), and the increase in training load was spread over several months from summer to winter. Another alternative explanation could simply be that measurements of acute leukocyte and stress hormone responses are not sensitive to changes in seasonal variations of the training and competition load in these athletes. Thus, a relationship between chronic and acute stress could still exist, but is not disclosed by our test variables. However, the immuno-endocrine variables measured in the present investigation are all known to be affected by both chronic and acute exercise stress (117;231)(28;45;70;96;163;167;173).

The slightly higher NE concentrations observed at the end of the in-season HI test compared with the off-season LO test is probably a result of larger spill-over of NE from sympathetically activated skeletal muscles (50). The longer time to exhaustion in the in-season HI test compared to the off-season LO test could possibly have contributed to this effect. We also observed an increase in IL-6 during the exercise test and for the first 30 min post-exercise in both the in-season HI-and off-season LO test (figure 4.2c). However, at 120 and 240 min post-exercise IL-6 did not return toward pre-exercise levels in the in-season HI test. Although time to exhaustion was 2.4 min longer, it is not likely that this can explain the higher IL-6 response in the late recovery phase on the in-season HI test. However, we do not believe that these small differences in NE and IL-6 levels have a biological significance.

4.2.1. Conclusion and implications

This study hypothesized that increased training and competition load would result in more pronounced stress responses in connection with an exhaustive exercise test. However, there were almost identical changes in blood concentrations of immuno-endocrine variables in response to a standardized exercise test with run to exhaustion, during periods with large variations in seasonal training and competition load among elite cross-country skiers. Thus, within the limits of this study, the hypothesis cannot be confirmed.

Nevertheless, one of the subjects in the present investigation presented signs of performance impairment towards the end of the ski season. Even though his training and competition schedule was reduced and the second in-season test was postponed for three weeks, irregularities

in some of his responses were evident when compared with the other tests. Interpretation based on a single case may only be speculative, but it is not unlikely that the irregular test responses in this skier may be linked to the preceding period of overload symptoms and performance impairment. One well-controlled study have compared resting values of hormonal, immune, and haematological variables in overreached and well-trained swimmers during an intensified training period and found no differences between these groups, apart from urinary norepinephrine secretion (117). However, new studies are warranted to examine if hormone and/or leukocyte profiles induced by a standardized exercise test could yield some information about the state of balance between training and recovery in athletes.

5. Study III: The impact of a previous bout of strenuous endurance exercise on the responses to a subsequent exercise bout the same day (papers 3, 4, 5 and 6).

5.1. Results

The results from the observed variables in the present study are illustrated in figures 5.1-5.3, and the statistical comparisons between the trial with prior exercise (trial TWO) and the trial without prior exercise (trial ONE) are summarized in table 5.1.

The main findings from the hormonal analyses may be summarized as follows: More pronounced increases in concentrations of E, NE, ACTH, cortisol and GH were observed during the second bout of exercise and early recovery in trial TWO compared with the responses to the single bout of similar exercise in trial ONE (figures 5.1a-e and table 5.1). Additionally, the urinary excretion of catecholamines was elevated during and after exercise trial TWO compared with trial ONE (paper 3). There was no difference in serum concentrations of IGF-1, SHBG, LH, and FSH between trials TWO and ONE during Ex-A and the subsequent recovery period (table 5.1). A larger decrease in insulin during Ex-A was observed in trial TWO compared with trial ONE (figure 5.1f), but there was no statistical difference in insulin responses between the trials during the combined exercise and recovery period. Total testosterone concentration decreased during recovery after the first and second bout of exercise in trial TWO compared with trial REST (figure 5.1g), but there was no statistical difference between the testosterone levels in trial ONE and TWO. Serum TSH increased during Ex-A, but decreased below resting values from 3h post exercise until the next morning (figure 5.1h). TSH plasma levels were higher in trial TWO than ONE during the first 3 h post-exercise, but not for the combined Ex-A and recovery period. Serum FT4 decreased during Ex-A in both trials (figure 5.1i). Even though FT4 levels were higher in trial TWO compared with trial ONE during the first 3 h post-exercise, there was no statistical difference for the combined Ex-A and recovery period.

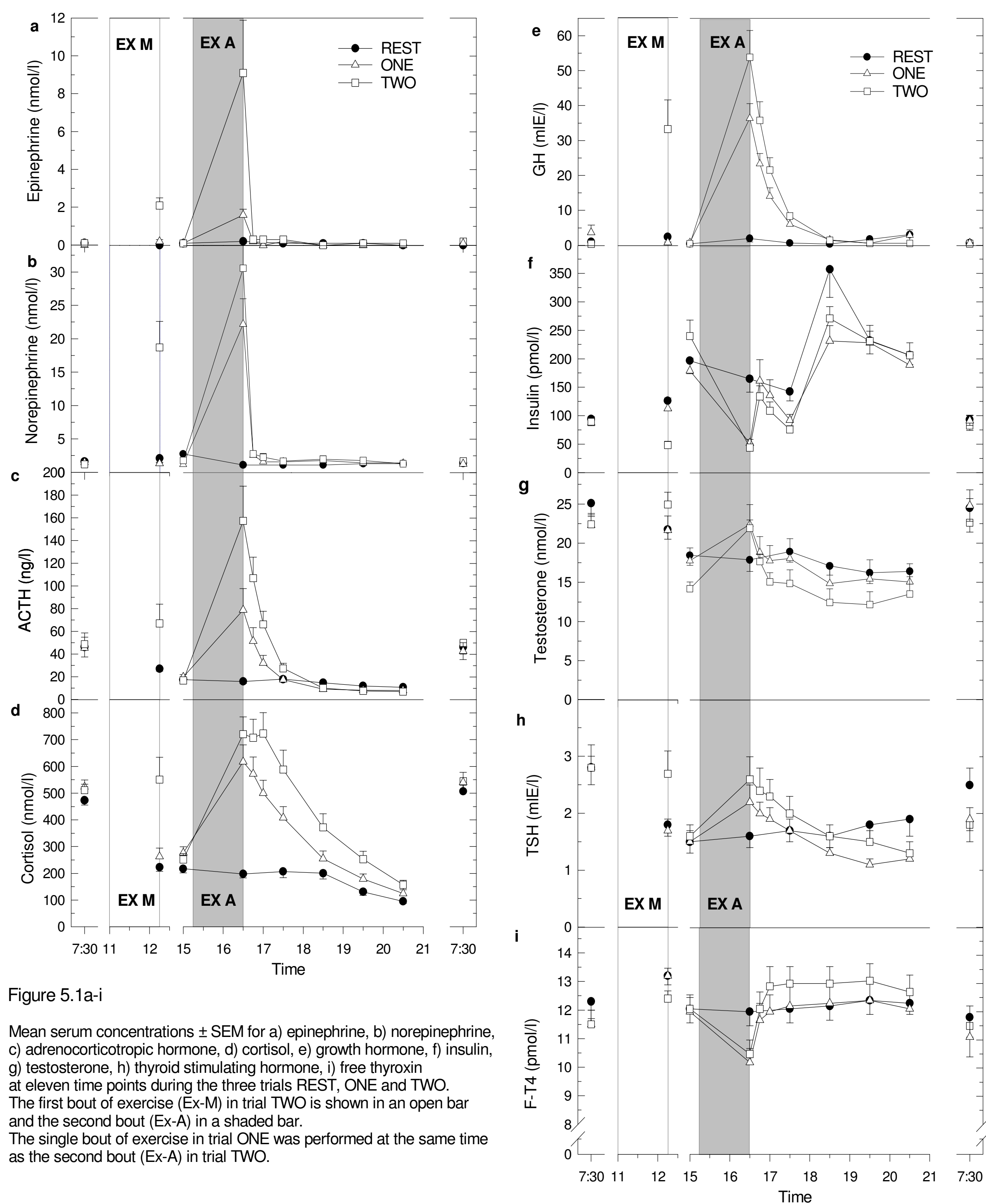


Figure 5.1a-i

Mean serum concentrations ± SEM for a) epinephrine, b) norepinephrine, c) adrenocorticotropic hormone, d) cortisol, e) growth hormone, f) insulin, g) testosterone, h) thyroid stimulating hormone, i) free thyroxin at eleven time points during the three trials REST, ONE and TWO. The first bout of exercise (Ex-M) in trial TWO is shown in an open bar and the second bout (Ex-A) in a shaded bar. The single bout of exercise in trial ONE was performed at the same time as the second bout (Ex-A) in trial TWO.

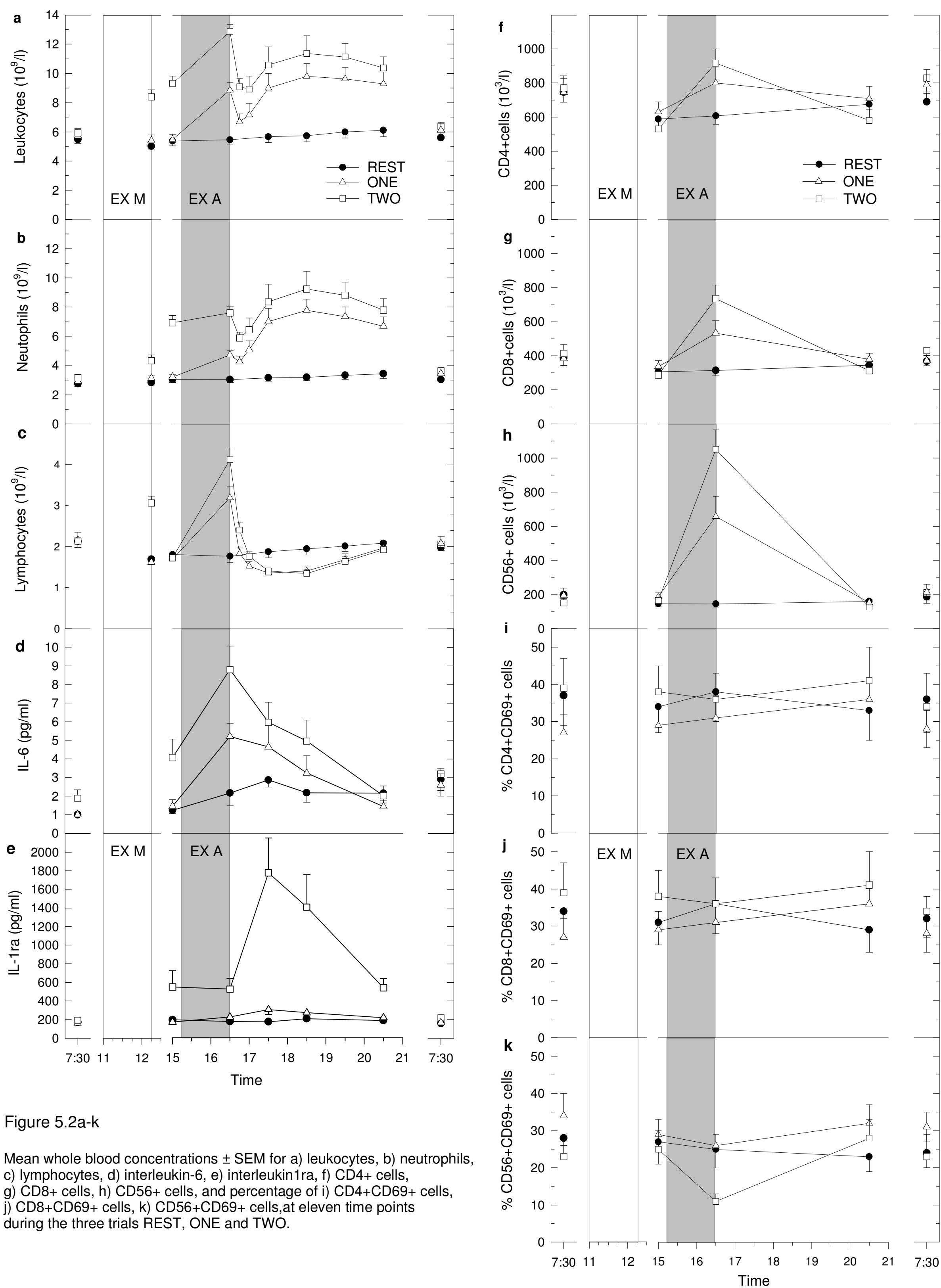


Figure 5.2a-k

Mean whole blood concentrations ± SEM for a) leukocytes, b) neutrophils, c) lymphocytes, d) interleukin-6, e) interleukin1ra, f) CD4+ cells, g) CD8+ cells, h) CD56+ cells, and percentage of i) CD4+CD69+ cells, j) CD8+CD69+ cells, k) CD56+CD69+ cells,at eleven time points during the three trials REST, ONE and TWO.

The levels of total leukocytes, neutrophils, lymphocytes, CD4+, CD8+ and CD56+ cells were augmented in connection with the second bout of exercise in trial TWO compared with the single bout in trial ONE (figures 5.2a,b,c,f,g,h and table 5.1). Furthermore, at the end of the second bout of exercise we observed a lower percentage of CD56+ cells expressing the activation marker CD69, and a decreased density of CD69 molecules on the CD56+ cells after stimulation with mitogen compared with the single bout in trial ONE (figure 5.2k). Regarding the changes in cytokines, we observed more pronounced increases in plasma levels of IL-6 during and IL-1ra after the second bout of exercise in trial TWO compared with the single bout in trial ONE (figures 5.2d and e).

Mean O2-uptake, EPOC, HR, lower RER (higher fat oxidation) was higher, and the increase in T_R was larger during and/or after the second bout of exercise in trial TWO compared with the single bout in trial ONE (figure5.3.c-h and table 5.1). After 14 h of recovery an increased O_2 uptake and a decreased RER was still evident in the two exercise trials compared with trial REST, but no differences between trial TWO and ONE were found. There was a trend towards a larger decrease in plasma glucose during Ex-A in trial TWO compared with trial ONE, but there was no significant difference in magnitude of change over the combined exercise and recovery period between the trials (figure5.3b). Finally, the changes in Hgb concentrations, reflecting the shifts in plasma volume during and after the exercise sessions in all three trials are illustrated in figure.5.3a. During Ex-A there was an 11% increase in Hgb concentrations in both trial ONE and TWO, but no difference in the change between trial ONE and TWO.

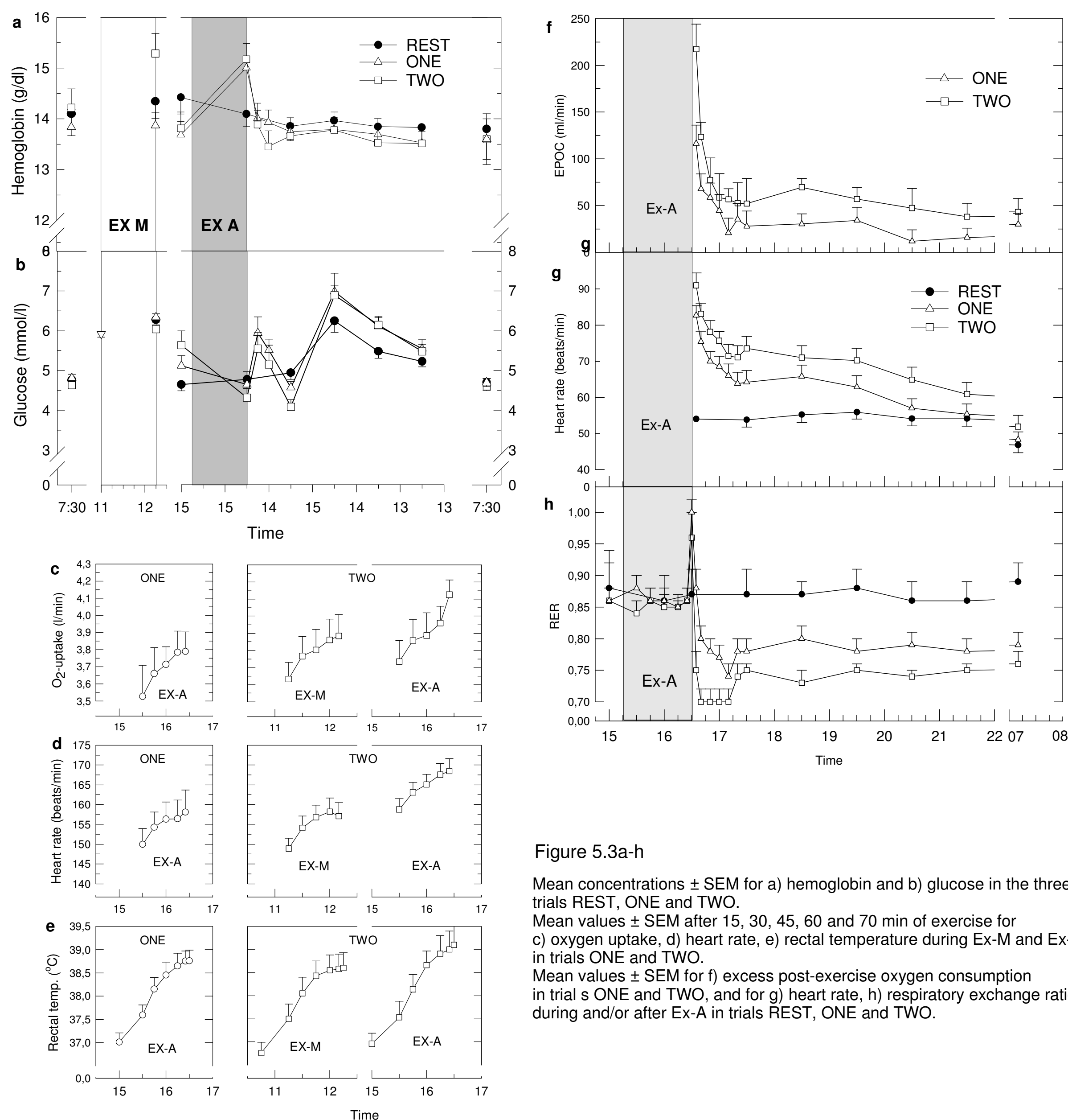


Figure 5.3a-h

Mean concentrations ± SEM for a) hemoglobin and b) glucose in the three trials REST, ONE and TWO.
Mean values ± SEM after 15, 30, 45, 60 and 70 min of exercise for c) oxygen uptake, d) heart rate, e) rectal temperature during Ex-M and Ex-A in trials ONE and TWO.
Mean values ± SEM for f) excess post-exercise oxygen consumption in trial s ONE and TWO, and for g) heart rate, h) respiratory exchange ratio during and/or after Ex-A in trials REST, ONE and TWO.

Table 5.1 Results of repeated measures ANOVA statistics for the comparison between trial TWO and trial ONE for each of the listed variables are given in this review table. Analyses of the changes occurring during Ex-A and subsequent 14 h recovery period, as well as for the combined period of Ex-A and recovery (15:00-07:00 next morning) were performed and are listed in separate columns (EXERCISE, RECOVERY and EXE + REC). Main effects of trial or interaction effect of trial by time are reported. Significantly higher levels of the variable in trial SHORT compared with trial LONG are indicated with ↑, significantly lower levels are indicated with ↓, and no difference between the trials with =, using an alpha level of 0.05. A trend (p=0.050-0.099) towards a difference between the two trials is indicated with (↑) or (↓). Where no measurement during exercise or recovery was performed, the space is left blank in the respective column. The exact p-levels for each variable are found in the original publications (papers 3, 4, 5, and 6). The abbreviations used in the table are explained separately at the front of the thesis (see abbreviations).

TRIAL TWO VS. ONE

Biological variable	Exercise	Recovery	Exe + Rec
Epinephrine	↑	↑	↑
Norepinephrine	↑	↑	↑
ACTH	(↑)	↑	↑
Cortisol	=	↑	↑
GH	(↑)	↑	↑
Insulin	↓	(↓)	↓
IGF-1	=	(↑)	(↑)
FSH	=	=	=
LH	(↓)	=	=
Testosterone	=	(↓)	=
TSH	=	↑	=
F-T4	=	(↑)	=
Glucose	(↓)	=	=
IL-1ra	=	↑	↑
IL-6	↑	↑	↑
Neutrophils count	↑	↑	↑
Lymphocytes count	↑	↓	↑
CD4+ cell count	↑	(↑)	↑
CD8+ cell count	↑	↑	↑
CD56+ cell count	↑	↑	↑
% CD4+CD69+ cells	=	=	=
% CD8+CD69+ cells	=	=	=
% CD56+CD69+ cells	↓	(↓)	↓
CD69 FIR of CD4+ cells	=	=	=
CD69 FIR of CD8+ cells	=	=	=
CD69 FIR of CD56+ cells	=	=	=
Mean oxygen uptake	↑		
Oxygen drift	↑		
EPOC		↑	
Mean HR	↑	↑	
Mean RER	↓	↓	
Rectal temperature	↑		
Mean Lactate	=		
Mean RPE	↑		

5.2. Discussion

The main hypothesis of this study was that changes in neuroendocrine, immune and metabolic variables would be more pronounced in connection with a second bout of exercise compared with a single (first) bout of similar exercise. The rationale for this hypothesis was that an incomplete recovery period after the first exercise session would result in a carry over effect of residual physiological stress from the first to the second bout of exercise. Consequently, stress-sensitive variables were expected to show a larger magnitude of change during and immediately after the second bout of exercise, despite the fact that the duration and absolute workload of exercise was identical to the first bout.

Most of our findings are in accordance with this hypothesis. Residual effects of a previous exercise session have not been properly examined with a study design controlling for diurnal variations and at the same time applying an exercise and recovery protocol similar to those used by endurance athletes. Moreover, by making frequent measurements throughout the recovery period after the exercise sessions, we were able to demonstrate complete normalization of almost all variables after the first exercise bout, before observing the augmented responses during and after the second exercise bout. Consequently, the residual effects of prior exercise is not simply a result of increased levels of these stress markers from the previous exercise session, but represents an altered responsiveness in several neuroendocrine, immune and metabolic functions. Furthermore, this indicates that complete homeostasis cannot be evaluated on the basis of normalization in key immuno-endocrine and metabolic variables. This discrepancy between normalization of plasma concentrations of signal molecules and functional markers on one hand, and the lack of complete homeostasis in the systems that they represent on the other hand, is also a novel finding of the present study.

5.2.1. Comparison with previous studies

Only a handful of investigations have addressed the issue of how the body reacts to repeated exercise sessions on the same day (chapter 1, table 1.4), but they have all failed to control for diurnal variations in their outcome variables. Another limitation is that they have not studied the recovery period after the second or third bout of exercise.

At the time we undertook the present investigation, only one study had used prolonged (>60 min) repeated exercise sessions (189), and to our knowledge only two more studies with

prolonged exercise have so far been published (68;216). The main findings of the previous studies using repeated bouts of exercise are summarized in table 5.2, and the comparisons of the last vs. the first bout of exercise are reported. Since there are substantial variations in study design and protocol, including the recovery procedures between the exercise sessions, caution must be used when comparing results. Not surprisingly, a rather equivocal picture appears when examining the results of these investigations collectively. Nevertheless, when comparing the last bout of exercise with the first, there is a tendency towards a more pronounced increase in concentrations of neutrophils, similar change in lymphocytes, variable changes in catecholamines, cortisol and GH, a decrease in insulin, and an increase in HR, TR, glucose and FFA utilization during the last exercise session.

Our results are in agreement with those studies showing augmented responses in neutrophils (Rhode 1997, Nielsen 1996, McCarthy 1992 and Field 1991) and lymphocytes (Nielsen 1996), increased epinephrine (Stich 2000, and Kaciuba-Uschilco 1992), increased GH (Kanaley 1997, and Kaciuba-Uschilco 1992), increased cortisol (Stich 2000, Kaciuba-Uschilco 1992), reduced insulin (Stich 2000, Kaciuba-Uschilco 1992, and Marliss 1991), reduced blood glucose (Galasetti 2001, Stich 2000, Marliss 1991), increased O_2 uptake and O_2 drift (Kaciuba-Uschilco 1992), increased T_R (Kaciuba-Uschilco 1992, and Sawka 1979), increased HR (Weltman 1998, Severs 1996, Kaciuba-Uschilco 1992, and Sawka 1979), and reduced RER (Stich 2000, Weltman 1998) during the last exercise session. However, in contrast to the previous investigations we also demonstrated increased CD4+, CD8+, CD56+ cells, reduced responsiveness and possibly cytotoxicity in CD56+ cells, increased concentrations of NE, a larger decrease in insulin and a larger increase in TR. Finally, we demonstrated changes during the recovery period that have not been reported before, such as a more pronounced neutrophilia and lymphocytopenia, increased concentrations of ACTH, increased EPOC and HR and reduced RER.

Table 5.2 The major outcome of the cited investigations on repeated bouts of exercise is listed according to their findings of immune, endocrine and metabolic changes. The results of the last bout of exercise are compared with the first bout of exercise.

IMMUNE CHANGES

Study	n	Major Immune Outcome of Last Exercise
Rhode 1997	8	inc neut,dec LAK act, sim lymp,CD4,CD16, CD56
Nielsen 1996	8	inc neut,lymp,CD4,CD16,IL-6, sim NKCA/cell
Severs 1996	11	sim neut, lymp,CD4,CD8,CD19,lym prol
Brenner 1996/97	11	sim CD16, CD56 and cytotoxicity
McCarthy 1992	8	inc neut, sim lymp,
Field 1991	12	inc neu,sim lymp, sim prolif and cytotox

ENDOCRINE CHANGES

Study	n	Major Endocrine Outcome of Last exercise
Galasetti 2001	8	red EPI, NE, GH,Glucagon, Cort,
Stich 2000	7	inc EPI, sim NE, red Ins, sim d-Ins, red GH, inc Cort
Kanaley 1997	6	incr GH (auc)
Brenner 1996/97	11	sim EPI, NE, Cort, GH
McCarthy 1992	8	sim EPI, NE, Cort,
Kaciuba-Uschilco 1992	10	inc EPI, NE, Cort, GH, red Ins
Marliss 1991	12	sim EPI, NE, red Ins

METABOLIC CHANGES

Study	n	Major Metabolic Outcome of Last Exercise
Galasetti 2001	8	incr Gluc infus rate and FFAox, sim RER, red Lactate,
Stich 2000	7	sim $V0_2$, red W-load, red RER, inc Glycerol and NEFA, red Glu
Weltman 1998	6	sim La^-, inc HR, red RER
Severs 1996	11	inc HR, sim Temp-r
Kaciuba-Uschilco 1992	10	inc HR, Temp-r, $V0_2$, sim RER
Marliss 1991	12	red p-Glu, inc peak Glu MCG, inc GluRd (ex) and GluRa(recov)
Sawka 1979	7	inc Temp-r and HR, sim $V0_2$

Abbreviations: inc = increased, sim = similar, dec = decreased, neut = neutrophils, lymph = lymphocytes, LAK act = lymphokine activated killer cell activity, NCCA = natural killer cell activity, prol = proliferation, cytotox = cytotoxicity, Glu = glucose, FFAox = free fatty acid oxidation, NEFA = non esterified fatty acids, MCR = metabolic clearance rate, Ra = rate of appearance, Rd = rate of disappearance. The rest of the abbreviations are found in the abbreviation list in the thesis.

5.2.2. Explanation of the findings

Intramuscular and hepatic glycogen contents are major determinants for the physiological response to prolonged strenuous exercise (17;31;54;80;92;93;103;240;242). We suggest that glycogen depletion after the first bout of exercise with subsequently decreased glycogen availability during the second bout of exercise may be the major contributor to the differences in

most physiological responses between the first and second exercise session in trials ONE and TWO, respectively. The following arguments and observations supports this hypothesis:

In the present investigation we did not obtain muscle biopsies that could determine muscle glycogen levels pre- and post-exercise, but on the basis of the earlier investigations a substantial reduction in glycogen stores should be expected after the first bout of exercise in trial TWO (37;61;91;120). Blom et al found that only 40-44% of the pre-exercise muscle glycogen content was present 4 h after an exercise session similar to ours, even when consuming adequate amounts of carbohydrates during recovery (16). Therefore, it can be assumed that muscle glycogen was incompletely restored during the rest period between the two bouts of exercise in the present study, even when the subjects consumed a 4000 MJ meal. This assumption is further supported by the observation of lower mean RER, i.e. decreased CHO oxidation relative to fat oxidation, found in trial SHORT compared with ONE. Moreover, Galasetti et al demonstrated a blunting of the neuroendocrine response from the first to the second bout of exercise when infusion of glucose was given during recovery between the two exercise sessions as well as during the second exercise (68). Finally, Steensberg et al demonstrated that exercising with a glycogen depleted leg resulted in increased plasma levels of catecholamines and decreased levels of insulin during the latter part of the 180 min exercise compared to exercise with the non-depleted leg (213).

Several studies have also found a strong association between increased CHO availability and attenuated cytokine responses during and after prolonged strenuous exercise (15;143;152;210). Perhaps the most convincing evidence for a role of muscle glycogen regulating the production of certain cytokines, has been demonstrated by Steensberg et al (211). They showed an inverse relationship between pre-exercise glycogen levels and exercise-induced IL-6 production in muscle and concentrations in the blood. Some studies also point to a relationship between changes in epinephrine and the cytokine IL-6 (43;131;203), but the exercise induced increase in IL-6 cannot be fully mimicked by epinephrine infusions (212). In sum, evidence for a mechanistic role of muscle glycogen content on the neuroendocrine and cytokine responses to endurance exercise is accumulating, and low glycogen stores may have been the major contributor to the augmented hormone and cytokine responses observed in connection with the second bout of exercise in the present study (166).

The increased neutrophil and lymphocyte counts during the second bout of exercise could also at least in part be explained by augmented levels of catecholamines, GH and cortisol, which again may be a result of decreased CHO availability. In support of this contention, two studies have found reduced neutrophil counts during and immediately after prolonged exercise with CHO as opposed to placebo supplementation (15;150). Moreover, Nieman et al also found reduced exercise induced lymphocyte and NK cell concentrations as well as reduced post-exercise lymphopenia with CHO supplementation vs. placebo during 2,5 h running (150). These changes were associated with increased levels of blood glucose and decreased levels of cortisol, which supports the view that CHO availability in the working muscle has a significant impact on the concentrations of circulating leukocytes during and after prolonged exercise. In the present study we observed altered NK cell *function* in the form of a larger reduction in activated CD56+ cells, estimated as the % of cells expressing the CD69 marker, in trial TWO compared with trial ONE. As an indicator of the functional capacity of neutrophils, the respiratory burst response of these cells was analyzed by chemiluminescence in a parallel investigation (21) (data not shown). The oxidative potentials per litre of blood, obtained by combining chemiluminescence values and cell numbers, yielded significantly higher values during and after Ex-A in trial TWO compared with trial ONE. This indicates that certain functional capacities of neutrophils and NK cells are affected by a previous bout of exercise on the same day. However, it is not possible to ascribe this effect to a particular factor. Bishop et al demonstrated reduced elastase release from stimulated neutrophils after an exercise trial with CHO supplementation compared with placebo (15), whereas and Nieman et al observed that their NK cell activity measurements were not influenced by CHO supplementation (150).

The more pronounced alterations in metabolic functions during and after Ex-A in trial TWO compared with trial ONE in the present study may also be linked to differences in glycogen levels in the liver and working muscles. As pointed out earlier, the lower mean RER found during Ex-A in trial SHORT compared with trial ONE --reflecting an increased reliance on fat oxidation-- indicates reduced intramuscular CHO availability, for which glycogen is the main source (37;91). Further evidence for glycogen effects on muscle substrate turnover has been provided by Weltan et al, who demonstrated that subjects with low pre-exercise glycogen stores showed increased fat oxidation during subsequent exercise compared with subjects having normal glycogen stores (243). Since the energy equivalent of O_2 (i.e. ATP yield) is lower when oxidizing fat compared with CHO, increased rate of fat oxidation during the second bout of

exercise can partly explain the elevated O_2 uptake and O_2 drift during Ex-A in trial TWO compared with trial ONE.

A 100% higher EPOC and elevated HR during the first 5 h of recovery after the second bout of exercise in trial TWO compared with the first bout in trial ONE also occurred when RER was lowered. A shift in substrate availability and increased fat oxidation could therefore in part explain the increased post-exercise metabolism during this period. An increased rate of energy demanding TG/FFA cycling (re-esterification of FFA) has also been shown to contribute to EPOC, particularly the prolonged component (7), whereas replenishment of O_2, and phosphagens, removal of lactate, increased circulation and ventilation and increased body temperature have been suggested to contribute to the rapid component of EPOC only (18). We observed a larger increase in body temperature during Ex-A in trial TWO compared with trial ONE, which could also be linked to the increased cytokine production seen during the second bout of exercise. Cytokines like IL-1 and IL-6 are known to induce elevated body temperature (48). Thus, increased concentrations of IL-6 and IL-1ra (reflecting the production of IL-1) observed during Ex-A in trial TWO, could have contributed to the larger increase in T_R during the second bout of exercise.

Based on the augmented levels of E, NE, ACTH, cortisol and GH as well as IL-6 and IL-1ra responses observed in connection with the second bout of exercise, it may be suggested that both the sympatho-adrenal system and the hypothalamo-pituitary-adrenal (HPA) axis as well as some cytokine pathways and immune cell functions have been triggered into a state of altered responsiveness during the first hours after prolonged, strenuous exercise. The occurrence of such alterations have also been discussed in the literature (229)(67;163). However, since the augmented responses in the present study were observed after the variables had retuned to baseline values between the two exercise sessions, the increased levels are not simply a "carry-over" effect of altered concentrations from the first exercise session. Rather, the present findings indicate that the signal systems involved in regulating several endocrine, immune and metabolic functions may be affected for a longer period of time after exercise than the blood concentration of the initial signal molecule may imply.

Apart from glycogen depletion, another possible explanation of our results may be found in altered responsiveness in the signal system involved in immunoendocrine and metabolic

functions. Modulation of the signal transduction cascade can occur anywhere from the initial step of the signal molecule-receptor binding to the ultimate change in protein synthesis that will lead to a specific biological response (241;254). Several studies have demonstrated an effect of acute exercise on adrenergic receptor sensitivity in different tissues, showing a decreased β-adrenergic sensitivity in different tissues and cells during the post-exercise period (19;39;59;59;60;141;215). Thus, the continuous stimulation of adrenoceptors and other neuroendocrine receptors during the first bout of exercise in trial TWO, may have affected the responsiveness of the receptor signaling system during the subsequent rest period and possibly all the way into the next exercise session. If the ultimate biological response to a specific signaling pathway is down-regulated, a possible compensatory mechanism could be to increase the production of the original signaling molecule in the pathway. Thus, higher secretion of catecholamines or other neuroendocrine factors may be a mean to achieve the same physiological response in the muscle and other tissue under high metabolic demands during the second bout of exercise. However, the present study was not designed to study receptor sensitivity, and the suggested mechanism needs to be tested in further studies.

5.2.3. Conclusion and implications

The present study hypothesized that a previous bout of strenuous endurance exercise would result in augmented immune, endocrine and metabolic responses to a subsequent exercise bout the same day. The findings in this investigation show that several immune, endocrine and metabolic exercise responses are more pronounced when a previous exercise session has been performed on the same day compared with no prior exercise. Thus, within the limits of this study the hypothesis is confirmed.

It is important to emphasize that the more pronounced changes in endocrine, immune and metabolically related variables observed during and after the second exercise session cannot be interpreted as either beneficial or detrimental to the athlete's health or performance. Increased stress reactions during and after a second bout of exercise could be an essential stimulus to achieve adaptive physiological changes in the various tissues and systems challenged during a second bout of exercise (77;202). More specifically, increased levels of signal molecules like hormones and cytokines can initiate augmented intracellular signalling, leading to increased activation of specific genes and subsequent altered protein synthesis (88;229). A change in protein expression inside a single cell is always a prerequisite for altered regulation and function

in a tissue (81;254). Thus, the desired adaptive outcome of an exercise session may need an above-normal physiological stress reaction, as was the case in the present investigation.

It is conceivable that an exercise stimulus (one single or repeated sessions) may be too large to elicit positive adaptive changes in various biological functions(44;63;136;202;232). We observed the athletes during a 24 h period and demonstrated an increased resting metabolism 14 h after the completion of the last exercise bout. Other studies have demonstrated that glycogen repletion is not completed from one day to the next with one daily exercise session consisting of 70 min running on 75-80% of VO2 max (37). This suggests that complete metabolic homeostasis may not be reached on consecutive days with two daily exercise sessions. Consequently, variation in exercise intensity, duration and mode should be carefully considered when composing a training program for elite endurance athletes with frequent exercise sessions (72).

Some implications of a more practical nature to athletes, coaches and sports scientists may be drawn from this study. When the total training load over a given period is planned, it is important to account for the interaction effect of a previous bout of exercise and not simply add up the stress load of single bouts of exercise (155). The results from the present study suggest that the total stress load of repeated exercise is more than the sum of stress from each individual training session. Integration of this knowledge into a well-composed periodization of the total training load could possibly prevent some cases of sustained fatigue and reduced performance in athletes (26;44). Finally, since heart rate was significantly higher after the second compared with the first bout of exercise, this indicates that heart rate is sensitive to the extra stress load that the second bout of exercise represents in these athletes. If measurements are performed under standardized situations, heart rate monitoring could therefore be a simple and reliable indicator of the progress in recovery after strenuous exercise.

6. Study IV: The impact of variation in recovery schedules between two daily exercise sessions on the responses to a second bout of exercise (papers 5, 6 and 7).

6.1. Results

The main results from the present study are illustrated in figure 6.1 and the statistical comparisons between trial SHORT with 3 h of rest and trial LONG with 6 h of rest are given in table 6.1. Regarding the hormone analysis, we found more pronounced changes in plasma concentrations of E, NE, ACTH, cortisol and insulin during and immediately after the second bout of exercise (Ex-A) in trial SHORT compared with trial LONG (figures 6.1a-e and table 6.1). Although there was a significant increase in serum GH, TSH, total testosterone, F- testosterone (total testosterone / SHBG) and decrease in F-T4 during the second bout of exercise, there were no differences in the concentration changes between trials SHORT and LONG for these hormones (table 6.1).

With regard to the immune-related variables we found higher concentrations in neutrophils (figure 6.1i) and lower CD4+ cell counts during Ex-A in trial SHORT compared with LONG (table 6.1). In the subsequent recovery period we observed a larger decrease (below baseline) in the total number of lymphocytes (figure 6.1j), including the CD4+ cells, in trial SHORT compared with LONG. For IL-6, there was only a trend towards higher concentrations during and after Ex-A in trial SHORT (table 6.1). Among the variables related to aspects of exercise metabolism we observed higher mean oxygen uptake, lower mean RER, a larger increase in rectal temperature and slightly higher HR during the second bout of exercise in trial SHORT compared with trial LONG. However, during the recovery period there was no significant difference in metabolic responses between trials SHORT and LONG (table 6.1).

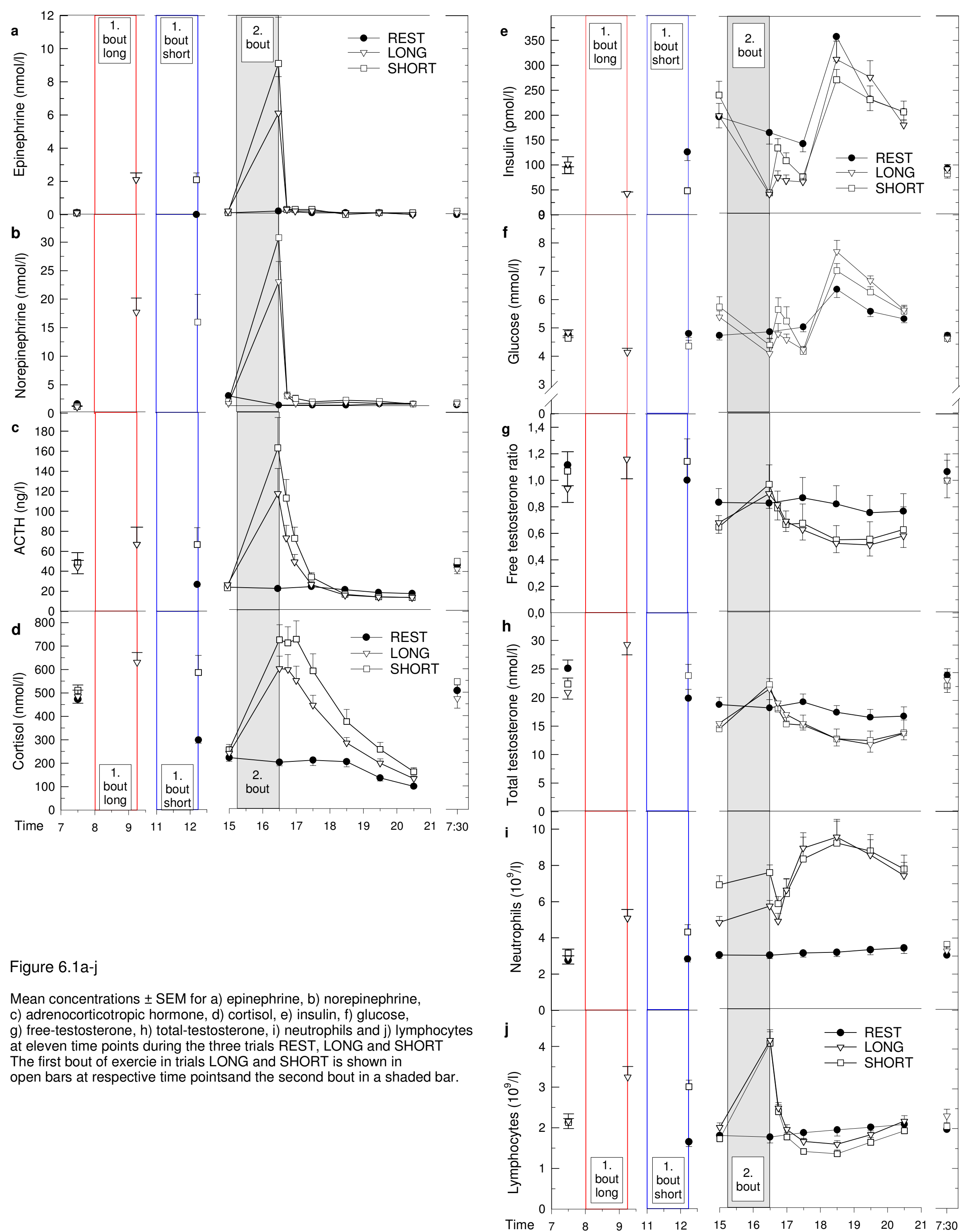


Figure 6.1a-j

Mean concentrations ± SEM for a) epinephrine, b) norepinephrine, c) adrenocorticotropic hormone, d) cortisol, e) insulin, f) glucose, g) free-testosterone, h) total-testosterone, i) neutrophils and j) lymphocytes at eleven time points during the three trials REST, LONG and SHORT The first bout of exercie in trials LONG and SHORT is shown in open bars at respective time pointsand the second bout in a shaded bar.

Table 6.1 Results of repeated measures ANOVA statistics for the comparison between trial SHORT and trial LONG for each of the listed variables are given in this review table. Analyses of the changes occurring during Ex-A and subsequent 14h recovery period, as well as for the combined period of Ex-A and recovery (15:00-07:00 next morning) were performed and are listed in separate columns (EXERCISE, RECOVERY and EXE + REC). Main effects of trial or interaction effect of trial by time are reported. Significantly higher levels of the variable in trial SHORT compared with trial LONG are indicated with ↑, significantly lower levels are indicated with ↓, and no difference between the trials with =, using an alpha level of 0.05. A trend (p=0.050-0.099) towards a difference between the two trials is indicated with (↑) or (↓). Where no measurement during exercise or recovery was performed, the space is left blank in the respective column. The exact p-levels for each variable are found in the original publications (papers 5, 6, and 7). The abbreviations used in the table are explained separately at the front of the thesis (see abbreviations).

TRIAL SHORT VS. LONG

Biological variable	Exercise	Recovery	Exe + Rec
Epinephrine	↑	↑	↑
Norepinephrine	↑	(↑)	↑
ACTH	↑	↑	↑
Cortisol	↑	↑	↑
GH	=	=	=
Insulin	↓	(↓)	↓
IGF-1	=	=	=
FSH	=	=	=
LH	(↓)	=	=
Testosterone	=	=	=
TSH	=	=	=
F-T4	(↑)	=	=
Glucose	=	=	=
IL-1ra	=	=	=
IL-6	(↑)	(↑)	(↑)
Neutrophils count	↑	=	=
Lymphocytes count	=	↓	=
CD4+ cell count	↓	↓	↓
CD8+ cell count	(↑)	=	=
CD56+ cell count	(↑)	=	=
% CD4+CD69+ cells	=	=	=
% CD8+CD69+ cells	=	=	=
% CD56+CD69+ cells	=	=	=
CD69 FIR of CD4+ cells	=	=	=
CD69 FIR of CD8+ cells	=	=	=
CD69 FIR of CD56+ cells	=	=	=
Mean oxygen uptake	↑		
Oxygen drift	(↑)		
EPOC 0-5		=	
Mean HR Ex-A and HR Rec 1-5	(↑)	=	
Mean RER Ex-A and RER Rec 1-5	↓	=	
Rectal temperature	↑		
Mean Lactate	=		
Mean RPE	(↑)		

Table 6.2 shows the correlation between exercise-induced changes in hormones, including interleukin-6, and some of the leukocyte and metabolic variables measured in trial ONE from study III and trial SHORT and LONG from study IV. When correcting for inter-trial dependency in our 27 observations (25 d.f.), an r-value (from Pearson's correlation analysis) of >0.44 gives a p-value of <0.05. Thus, all correlations in the table showing r-values of >0.45 are statistically significant.

Table 6.2. Results of correlation analyses (Pearson's correlations coefficient) between changes from pre- to post-exercise (delta values) in hormones, including IL-6 and some of the leukocytes and metabolic variables measured during exercise and recovery in trials SHORT and LONG as well as in trial ONE from study III. The degree of inter-trial dependency for these observations was corrected according to the formula given in the result section in this chapter. All correlations in the table showing r-values >0.45 should be considered statistically significant and these are highlighted in bold. The abbreviations used in the table are explained separately at the front of the thesis, except for d = delta (see abbreviations).

Pre-post Ex-A	d-EPI	d-NE	d-GH	d-ACTH	d-CORT	d-INS	d-IL-6
EPI							**0,46**
NE							0,17
GH							**0,46**
ACTH							0,25
Cortisol							**0,58**
Insulin							0,27
Glucose	-0,18	0,10	-0,10	0,02	-0,03	**0,55**	0,29
Neutrophils	-0,14	0,28	-0,22	0,19	-0,31	-0,13	-0,16
Lymphocytes	**0,49**	0,26	0,34	-0,16	0,32	-0,01	**0,55**
CD56+	0,39	0,09	0,22	0,07	0,13	-0,36	0,39
Heart rate	0,18	0,18	0,32	0,37	0,51	0,24	0,15
Temperature	**0,49**	0,28	**0,63**	**0,63**	**0,49**	-0,13	0,19
O_2 uptake	0,39	0,15	0,43	0,33	**0,49**	0,02	**0,46**
O_2 drift	0,29	0,19	0,25	-0,03	0,22	0,10	0,18
RER	-0,21	-0,24	-0,13	-0,12	-0,23	0,25	0,09
Recovery							
d-neutrophils	**0,66**	0,26	0,54	0,31	**0,48**	0,09	**0,51**
d-lymphocytes	**-0,57**	-0,28	**-0,47**	-0,40	**-0,50**	0,06	**-0,53**
EPOC 0-5h	**0,22**	-0,02	0,16	0,28	-0,06	**0,47**	-0,08
HR 1-5h	**0,68**	0,34	**0,54**	**0,46**	0,45	-0,35	0,34
RER1-5h	-0,26	-0,32	0,01	-0,06	-0,06	0,33	0,16

6.2. Discussion

Several investigations have demonstrated that prolonged strenuous exercise strongly activates the neuroendocrine system (71;108;180) and that such neuroendocrine activation leads to substantial, yet temporary changes within the immune system (153;163). In study III we presented new data

that demonstrate additional alterations in neuroendocrine, immune and metabolic functions when the same bout of endurance exercise is repeated within a few hours. The main findings in study IV were that several hormones (EPI, NE ACTH and cortisol) and leukocytes (neutrophils and lymphocytes) showed larger perturbations when the second bout of exercise (Ex-A) was performed after a short compared with a long recovery period between the two exercise sessions. Furthermore O_2 uptake and T_R were higher and RER lower during Ex-A in trial SHORT compared with trial LONG. However, the difference in recovery time does not seem to have affected post-exercise O_2 consumption (EPOC) or substrate turnover (RER). In other words, increased recovery time between the first and second bout of exercise results in attenuated neuroendocrine, immune and metabolic responses during the second exercise session.

However, it is important to emphasize that we cannot ascribe the observed differences between trial SHORT and LONG to the time factor alone (i.e. the extra 3 h of rest given in trial LONG), because the subjects were also given an additional 1 MJ meal between the two exercise sessions in trial LONG. Nevertheless, recovery time and diet are both known to influence the degree of glycogen repletion after prolonged strenuous exercise, and restoring muscle glycogen after such exercise is a continuous process during the first 12-24 h of recovery (13;16;37;119;213). Thus, with only 3 h of rest and a single meal in trial SHORT, glycogen stores must have been lower, compared with trial LONG when the subjects were given more food and rest.

In study III we presented glycogen depletion as a plausible explanation for at least part of the more pronounced neuroendocrine, immune and metabolic responses observed in connection with the second as opposed to the first bout of exercise. In keeping with this hypothesis, it is possible that the observed differences in endocrine, immune and metabolic responses between trials SHORT and LONG are at least partly linked to a difference in glycogen levels before as well as during the second bout of exercise. Further support for this explanation is found in two studies by Weltan et al who examined the impact of different pre-exercise glycogen levels on metabolic regulation during subsequent exercise (242;243). They demonstrated higher concentrations of NE, lower concentrations of insulin, and a lower RER in subjects with low compared with high muscle glycogen levels prior to exercise. Our observations correspond with these findings and thus suggest a role for muscle glycogen in regulating several physiological responses during the second bout of exercise in our study.

To our knowledge there is only one investigation that has specifically examined the impact of different rest periods between the exercise sessions on immuno-endocrine or metabolic variables, resulting in two publications (98;244). The study used three bouts of 30 min exercise at 70% of maximal O_2 uptake separated by 1 h and 3,5 h of rest on two separate trial days. However, in contrast to our findings, they did not observe differences between their short and long rest trials in any of the metabolic or endocrine variables. Since the exercise and recovery protocols were different, it is difficult to compare the results.

6.2.1. Endocrine changes

EPI, and to a lesser degree NE, are important stimuli for increased energy substrate turnover in the form of muscle glycogenolysis and glycolysis (53;183;207), hepatic glycogenolysis and gluconeogenesis (34;249) and lipolysis in adipose and muscle tissue (25;209), The catecholamine effects are mediated directly through α- and β-adrenergic receptors in muscle, liver and adipose tissues, but also indirectly by regulating the secretion of other metabolically active hormones such as insulin (70;180). In the present study, the more pronounced increase in EPI during Ex-A in trial SHORT coincided with a larger decrease in insulin compared with trial LONG, which underlines the inhibitory effect of EPI on insulin. Perhaps the most important physiological effect of this EPI driven suppression of insulin is to reduce the inhibitory effect of insulin on hormone sensitive lipase (HSL) and thereby facilitate increased lipolysis during exercise (71). Such a shift towards increased fat oxidation becomes increasingly important when severe glycogen depletion is evident towards the end of prolonged exercise or in this case a second exercise session.

In chapter 3 (and paper 3) we argued that the more augmented catecholamine response observed in connection with the second bout of exercise compared with the first bout of exercise could be related to desensitization of adrenergic receptors in metabolically active tissues. Recently, beta-adrenergic receptor desensitization in human adipose tissue has been demonstrated during the first hours after a single bout of exercise (19)(123) and after epinephrine infusion (208). Changes in adrenergic receptors density and responsiveness have also been found after exercise in other tissues and cells like cardiac muscle (60), mononuclear cells (59) and neutrophils (39), as well as after exposure to adrenergic agonist in liver (177), smooth muscle cells of the vascular wall (215), and skeletal muscle (112).

Assuming a time dependent reduction in adrenergic desensitization during the post-exercise period, the difference in duration of rest between the two exercise sessions should therefore affect the degree of receptor desensitization during a second exercise session on the same day. Thus, it is reasonable to suggest that the more pronounced increase in EPI and NE levels in trial SHORT compared with trial LONG could in part reflect an increased desensitization in several tissues. Consequently, a stronger hormonal stimulus is needed to provide the same rate of substrate turnover through glycogenolysis, glycolysis, lipolysis and gluconeogenesis. Since also desensitization of corticotropic and 5-HT receptor of the hypothalamus have been demonstrated after exposure to specific receptor agonists (174;179), it is conceivable that a decreased receptor responsiveness caused by prior exercise may have contributed to the higher levels of ACTH and cortisol in trial SHORT than trial LONG in the same fashion as with catecholamines.

Hypoglycemia is a strong stimulus for increased EPI secretion (41;50;70). The decrease in blood glucose from pre- to post-exercise in both trial SHORT and LONG could thus have contributed to the substantial increase in catecholamines during the second bout of exercise. Yet, plasma glucose levels did not fall below 4 mmol/L at the end of Ex-A in any of the trials, which suggests that there was no severe hypoglycemia (71). Therefore, it is not likely that the observed reduction in plasma glucose contributed significantly to the large EPI response. Moreover, there was no correlation between the decrease in plasma glucose and increase in EPI from pre- to post-exercise (table 6.2), and we observed different pre- to post-exercise changes in EPI but similar changes in glucose in trials SHORT and LONG.

Several of the hormones with metabolic and/or immuno-modulating action that were measured in this study are bound to specific proteins in the blood compartment. However, it is the small free fraction of the hormone that exerts the biological effect on different tissues and cells by binding to specific receptors on the cell membrane or inside the cytosol, depending on its lipophilic or hydrophilic properties. Testosterone is a steroid hormone largely bound to the plasma protein SHBG, with only a small free fraction (F-testosterone) that is biologically active. Figures 6.1g and h illustrate that total testosterone and F-testosterone concentration are similarly affected by exercise, and different recovery protocols result in parallel changes in trials SHORT and LONG. Thus, exercise-induced changes in total testosterone seem to reflect concomitant changes in the biologically active fraction of the hormone.

6.2.2. Metabolic changes

The more pronounced alterations in the metabolic variables during the second bout of exercise in trial SHORT could in part reflect a more severe glycogen depletion with a subsequent shift towards increased fat oxidation compared with trial LONG. A lower RER during Ex-A in trial SHORT than LONG is in itself indicative of a greater reliance on fat oxidation. This shift in substrate turnover towards utilization of less CHO fuels and more fat fuels will lead to a higher metabolic cost in the production of ATP. Our observation of a higher mean O_2 uptake in trial SHORT compared with trial LONG supports this argument. Thus in short, the presumably lower glycogen content prior to the second bout of exercise in trial SHORT was followed by a proportionally greater oxidation of fat fuels (as evidenced by the lower RER), which has caused a higher metabolic rate (as evidenced by the increased mean oxygen uptake).

Since the rise in core temperature is strongly linked to the rate of energy turnover during exercise under otherwise stable environmental conditions (132), it is likely that the larger increase in T_R in trial SHORT compared with trial LONG is a reflection of the increased metabolic rate during Ex-A in this trial (252). From the correlation analysis between changes in hormones and metabolic variables (table 6.2), we found that the rise in T_R was associated with the exercise-induced increase in EPI, GH, ACTH, and cortisol. Of these hormones, it is only GH that has been causally linked to a rise in core temperature (99;176). It is acknowledged that the correlations shown in table 6.2 are purely explorative. However, having made the proper correction for inter-trial dependency (see chapter 2), we find it is justified to use the opportunity to explore our data in this manner and thereby shed some light on possible links between changes in variables reflecting different physiological functions.

6.2.3. Leukocyte changes

The changes in neutrophil and lymphocyte concentrations during exercise and recovery in trials SHORT and LONG (figures 6.1i-j) followed the general pattern of cell trafficking described in chapter 5. However, a novel finding from the present study was the more pronounced neutrophilia during the second bout of exercise and a more pronounced lymphocytopenia during the subsequent recovery period in trial SHORT compared with trial LONG. It is evident from figure 6.1i, that the higher concentrations of neutrophils during Ex-A are preceded by elevated levels of these cells during rest prior to the second bout of exercise. Thus, it seems that the larger neutrophilia during Ex-A in trial SHORT compared with trial LONG is due to the residual

elevation of neutrophil concentrations from the first bout of exercise. A similar magnitude of increase in neutrophils in trials SHORT and LONG during Ex-A, coinciding with differences in neuroendocrine responses in these trials, also suggest that endocrine factors do not contribute substantially to the increased neutrophilia in trial SHORT. This does not necessarily imply that hormones like EPI, GH and cortisol have no role in the exercise-induced neutrophilia per se, because infusion studies at rest have confirmed the contribution of these hormones (100;224;227). However, the significance of this hormone-driven neutrophilia may be somewhat less during exercise than in under resting conditions. The results of the correlation analyses of all exercise trials given in table 6.2 also suggest that changes in EPI, GH, cortisol or any of the other measured hormones are not significantly related to the neutrophilia observed during exercise. An alternative explanation focusing on the impact of an exercise-induced increase in shear forces on the neutrophil concentrations in the vascular compartment has been discussed in chapters 1 and 5.

Since the concentrations of lymphocytes before Ex-A was similar in trials SHORT and LONG, it is clear that the larger lymphocytopenia found after the second bout of exercise in trial SHORT compared with trial LONG is not due to residual alterations in lymphocyte counts from the first bout of exercise. The correlation analysis of the concentration changes in neuroendocrine factors during Ex-A and the observed decrease in lymphocyte counts from 0-2 h post-exercise (lymphocytopenia) indicates that the efflux of lymphocytes from the blood after exercise may be related to increases in several hormones, including EPI, GH, cortisol as well as IL-6 (table 6.2). Previous infusion studies have so far confirmed a mechanistic role for increased cortisol levels in the efflux of lymphocytes from the circulation (51;227), but not for EPI, GH or IL-6. In contrast to the changes in the latter factors, increased cortisol levels also persisted for the entire period of the lymphocytopenia in trial SHORT and thus showed a temporal relationship with the changes in lymphocytes during recovery. This supports that cortisol was a major contributor to the more pronounced lymphocytopenia in trial SHORT compared with trial LONG. Furthermore, the post-exercise neutrophilia observed during the same time period also showed a significant correlation to the exercise-induced increases in EPI, GH, cortisol and IL-6 (table 6.2). Overall, this suggests that the changes in leukocyte trafficking in and out of the circulation during recovery --as opposed to during exercise-- may be related to the degree of change in several neuroendocrine factors from the preceding exercise session. However, it is important to underline that such correlations do not imply a causal relationship between changes in these neuroendocrine factors and leukocyte sub-populations.

6.2.4. Conclusion and implications

The present study hypothesized that the changes in neuroendocrine, immune, and metabolic variables elicited by the second bout of exercise would be more pronounced when the rest period between the exercise sessions was 3 h compared with 6 h. The findings of this investigation show that several immune, endocrine and metabolic responses are more pronounced during and after the second bout of exercise when the recovery schedule between the exercise sessions consists of a 3 h short rest and a single meal compared with a 6 h long rest and two meals. Thus, within the limits of this study, the hypothesis is confirmed.

In keeping with the hypothesis that augmented stress responses may be beneficial and/or detrimental to training adaptation, it is not possible to determine which of the two recovery protocols (SHORT or LONG) that may be recommended. On one hand, if the purpose of a second exercise session on the same day is to avoid augmented stress responses in the athlete, a minimum of 6 h of rest and two meals should perhaps be suggested. On the other hand, if the aim is to create such augmented stress reactions, a short 3 h rest including only one meal between the exercise sessions may be favourable. Thus, incomplete recovery may be a method of initiating desired training adaptations and ultimately improved performance in an athlete. Further research should aim at what methods and measures that may have the potential to enhance recovery after a second bout of exercise, but also how different recovery regimes between daily exercise sessions may affect the adaptation to training on a cellular level (3;38;83;251).

The results of this study suggest that complete homeostasis of a functional system like the neuroendocrine, may not be evaluated on the basis of measuring plasma concentrations of its signal molecules. In practical terms, this means that great caution should be employed when interpreting the biological significance of concentrations of hormones, cytokines and other stress sensitive components in blood tests from athletes. Normalization of such variables after exercise does not necessary imply that all recovery processes have been completed (239).

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