Physiological responses to acute physical and psychosocial stress

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Physiological responses to acute physical and psychosocial stress-

relation to aerobic capacity and exercise training

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Abstract

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Exercise training is an effective method to promote health and to prevent development of disease. Both physical and mental health have been shown to benefit from exercise training. It has also been speculated that physical exercise might affect responses to acute psychosocial stress. In an acute stress situation, several physiological systems respond to ensure survival and it is suggested that exercise training may influence these stress systems.

The main purpose of this thesis was to study physiological responses to acute physical and psychosocial stress and possible associations with aerobic capacity and exercise training. The thesis is based on four papers analysing data from a randomized controlled trial (RCT). The participants were healthy individuals who reported themselves as untrained at screening. The RCT included testing of acute physical and psychosocial stress. Before and after the tests, hormonal and autonomic responses were assessed. After initial testing, the participants were randomized to either an intervention- or a control group. The intervention consisted of regular aerobic exercise training conducted for six months. At follow-up, the same tests were repeated for both groups.

The main findings were that most participants showed an increase in the studied variables in response to acute stress. Aerobic capacity did not seem to have any relation to hormonal or blood pressure responses to acute psychosocial stress. Neither did the subjective perception of stress at the psychosocial stress test correlate with the actual physiological response. Due to methodological issues, it was not possible to evaluate the effects of exercise training.

Thus, in healthy individuals, the stress systems seem to respond adequately to acute stress, irrespective of level of aerobic capacity or type of stressor.

Svensk sammanfattning

Fysisk träning är ett av våra viktigaste redskap för att bibehålla hälsa, och bevisen för dess välgörande effekter är många. Flera av våra vällevnadssjukdomar är möjliga att förebygga, såsom hjärt-kärlsjukdom och diabetes typ II. Det har även spekulerats i huruvida fysisk träning kan påverka kroppens svar på akut psykosocial stress.

Stress är ett växande samhällsproblem och allt fler individer upplever ökade nivåer av stress. Våra kroppar är emellertid väl rustade för att hantera akuta stressreaktioner. Flera olika system borgar för att upprätthålla kroppens inre balans och att livsviktiga funktioner vidmakthålls.

Syftet med denna avhandling var att studera fysiologiska reaktioner på akut fysisk och psykosocial stress, och om/hur dessa relaterar till och påverkas av aerob kapacitet och fysisk träning. Genom fyra delstudier har olika aspekter av ämnet belysts. Samtliga delstudier bygger på data från en randomiserad kontrollerad studie (RCT) genomförd på Institutet för Stressmedicin i Göteborg. Vi sökte otränade och friska kvinnor och män i åldern 20 till 50 år. Studien innefattade baslinjemätningar bestående av ett maximalt uthållighetstest på ergometercykel samt ett standardiserat psykosocialt stresstest. Före och efter de båda testerna mättes hormonerna adrenokortikotropt hormon (ACTH), kortisol, dehydroepiandrosteron (DHEA) och dehydroepiandrosteronsulfat (DHEA-S). Autonoma reaktioner mättes som systoliskt och diastoliskt blodtryck samt hjärtfrekvens. Efter de inledande mätningarna randomiserades deltagarna till antingen en interventionsgrupp eller en kontrollgrupp. Interventionsgruppen skulle under ett halvår komma ingång med regelbunden konditionsträning, samtidigt som kontrollgruppen skulle fortsätta på samma aktivitetsnivå som tidigare. Efter sex månader genomfördes samma tester som vid baslinjemätningarna.

Delstudie I är en beskrivning av själva studieprotokollet, men innehåller också en metodologisk diskussion om två avgörande aspekter i genomförandet av en RCT. Den första aspekten är kopplad till ett av inklusionskriterierna, att deltagarna skulle vara otränade vid tidpunkten för inklusion. Det visade sig att konditionsvärdena (mätt vid ett maximalt konditionstest) var högre än väntat utifrån den självskattade aktivitetsnivån som rapporterats vid screeningtillfället. Den andra aspekten var den fysiologiska responsen vid akut stress, där en minskad respons var förväntad för de deltagare som genomgått träningsinterventionen. Förutsättningarna för att kunna observera en sådan nedgång var en synlig respons vid den första mätningen, men för 13 av deltagarna kunde ingen positiv respons påvisas.

Delstudie II jämförde fysiologiska reaktioner vid akut fysisk och psykosocial stress. Den hormonella responsen samt pulsreaktionen var högre vid fysisk stress än vid psykosocial stress, medan det omvända gällde för systoliskt och diastoliskt blodtryck. Det fanns en korrelation mellan kortisolresponsen vid fysisk och psykosocial stress, det vill säga att de deltagare som reagerade med hög kortisolrespons under det fysiska testet reagerade med hög respons även under det psykosociala testet. Däremot sågs inget samband för autonoma reaktioner eller vid vilket tidpunkt högsta värdet inträffade. Inget samband kunde heller fastställas mellan hur stressande testet upplevts och storleken på den fysiologiska reaktionen.

Delstudie III presenterade resultat från de uppföljande mätningarna, där den fysiologiska responsen på akut psykosocial stress jämfördes mellan interventions- och kontrollgruppen. I interventionsgruppen ökade konditionsvärdet signifikant (+9,5 %) jämfört med baslinjemätningen, samtidigt som konditionsvärdet i kontrollgruppen minskade (-3 %). Båda grupperna fick en minskad reaktion på stresstestet, vilket tyder på att deltagarna fått en tillvänjning till testet och/eller testsituationen. Det går därför inte att uttala sig säkert om effekterna av träningsinterventionen.

Delstudie IV innehöll tvärsnittsanalyser av DHEA och DHEA-S och dess samband med aerob kapacitet, och också hur respons i DHEA och DHEA-S på akut psykosocial stress påverkas av aerob kapacitet. Inga samband kunde dock ses.

Sammanfattningsvis kan sägas att det inte verkar finnas något samband mellan aerob kapacitet och hormonell respons vid akut fysisk och psykosocial stress. Det verkar inte heller som att upplevelsen av en stressituation är avgörande för den fysiologiska responsen. Hos friska individer ser stressystemen ut att fungera väl, oavsett vilken aerob kapacitet individen besitter. Resultat från tidigare studier har visat på varierande resultat gällande effekterna av aerob kapacitet på akuta stressreaktioner, och denna avhandling adderar således till gruppen av studier som inte kunnat påvisa samband. Fler longitudinella studier av hög kvalitet är dock önskvärda för att med säkerhet kunna fastställa resultaten i denna avhandling.

Contents

Tables and figures

Included publications and manuscripts

One paper included in this thesis has been published and is reprinted with permission from the publisher.

I. Arvidson E, Sjörs A, Gullstrand L, Börjesson M, Jonsdottir IH. Exercise training and physiological responses to acute stress: study protocol and methodological considerations from a randomized controlled study. BMJ Open Sport & Exercise Medicine 2018;4: e000393. doi:10.1136/bmjsem-2018-000393

II. Arvidson E, Sjörs A, Gullstrand L, Börjesson M, Jonsdottir IH. Physiological responses to acute physical and psychosocial stress in healthy women and men (In manuscript).

III. Arvidson E, Sjörs A, Gullstrand L, Börjesson M, Jonsdottir IH. The effects of exercise training on HPA-axis reactivity and autonomic response to acute stress – a randomized controlled study. (Submitted)

IV. Arvidson E, Börjesson M, Jonsdottir IH, Lennartsson A. DHEA and DHEA-S response to acute psychosocial stress and the relation to aerobic capacity in healthy women and men (In manuscript).

Abbreviations

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
AUC_i	Area under the response curve with respect to increase
Bpm	Beats per minute
DBP	Diastolic blood pressure
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
HR	Heart rate
ISM	Institute of Stress Medicine
RCT	Randomized Controlled Trial
SBP	Systolic blood pressure
TTE	Time-to-exhaustion
VO_2 peak	Peak oxygen uptake
W	Watts

Background

Exercise training is known as one of the most effective ways to promote overall health and well-being (1). Regular exercise training can contribute to the prevention and treatment of many common diseases, such as cardiovascular disease (2), diabetes type II (3, 4) and stroke (5). Exercise training has also been shown to have positive effects on mental health (6) and is used to treat mild to moderate depression (7) and long-term stress (8).

Feeling stressed has become a common part of everyday life in Western societies. In both working life and private life, the possibility of being constantly online and available is only one of many factors that probably has contributed to the increased stress levels. However, the human body is well equipped to physiologically respond to stress, which is essential for survival. Several systems act to prepare the body and mobilize energy during stressful situations. Two of the most commonly studied systems are the hypothalamic-pituitary-adrenal (HPA) axis, acting through the release of the catabolic hormone cortisol, and the autonomic nervous system (ANS), which, among other things, increases heart rate and blood pressure. Two other hormones that also respond to acute stress are dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S). In contrast to the catabolic effects of cortisol, DHEA and DHEA-S have anabolic effects.

Definition of stress

What is stress?

First of all, *"stress"* is a difficult concept. The word "stress" is commonly used in everyday life, and interpretations vary considerably. But what does "stress" mean? Is there a clear definition of the word? There may actually be several answers to these questions. Outside research, stress is often understood as synonymous with having too much to do or being short of time. In this case, the cause of stress, the so-called "*stressor*", is in focus. The stressor might be having a tight time-schedule, having too many work tasks or being in a traffic jam on the way to a meeting. Stress can also be described in terms of a feeling, reflecting how an individual perceives a stressful situation. It can include feelings of anxiety or fear (9, 10). In research, though, the emphasis can be on physiological responses and bodily reactions to stress (11). For example, it is common to assess physiological responses to stress as hormonal reactions or cardiac reactivity (12).

But why is it necessary to define stress? Because clear definitions and common interpretations of concepts are important to ensuring clear communication in research. During the last 100 years, several scientists have tried to find a unifying definition of stress, but the word is still used differently in different research areas. The well-known stress researcher Hans Selye, who in the 1920s contributed to developing the stress concept, defined stress as

> a nonspecific response of the body to any demand made upon it.

Since then, this definition has been modified several times, often based on the orientation of the research. More recent adjustments have included additional aspects, as in Dhabhar and McEwen's 1997 definition:

> An integrated definition states that stress is a constellation of events, consisting of a stimulus (stressor), that precipitates a reaction in the brain (stress perception), that activates physiological fight or flight systems in the body (stress response)(13).

This definition considers not only the unspecified response that is central in Selye's definition but also the mental process involved. But while Selye describes a more general stress response, the later explanation more clearly relates to the instant stress response, or as it is more commonly termed, *"acute stress"*.

In everyday life, situations that can elicit an acute stress response of varying degrees might occur on a daily basis. This include public speaking, running to catch a bus, getting stuck in an elevator, and discovering that there is not enough money in one's account when trying to pay in a store. The physiological responses are often immediate, with increased heart rate and blood pressure as the most noticeable effects (14). These reactions aims to preserve *"homeostasis"*, defined as the maintenance of a steady state of body fluids, circulation, blood pressure, and a number of other variables (15). Sterling and Eyer (16) called the physiological adaptations to a new situation *"allostasis"* which they described as

> active processes by which the body responds to daily events and maintains homeostasis (16).

For the most part, acute stress reactions are not threatening to our health because of the body's physiological ability to cope with stressful situations. However, if the stressful situations are frequent and continue for a longer period, the systems might not have a chance to recover and the risk of deteriorating health increases (17, 18). This condition is often defined as *"long-term"* or *"chronic stress"* although the terms might differ depending on the research field. McEwen used the term *"allostatic overload"* to describe the condition in which the body fails to turn the systems involved on and off adequately (19). This thesis will focus on the acute stress reaction only. To learn how to prevent and treat the effects of long-term stress in patients, it is necessary to increase our understanding of how healthy individuals respond to acute stress. Theoretically, if it is possible to affect the physiological response to acute stress, it may also be possible to affect the degree of stress developed over time by reducing the pressure on the stress systems.

Stress physiological systems

Several bodily systems react to stress. The initial stress response starts in the brain, which is the central organ for the stress reaction (19). After evaluating the situation, necessary interventions are initiated (19). The allostatic processes activate many reactions, such as neuroendocrine and autonomic responses (20, 21). Here, the two main response systems will be studied, namely the HPA axis and the ANS. The thesis also includes studies of the anabolic hormones dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S).

Hypothalamic Pituitary Adrenal axis

One of the most important systems involved in the acute stress reaction is the HPA-axis. The response is relatively slow compared to the autonomic nervous system in that it takes some minutes after exposure to a stressor before a response is detected. At the onset of stress, corticotropin-releasing hormone (CRH) is released from the hypothalamus, stimulating the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary into the blood. ACTH, in turn, triggers the release of cortisol from the adrenal cortex. This chain of reactions is regulated by negative feedback: that is, sufficient levels of cortisol in the blood will decrease the release of CRH in the brain (20)(see figure 1).

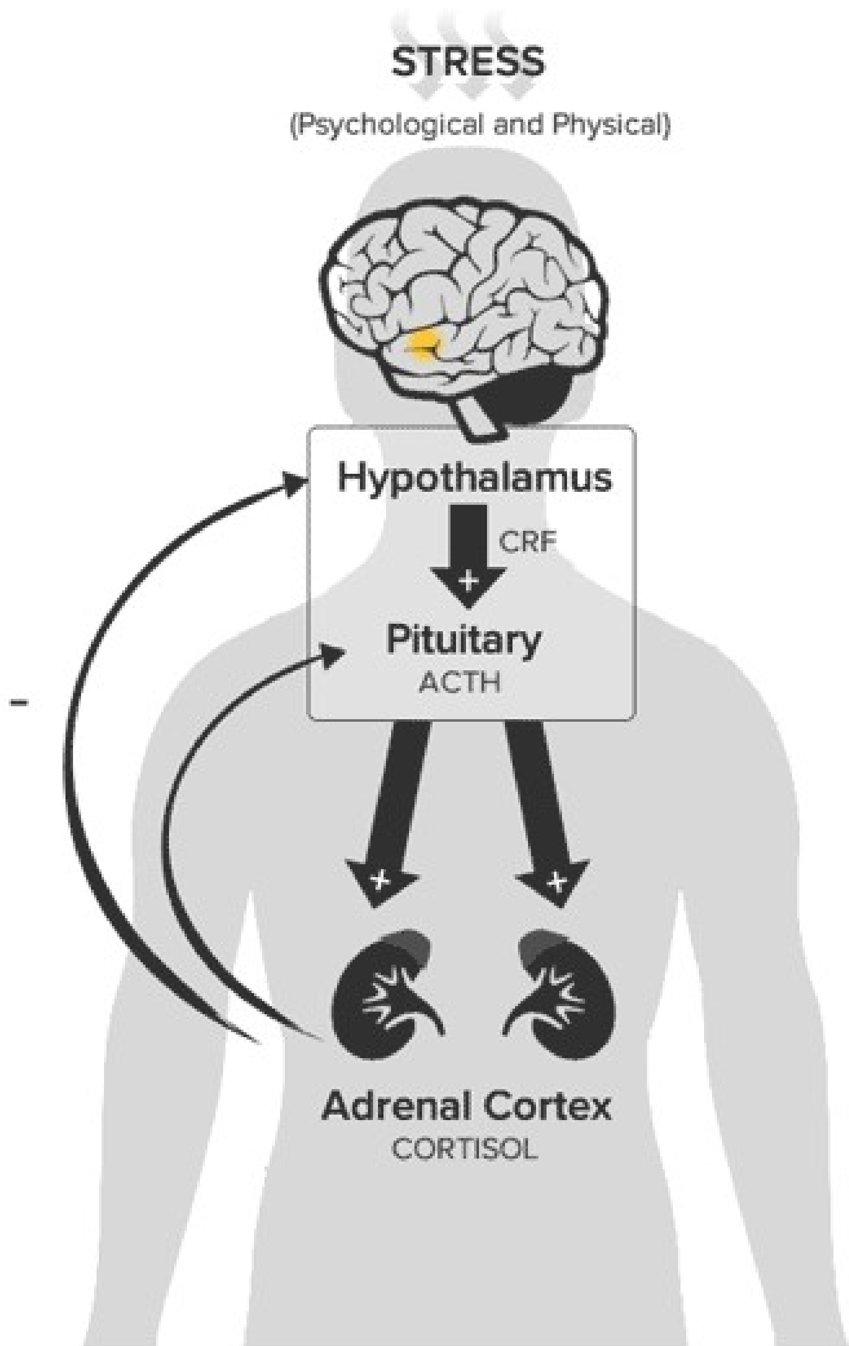
Cortisol is considered an important stress hormone due to its different features to adjust bodily functions during acute stress, in order to preserve homeostasis. The increased metabolic demands are regulated through the stimulation of gluconeogenesis (breakdown of lipids to glucose) that increases blood glucose concentrations. Cortisol also promotes mobilization of fatty acids from adipose tissues as well as the breakdown of protein to mobilize energy for the acute muscular stress reaction (22), and it is therefore defined as a catabolic hormone.

The levels of cortisol vary throughout the day in a circadian rhythm, with the lowest level seen during the night, the highest level before awakening and declining levels throughout the day. During stressful events or acute exercise, the levels rise and the time to recover is dependent on the duration and intensity of the stressor, which also determines the magnitude of the cortisol response (22). Seasonal variations have been observed indicating higher levels of salivary cortisol on awakening during winter and lower levels in the late summer (23, 24). However, the populations in these studies were small, and the studies included no information whether acute cortisol responses during the day were also affected. Thus, more studies are needed to confirm the results.

Prolonged activation of the HPA-axis, without enough recovery time, has been shown to lead to heightened basal levels of hair cortisol (25, 26). Elevated cortisol levels are thought to have neurotoxic effects, especially in a part of the brain called the hippocampus, which is important for learning and cognition (19, 27).

Figure 1.

Schematic picture of the hypothalamic-pituitary-adrenal axis



CRF: Corticotrophin releasing hormone, ACTH: Adrenocorticotrophic hormone

Autonomic nervous system

The ANS is largely controlled by areas in the spinal cord, brain stem and hypothalamus, and it controls subconscious functions in visceral organs (21). In contrast to the HPA-axis, communicating through the bloodstream, the ANS sends signals through afferent and efferent nerves, which results in a much more rapid response compared to the HPA-axis. When the ANS responds to a stressor, it acts through activation of the adrenal medulla and the sympathetic and parasympathetic nervous systems (28). The sympathetic activation in response to acute stress, also referred to as the "fight-and-flight" response, has the capability to respond within seconds of the emergence of a stressor. It increases heart rate, blood pressure and frequency of breathing (20), and also redistributes blood from the general circulation and gastrointestinal areas to the muscles. Additionally, the coagulation capacity in the blood increases in case of potential damage and blood loss. These actions prepare the body to either fight against or flee from the potential danger (21). The parasympathetic nervous system can act through a "playing dead" or "freeze" reaction to avoid attention (14).

Allostatic overload in the ANS often depends on increased activity in the sympathetic nervous system with a simultaneous decrease in parasympathetic activity (19). This might lead to insufficient recovery in the affected organs, such as the heart and blood vessels. Long-term stress has been associated with an increased risk of developing hypertension and stroke (29, 30).

Dehydroepiandrosterone

DHEA and DHEA-S are, in contrast to cortisol, endogenous anabolic steroid hormones released from the adrenal cortex, also in response to ACTH. They act as precursors of testosterone and oestrogen and have been shown to have anti-glucocorticoid and neuroprotective effects (31). DHEA and DHEA-S also play an important role for nerve growth and are suggested to have both anti-oxidative and anti-inflammatory properties (32). One anabolic effect of DHEA and DHEA-S is an increase in the synthesis of protein.

Unlike DHEA, no circadian rhythm is seen for DHEA-S, probably because of its larger quantities and slower clearance rate (33). DHEA and DHEA-S are the most abundant hormones in the body, highly dependent on sex and age, with men having higher levels than women (34). The highest levels are seen in early adulthood; thereafter, they decline every year and are only at 20 % of peak values by the ages of 65-70 years.

It has previously been shown that prolonged stress leads to decreased levels of DHEA-S, and to attenuated levels of DHEA-S in response to acute psychosocial stress (35, 36).

Different types of stressors

It is important to distinguish not only between acute and long-term stress, but also between different types of stressors. The general perception of "stress" often emphasizes the mental aspect. However, stressors can be of several origins. In this thesis, two types of stressors will be studied: psychosocial and physical stressors. Both types of stressors elicit a reaction of the stress systems and the physiological reactions share many similarities, although the physical demands often diverge.

Psychosocial stress

In 2015, Kogler et al. defined psychosocial stress as follows:

> Psychosocial stress is induced by situations of social threat including social evaluation, social exclusion and achievement situations claiming goal-directed performance (37).

This definition includes the mental strain experienced in social interactions, especially in socially demanding situations. Psychosocial stress has been evaluated with several different methods, including the Montreal Imaging Stress Task, developed to study responses in the brain with functional magnetic resonance imaging or positron emission tomography (38), and the Trier Social Stress Test (TSST)(39), which is used in this study (see description of the test in the Methods section, p. 38). In the Results and Discussion sections of this thesis, descriptions of reactions to acute "psychosocial stress" are referring to responses to the TSST.

Physical stress

Physical stress can be triggered by a number of factors, for example a cold pressure test (40) or electric foot shock (used on animals) (41). In this thesis, acute physical stress is elicited using an exercise test performed to exhaustion. The most prominent difference between psychosocial and physical stress is the bodily demands, among them metabolic requirements. During exercise, the muscles and tissues need to be supplied with oxygen and nutrients to maintain homeostasis. This is executed by the release of hormones that, in turn, increase the heart rate to provide the working muscles with energy and increase breathing to meet the demands of oxygen supply (42). Throughout this thesis, "physical stress" refers to responses to an acute exercise bout.

Aerobic capacity

Aerobic capacity can be defined as:

> the maximal amount of physiological work that an individual can do as measured by oxygen consumption. It is determined by a combination of aging and cardiovascular conditioning and is associated with the efficiency of oxygen extraction from the tissue (43).

Aerobic capacity is ideally assessed as peak oxygen uptake (VO_2 peak) (44), achieved by an exercise test that includes gradual increase in resistance and is performed to exhaustion (45).

Another plausible method to assess aerobic capacity is to use submaximal tests and estimate a peak value (46, 47). However, a submaximal test can never be as reliable as a peak performance test, and often overestimate the values compared to maximal testing (46, 47).

Exercise training

It is possible to improve aerobic capacity through regular aerobic exercise training. According to the World Health Organization (WHO), *"exercise training"* is a subcategory of the overarching concept *"physical activity"*. Physical activity is defined as

> any bodily movement produced by skeletal muscles that requires energy expenditure- including activities undertaken when working, playing, carrying out household chores, travelling, and engaging in recreational pursuits (48).

Exercise training, on the other hand, is described as activity that is

> planned, structured, repetitive, and aims to improve or maintain one or more components of physical fitness (48).

It is common to distinguish between "aerobic" exercise, which aims to increase oxygen uptake, and "resistance" exercise, which focuses on improving in muscular strength. Aerobic and resistance exercise have been shown to affect the body differently (15, 49). In this thesis, "exercise training" refers to aerobic exercise only.

Regular exercise training leads to physiological adjustments that occur on several levels. One example is cellular adaptations that increase biosynthesis and storage of essential neurotransmitters such as epinephrine and norepinephrine. Other adaptations include changes in neural communication and functional adaptations in "end-organs" such as the heart or the muscles. For example, one effect of aerobic exercise is increased oxidative capacity in the muscles due to enlarged mitochondrial volume and increased utilization of free fatty acids. This adaptation results in increased endurance and more effective use of energy (42). Other adaptations are increased wall thickness and heart volume, improved cardiac output, increased VO_2 peak and increased blood supply to the heart itself. Due to increased vagal activity, decreased sympathetic activity and increased heart size, a decreased resting heart rate is seen as well as lower heart rate at submaximal work as a result of aerobic exercise training of sufficient intensity and duration (42).

The cross-stressor adaptation hypothesis

It is believed that physiological adaptations to exercise training may also affect reactions to psychosocial stress. The so called "cross-stressor adaptation hypothesis" is a theory originally described by Sothman et al. (50) in the mid-1990s. Since then, a number of studies addressing this theory have emerged, but no consensus has been reached yet, and the hypothesis has not been fully confirmed. Their hypothesis is based on the idea of the stress response as non-specific, causing similar actions in the stress systems independent of the origin of the stressor. They suggest that the physiological adaptations that are seen after regular exercise, would also be seen in responses to psychosocial stress.

An important result of an adaptation is that it may lower the physiological "cost" for a response, that is, the total physiological arousal might be weaker. According to the hypothesis, exercise training will increase coordination between the different systems to diminish the risk of disturbed homeostasis. During acute exercise, a decreased HPA-axis and ANS response has been observed at a given work load in trained individuals compared to untrained individuals (42, 51). If these adaptations are transferable to non-exercise stressors, it would be beneficial in every-day life when commonly recurring stressors might put great pressure on the individual. However, the mechanisms behind the plausible effects are yet not fully known.

Summary of previous studies of the cross-stressor adaptation hypothesis

Although the paper defining the cross-stressor adaptation hypothesis was published in 1996, the plausible connections between physical and psychosocial stress were studied many years earlier. The first review of articles studying the effects of exercise training and aerobic capacity in response to psychosocial stress was a meta-analysis by Crews and Landers, published in 1987 (52). The paper comprised 34 studies, including both published and unpublished work. Outcome measures were heart rate, blood pressure, temperature, hormonal changes and subjective assessment. The stressors used were categorized as cognitive performance, physical performance, active physical performance and passive response, which imply a great diversity of both stressors and evaluative methods. Heart rate was the only variable assessed in all studies and was therefore used as a comparable factor of arousal achieved at the stress tests. An increase in heart rate above or below 30 beats per minute (bpm) was classified as a high and low response, respectively. The authors concluded that exercise training resulted in a lower response to psychosocial stress. However, in 24 of the 34 studies, the stress test caused a low response in heart rate (lower than 30 bpm), which raises questions of the adequacy of the stress tests. Also, the heterogeneity in the different stressors and outcomes leads to doubts about the authors conclusions.

Another review, also published before Sothman et al. presented their hypothesis, reported a conclusion that was opposite to the meta-analysis above (53). The authors used the term "fitness" to distinguish between individuals with high or low aerobic capacity and questioned the absence of a clear definition of fitness in earlier studies. Also, they claimed that, to be valid, fitness must be assessed using a VO_2 peak test, which precluded several studies from comparative analyses. They concluded that it was not possible to predict the physiological response to acute psychosocial stress based on the individual's level of fitness. They also questioned the comparison of response to physical and psychosocial stressors, given the different mechanisms involved.

In 2006, Jackson and Dishman published a review including a meta-regression analysis of 73 studies of both cross-sectional and longitudinal design (54). The aim of the review was to study the influence of cardiorespiratory fitness, defined as VO_2 peak, on cardiovascular responses to acute psychosocial stress. In cross-sectional studies, the VO_2 peak was between 40 and 59 mL/kg/min. In the studies that included an exercise training intervention the VO_2 peak was between 35 and 42 mL/kg/min, with an average increase of approximately 13 % following the intervention. The results revealed a slightly higher cardiovascular reactivity in individuals with higher cardiorespiratory fitness, but the effects were smaller in studies where fitness was measured using a VO_2 peak test than in studies using submaximal testing. There were also smaller effects in better controlled studies.

The same year, a meta-analysis by Forcier et al. was published, studying the effects of physical fitness on cardiovascular reactivity (55). The 33 included studies compared fit (high initial fitness, objectively measured as resting HR, VO_2 peak or treadmill tests) and unfit (low initial fitness) participants and their responses to psychosocial stressors. Stress reactivity was measured as at least 5 bpm increase in heart rate or at least 5 mmHg increase in blood pressure. Nineteen of the studies induced a reactivity of less than 10 mmHg or bpm, while 14 resulted in reactivity of more than 10. The authors concluded that fitness had a significant effect on heart rate and systolic blood pressure reactivity, and on heart rate recovery after psychosocial stress. However, as with the study by Crews and Landers, it is reasonable to question a stress test inducing an increase in heart rate or blood pressure less than 10 bpm or mmHg. Moreover, only a few of the longitudinal studies included a non-training control group, which makes it difficult to assume that the results are an effect of exercise training alone.

The most recent review found was published in 2018 (56). The review evaluated effects of exercise training and cardiovascular fitness on physiological responses to acute laboratory stress, measured with the Trier Social Stress Test (TSST; for a description, see the Methods section). The main outcome measures were cortisol, heart rate and psychological stress reactivity. Physical activity and fitness were measured both objectively and subjectively. Seven out of twelve studies reported attenuated responses in cortisol, and 4 out of 9 showed lower reactivity in heart rate, in groups performing a greater amount of physical activity or with higher levels of fitness. In contrast to the review by Jackson and Dishman (54), which found smaller effects if fitness was objectively measured, this study reported the opposite finding. In light of previous reviews and meta-analyses, the use of the same test to elicit stress increases the comparability between studies. However, like many of the earlier studies, it is problematic that physical activity and fitness are measured and defined differently across studies.

Only one RCT has been found examining the cross-stressor adaptation hypothesis for both HPA-axis and ANS responses. Klaperski et al (57) conducted an exercise training intervention study, comparing 12 weeks of aerobic exercise to relaxation training or a control situation. The result showed a reduced response in cortisol in the exercise training group compared to the control group, but no significant differences could be seen compared to the relaxation training group. However, although the exercise training group increased their level of exercise training, the level of daily activities was reduced. At the same time, the relaxation group increased both the level of exercise and level of daily activities, potentially influencing the results.

No reviews, meta-analyses or studies were found that explore the cross-stressor adaptation hypothesis addressing the influence of DHEA and DHEA-S, although the anabolic effects might constitute a role in the theory.

In summary, results from earlier reviews and meta-analyses are not unequivocal. The support for the cross-stressor adaptation hypothesis is therefore not clear, but taking into account the methodological diversity in study designs over the years, this is perhaps not so surprising. The only RCT found confirmed the hypothesis, but the unclear results regarding the relaxation group makes the interpretations unsure. Thus, further studies are needed, especially in the form of well performed RCT: s, elucidating the plausible role of exercise training on affecting the acute physiological stress response.

Aims

The overall aim of this thesis was to study, from different perspectives, physiological reactions to acute physical and psychosocial stress in healthy women and men.

Aims for each paper:

Paper I: To describe the protocol of an RCT designed to explore the effect of exercise training in physiological reactions to acute psychosocial stress. The aim was also to discuss relevant methodological issues related to conducting an exercise intervention study with acute stress responses as outcome measures.

Paper II: To study the physiological reactions to acute physical and psychosocial stress in terms of HPA-axis response and autonomic reactions in women and men. The paper also aimed to study differences and/or associations between the responses to physical and psychosocial stress, and whether the responses correlated to perceived stress.

Paper III: To study the effects of a six-month exercise training intervention on HPA-axis response and autonomic reactions to acute psychosocial stress in healthy but untrained individuals.

Paper IV: To study physiological responses to acute psychosocial stress in women and men, focusing on levels of DHEA and DHEA-S. The aim was also to study whether aerobic capacity correlated to levels of DHEA and DHEA-S.

Methods

Table 1.

Overview of methods and number of participants for each included paper

	Number of participants	Study design	Measurements
Paper I	119	study protocol/ cross sectional	VO_2 peak, ACTH, cortisol
Paper II	119	cross sectional	ACTH, cortisol, BP, HR, perceived stress
Paper III	81	RCT, longitudinal	VO_2 peak, TTE, ACTH, cortisol, BP, HR
Paper IV	88	cross sectional	VO_2 peak, DHEA, DHEA-S, cortisol

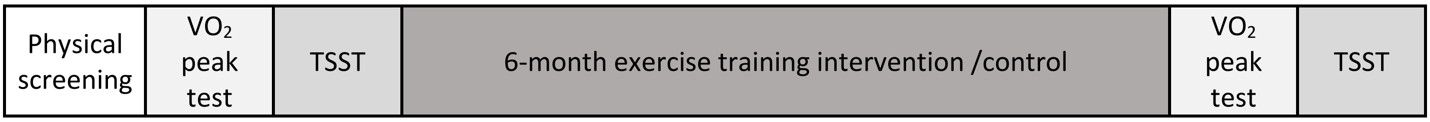
VO_2 peak: peak oxygen uptake, ACTH: adrenocorticotropic hormone, BP: blood pressure, HR: heart rate, DHEA: dehydroepiandrosterone, DHEA-S: dehydroepiandrosterone sulphate, RCT: randomized controlled trial, TTE: time-to-exhaustion

Study design

This thesis is based on an RCT, called "Acute Stress and exercise Training Intervention" (ASTI). The study was conducted at the Institute of Stress Medicine (ISM) in Gothenburg, Sweden, from 2013 to 2016 and registered at clinicaltrials.gov, ID NCT02051127. The aim of the RCT was to explore the effects of exercise training on physiological responses to acute psychosocial stress. Only selected parts of the original study are included in this thesis. The participants went through a physical stress test (VO_2 peak test) and a psychosocial stress test (TSST) and were then randomized to either an intervention group, which performed aerobic exercise training during the intervention period, or a control group. Six months later, both groups were followed up using the same procedures as at baseline (see details below) (Figure 2).

Figure 2.

Flow through the AST-study



VO_2 peak: peak oxygen uptake, TSST: Trier Social Stress Test

Study procedures

The study protocol was extensive and required considerable resources. The planning of the study began in 2012, and the actual testing started in spring 2013 as a small pilot study that included eight participants. In the autumn of the same year the original study started. The testing periods were divided into several "test weeks". Once a month (excluding January, June, July and December) a group of 8-12 participants were tested. The groups were generally a mix of participants performing either baseline- or follow-up measurements. In total, there were 28 test weeks from April 2013 to April 2016, with the last follow-up in October 2016.

Every participant made at least five visits during the study (see table 2). The first visit was a physical screening. Individuals considered eligible for the study were then booked for the second visit.

Table 2.

Activities at the five visits for each participant during the study.

Visit 1	**Visit 2**	**Visit 3**	**Intervention**	**Visit 4**	**Visit 5**
App. 30 min	*App. 4 h*	*App. 2.5 h*	*6 months*	*App. 4 h*	*App. 2.5 h*
Screening	Questionnaires	TSST	Exercise or	Questionnaires	TSST
BP, ECG,	Cognitive tests		control group	Cognitive tests	
blood tests	Vo2 peak test			Vo2 peak test	

BP: blood pressure, ECG: electrocardiography, TSST: Trier Social Stress Test, VO_2 peak: Peak oxygen uptake

Visits 2 and 4

The second and fourth visits took place at the Centre for Health and Performance, University of Gothenburg. At the start of the second visit, participants were given verbal and written information about the study and they were invited to ask questions. A written informed consent form was signed by each participant and the researcher. At both visit 2 and visit 4, a standardized meal was served containing controlled amounts of protein (15 g), carbohydrates (65 g) and fat (20 g). All meals were frozen ready meals (from e.g. Findus and Felix), and all participants were served the same amount of food. After lunch, each participant was taken to a quiet room to perform a computerized cognitive test, which took approximately 30 minutes to complete. Thereafter the participant was shown to a room outside the test lab and met the nurses who would take the samples. The participant filled out questionnaires and was prepared for the physical stress test. A peripheral venous catheter (BD Venflon Pro, Becton Dickinson Infusion Therapy, USA) was inserted in an antecubital vein by a nurse, and an automatic blood pressure cuff was put on.

Two hours after the lunch was ingested the first blood sample was drawn (-10 minutes) and the participant entered the test lab to perform the VO_2 peak test. The participant was provided with a pulse sensor and was informed of the test procedures. After a five-minute warm-up on the bicycle, a tight mask was put on to collect expired gases during the test. Some degree of discomfort was experienced by most of the participants, but the mask had to sit tight in order to avoid air leakage. When the equipment was calibrated and the participant was ready to start, the second sample was drawn (-0 minutes), the blood pressure cuff was turned off and the test started (for a description of the test protocol, see page 37).

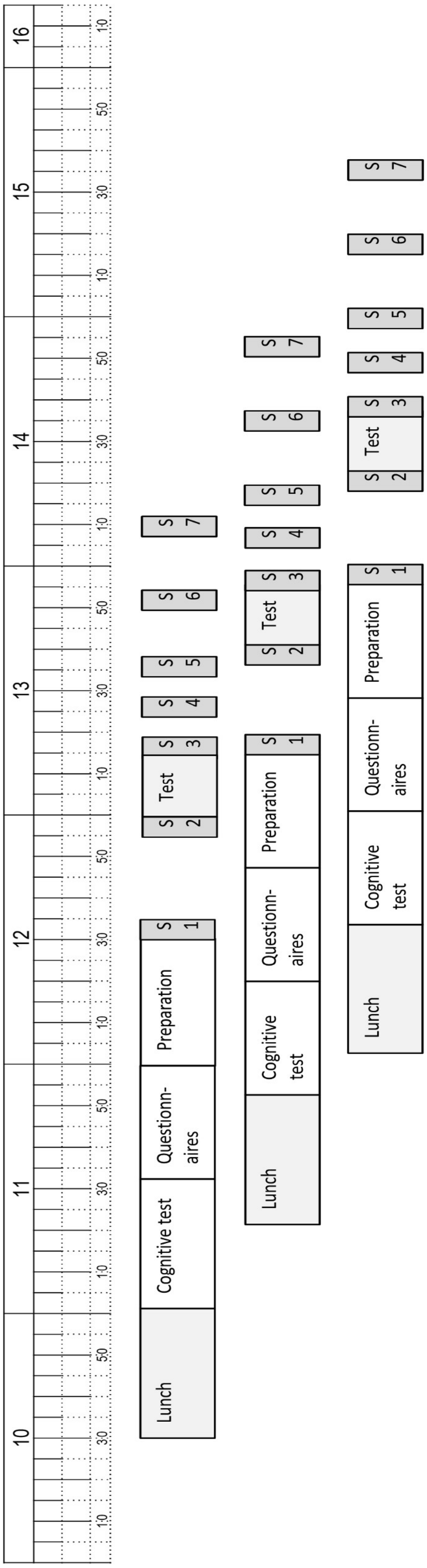
Directly after the test, the participant was released from the mask and sat down on a chair when the third (+0 minutes) sample was drawn and the blood pressure cuff was turned on again. Several participants had slight vertigo some minutes after the test and were therefore supervised by the nurses taking the samples. The participant rested for one hour in a sitting position while the remaining samples were taken (10-, 20-, 40- and 60-minutes post-test). They were allowed to drink water, but no other intake of food or beverages was permitted. Following the last sample, the nurse removed the inserted catheter and supplied the participant with a frozen ready meal that was to be eaten before the stress test scheduled for the following week.

Two to three participants were booked for each test day. To manage the logistics for personnel and use of the test rooms, the time for arrival was set to 45 minutes between participants (protocol for the logistics are shown in figure 3). On most of the testing days, three personnel were in place.

Samples that were to be cold spun were put on ice and taken care of immediately. Samples that were to be centrifuged at room temperature were taken care of after the last participant had completed the samplings.

Figure 3.

Overview of logistics for the physical stress test, a testing day including three participants



S 1-7: time point for samplings 1-7

Visits 3 and 5

On the testing days for the TSST, the participants had ingested their lunch before arrival, again two hours before the test was scheduled to start. The vein catheter was inserted by the nurse directly on arrival, and the participant had a short rest before the first blood sample was drawn (-10 minutes). After the second sample (-0 minutes), the participant started the test (for a description of the test protocol, see page 38).

The samplings after the stress test followed the same protocol as after the physical stress test (+0-, 10-, 20-, 40- and 60 minutes post-test). Following the TSST sampling procedures at the third visit, the participants were randomized. Each participant, regardless of whether he or she was randomized to the intervention- or the control group, received information on what was to take place during the time between baseline measures and follow-up. Participants randomized to the intervention group booked time for a group meeting the week after the stress test. At that meeting, the research staff gave the participants information about the intervention, handed out pulse watches and gave instructions on how to use the watch and the training log. The participants also received information on the positive effects of exercise training and a voucher giving them access to the training centres during the intervention period. Participants randomized to the control group were asked to maintain their current level of physical activity.

At the last visit (visit 5), all participants were thanked for their participation. Participants in the control group were invited to a motivational group meeting the week after the test. They received information on the positive effects of exercise training and a 12-month voucher to a training centre and were introduced to the same training log as the intervention group.

Preparations between the test weeks

In the time between the test weeks, data and results from the tests were entered in SPSS, and protocols for the coming tests were prepared. Labels for all the tubes for sampling (28 for each participant) and micro tubes for storing of samples (72 for each participant) were printed. Medical journals and questionnaires were prepared for new participants, and physical screenings were done for potential participants. Additionally, participants randomized to the intervention group got started on their exercise training.

Participants

The participants were healthy volunteers, 20 to 50 years of age, both women and men, with a self-reported sedentary lifestyle, working or studying at least 50 % of full time and living in the Gothenburg area. Participants were recruited through two local newspapers, notice boards around the city of Gothenburg, and social media. Individuals interested in participating sent an e-mail to the study coordinator, who sent a screening form that included questions regarding psychological health and medication, and a screening for heart disease with the Physical Activity Readiness Questionnaire (PAR-Q) (58). They reported their current level of physical activity with the Saltin-Grimby Physical Activity Level Scale (SGPALS)(59), a four-graded single-item request: "Mark the alternative that best describes your physical activity level in the last year." The alternatives were as follows: 1) Mostly sedentary, sometimes walking, light gardening or comparable activities, 2) Light physical activity at least two hours per week, such as walking or bicycling to work, dancing, ordinary gardening or comparable activities, 3) More strenuous activity at least two hours per week, like playing tennis, swimming, running, gymnastics, bicycling, dancing, playing football or indoor hockey, heavy gardening or comparable activities, 4) Regular hard exercise several times per week for at least five hours, with a high physical effort.

Individuals reporting level 1 were defined as untrained and were invited to a physical screening at the ISM. The physical screening included assessments of weight and height, blood tests (HbA1c, glucose, insulin) and ECG. Individuals with diverging levels of glucose, HbA1c or blood pressure, abnormal resting ECG, anaemia, under- or overweight, medication with beta blockers, psychopharmacological drugs or asthma medicine, or inability to exercise at a relatively high intensity were excluded due to the exclusion criteria. Individuals with an abnormal ECG were further examined by a cardiologist before inclusion or exclusion in the study.

Oxygen uptake test

At both baseline and follow-up, the participants went through a bicycle ergometer test to assess their peak oxygen uptake, peak heart rate and time-to-exhaustion (TTE). The protocol was adapted to this specific group of untrained, healthy adults in the form of a ramp test (45). The test leader was the same person (the author) for almost all tests at both baseline and follow-up.

The participants warmed up for five minutes on the bicycle ergometer (Monark 828 E, Monark Exercise AB, Vansbro, Sweden). The relatively short time was set due to the risk of fatigue even at a low resistance. The cadence was set to 70 revolutions per minute, since a higher speed can be difficult to keep for a person unaccustomed to ergometer cycling, and a lower speed increases the risk of early fatigue in the legs. The initial load was 87.5 watt (W) for women and 105 W for men, increasing by 17.5 W (0.25 kilo pounds) every minute until exhaustion. The participants were verbally encouraged by the test leader during the test, with increasing frequency at the end of the test. Previous studies have shown that individuals, especially untrained individuals, can increase their performance when verbal encouragement is given (60). The test ended when the participant reached a plateau and/ or a decrease in oxygen uptake, had a respiratory exchange ratio above 1.1, hyperventilated and could not keep the required cadence on the bicycle, or chose to stop for other reasons. Oxygen uptake was measured as mL/kg/min with the Jaeger Oxycon Pro metabolic chart (Carefusion, Hoechberg, Germany) in a mixing chamber mode. The device was calibrated before each measurement according to the manufacturer's manual. HR was monitored with a pulse sensor (Polar 300 RS, Polar, Finland).

Trier Social Stress Test

One week after the VO_2 peak test, the participants performed a psychosocial stress test. The test used was the Trier Social Stress Test (TSST), which has been shown to strongly activate both autonomic and neuro hormonal stress responses during and after the test (39). It has been widely used in previous studies in this research area and is well established as a reliable and valid test (12, 61). The TSST is based on two parts: 1) a free speech and 2) an arithmetic task, both parts in the presence of a committee consisting of two men and one woman. The participant enters the test room and is given information about the procedures and is instructed to give a five-minute presentation of him- or herself in a fictitious job interview for his or her dream job. The participant is told that the test will be recorded both by video- and audiotape, and that the members of the committee are specialists in studying behaviour. The participant leaves the room for a preparation period of five minutes. Thereafter the participant re-enters the test room and starts the first part of the test- the free speech. The members of the committee give no form of encouragement during the speech. If there is still time when the speech is over, the chairman of the committee encourages the participant to continue. If the participant has no more to say, it will be quiet in the room for the rest of the time. In the second part of the test, the participant is given a task to count down from 1637 in steps of 13. If the participant fails to give the right number, he or she must start from the beginning. This part lasts for five minutes. After the test the participant leaves the room.

No debriefing was held after the baseline TSST since almost the same test was performed at follow-up. There was a small change in the instructions given the second time, in that participants would instead apply for a job they had dreamed about as a child. After the follow-up TSST, the participants received a short debriefing and were informed that nothing from the test had been recorded and that the members of the committee were not experts in behaviour.

Randomization

After completing the psychosocial stress test, the participants were randomized to the intervention or control group by picking a sealed envelope. The randomization rate was 50 % to the intervention group and 50 % to the control group. Due to higher numbers of drop-outs than expected in the intervention group, the rate was changed to 70 % to the intervention group and 30 % to the control group during the last year of inclusion.

Outcomes

The main outcomes of the RCT were ACTH and cortisol response to acute psychosocial stress. In this thesis, DHEA, DHEA-S, blood pressure and heart rate were also assessed. Paper II included responses to physical stress as well.

Assessments

ACTH, Cortisol, DHEA, DHEA-S

Identical protocols for collection of blood samples of ACTH and cortisol were used for both the physical and the psychosocial stress tests with samples drawn 10 minutes before the test (-10), directly before the test (-0), directly after the test (+0), and thereafter 10, 20, 40 and 60 minutes after the test was finished.

DHEA and DHEA-S samples were drawn at -10, +0, 10, 20 and 60 minutes post-test. A total of 220 mL of blood were taken at each test session.

Plasma samples, collected in EDTA tubes, were used to assess ACTH, DHEA and DHEA-S. To separate plasma, the tubes were cold spun at 3500 revolutions per minute for 15 minutes and stored in micro tubes at -80 º C until analysed. Cortisol was assessed in serum and collected in Serum Sep Cloth Activator tubes. To separate serum, the tubes were spun at 20 º C for 10 minutes at 3500 revolutions per minute and stored at 6 º C until analysis the day after the test. Plasma concentrations of ACTH were assessed by immunoradiometric assay (limit of detection, 0.4 pmol/L) (CIS bio International, Gif-sur-Yvette Cedex, France). Serum concentrations of cortisol were assessed by electro chemiluminescence immunoassay (limit of detection, 0.5 nmol/L) (Roche Diagnostics GmbH, Mannheim, Germany). Serum concentrations of DHEA were determined using a Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (limit of quantitation 175 pmol/L), and serum concentrations of DHEA-S were assessed by radioimmunoassay techniques (RIA) (limit of detection 0.14 µmol /L, Diagnostic Products Corporation, Los Angeles, CA, USA).

Blood pressure and heart rate

The participants wore an automatic blood pressure cuff (Welch Allyn, ABPM 6100, USA) from 10 minutes before the tests started to 60 minutes after the tests were finished. The device assessed systolic and diastolic blood pressure (SBP and DBP, respectively) and heart rate (HR) every five minutes at the TSST. At the VO_2 peak test the device assessed every 10 minutes, but it was turned off during the test and started again directly after the test was finished.

Perceived stress

Immediately after the psychosocial stress test, the participants rated their perceived stress according to an adapted version of the Borg CR 10 scale of Perceived Stressfulness (62). It is a 13-grade category scale, ranging from "nothing at all" to "maximal", modified to fit the rating of stressfulness during the psychosocial stress test.

Exercise training intervention

The week after the psychosocial stress test, participants randomized to the intervention group were instructed to start regular aerobic exercise during the intervention period. The goal was to reach a frequency of three times per week with a duration of 45-60 minutes at each session. The goal of intensity was to reach an average heart rate of at least 75 % of peak heart rate, measured at the peak oxygen uptake test, and sustain it during at least 80 % of the session. To measure the duration and intensity of exercise, the participants wore a pulse sensor (Garmin 210) at each session. Data was transferred from the sensor to a web-based training log (www.funbeat.se). In the training log, the participant manually recorded the type of activity performed. The data was registered to be further analysed in terms of frequency, duration, intensity and type of activity. For untrained individuals, an increase in exercise level from no exercise training at all to three times per week is challenging. Therefore, the participants were encouraged to increase their activity level gradually, starting at 30 minutes two times per week to reach the final level after 6-8 weeks.

Participants were free to choose the type of aerobic exercise they would do, as long as they reached the intended level of intensity. During the intervention, the participants received free access to a commercial fitness establishment (Nordic Wellness) with several facilities in the Gothenburg area. The participants were instructed to avoid resistance training, since it is thought to affect the hormonal systems differently than aerobic exercise (49).

To support the participants in their lifestyle change, they were offered four sessions with a coach, trained in motivational interviewing. The sessions were guided by Self-Determination Theory, which provides guidelines for the interviewer supporting participants as they increase their level of exercise (63). The coach had access to the training log and referred to it during the sessions.

Control group

Participants allocated to the control group were instructed to maintain their current level of exercise, that is, to not increase or decrease their degree of physical activity. After follow-up measures were taken, they were encouraged to start to exercise. They were called to a motivational group meeting and received one-year access to the same fitness establishment as the intervention group.

Ethics

The original study was registered at clinicaltrials.gov, ID NCT02051127, and designed according to the Consolidated Standards of Reporting Trials (CONSORT) Statement (64). All participants gave written informed consent before entering the study and were informed that they could withdraw their participation at any time. The study was conducted according to the 1964 Declaration of Helsinki and approved by the Regional Ethical Board, Gothenburg, Sweden, Dnr 917-12, and supported by funding from the Swedish Research Council for Health, Working and Welfare.

Data handling

Pre-test values in papers I-III were calculated as the mean value of the -10- and -0-minute values. In paper IV the -10-minute value was defined as the pre-test value. For all variables, peak value was the highest value measured during or after the stress test. The lowest value after the peak was also identified.

In papers II, III, and IV, reactivity values are presented, calculated by subtracting the pre-test value from the peak value. Percental change was calculated by dividing the absolute change from pre-test to peak by the peak value. Similarly, recovery values were calculated by subtracting the lowest value from the peak value, and percental change was calculated by dividing the difference by the peak value.

In paper IV, ratios for cortisol and DHEA and cortisol and DHEA-S were calculated by dividing the cortisol value by the value for DHEA and DHEA-S at pre-test and peak (65).

The area under the response curve with respect to increase (AUC_i) was calculated in accordance with Fekedulegn et al. (66). AUC_i is a suitable method to analyse total arousal over a limited time period and may simplify analysis of multiple assessments. It provides information on both changes over time and overall intensity of the response (66).

The rating of perceived stress during the TSST was dichotomized into two groups representing low stress and high stress, respectively. Ratings from 0 to 4 (“not at all” to “somewhat strong”) were considered low perceived stress, and 5-10+ (“strong” to “maximal”) were considered high perceived stress.

Statistics

A power analysis for the RCT was done before the study was started. The sample size calculation for the main outcome measure, cortisol, showed that 39 subjects in each group were needed to enable a detection of an effect size of Cohen's $f = 0.25$, with power ≥ 0.80 and $\alpha = 0.05$. It was anticipated that a number of subjects could drop out or withdraw their participation, which resulted in a goal to include at least 50 participants in each group.

Several statistical methods were used in the four papers included in this thesis (see table 2). First, to check whether the variables were normally distributed, Kolmogorov-Smirnov tests were used. In papers I, II and IV, group differences at baseline were analysed with an independent samples t-test. For categorical data, $\chi 2$ tests were used.

A mixed between-within subjects analysis of variance (ANOVA) method was used in papers II and III. It combines a repeated-measure design with a between-subjects design in the same analysis and also presents possible interaction effects (67).

In paper II, AUC_i for ACTH and cortisol as well as reactivity and recovery in SBP, DBP and HR were analysed with mixed between-within subjects ANOVA for both physical and psychosocial stress. Correlations were analysed with Pearson correlation coefficient analysis for normally distributed data, or Spearman's rank order correlation coefficient for data that was not normally distributed.

Paper III compared pre-test, peak and recovery values from baseline to follow-up with mixed between-within subjects ANOVA. Correlations were analysed for amount of training and response to the stress test using Pearson correlations analyses. Finally, recovery values were analysed with mixed between-within subjects ANOVA.

In paper IV, independent samples t-tests were used for pre-test values. Pearson correlation analyses were used to evaluate associations between variables, and paired samples t-tests were used to analyse physiological responses to acute psychosocial stress.

Table 3.

Statistical methods used in each paper.

	Independent samples t-test	Paired samples t-test	Chi-square test	Mixed between-within subjects ANOVA	Pearson correlation coefficient /Spearman's rank order correlation coefficient	Kolmogorov-Smirnov test
Paper I	x		x			
Paper II	x		x	x	x	x
Paper III				x	x	x
Paper IV	x	x	x		x	x

Statistical methods used in each paper.

ANOVA: analyses of variance

Results

Since all included papers are based on the same study, general results are presented first. Specific results for each paper are presented below.

Participants

A total of 416 individuals responded to the advertisement recruiting participants for the study. Of these, 170 fulfilled the inclusion criteria and were invited to the physical screening. Twenty-four individuals were excluded due to the exclusion criteria, and another 22 declined participation. Five participants did not complete the baseline measurements and were therefore excluded. The final number of participants at baseline was 119 (see study flow diagram, figure 3). Of these, 89 participants (75 %) worked full time, 21 (18 %) worked 50 to 90 % of full time and 8 participants (7 %) were studying. The number of participants included during spring (February to May) was 56 (47 %). In autumn (August to November), 63 participants (53 %) were included. Alcohol was used by 93 % of the participants; of these, 54 % reported a frequency of 2-4 times per month. Tobacco was used by 19 participants (16 %); four were smokers and 14 used snuff, and one participant used both cigarettes and snuff. Nearly three-quarters (n = 86, 72 %) of the participants reported an educational level of at least three years of post-graduate education. A majority (n = 97, 82 %) of the included participants were living in a relationship.

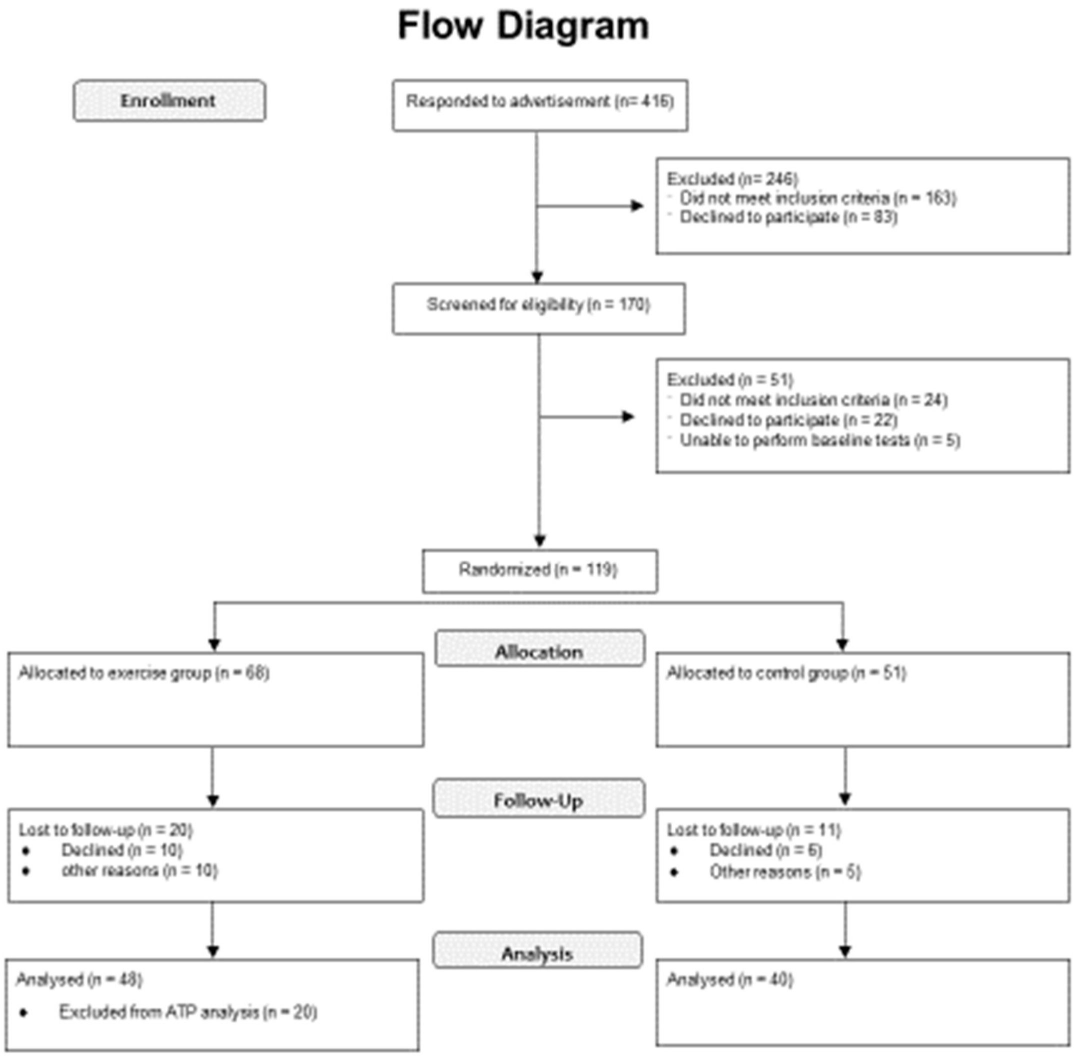
Activity level was self-reported as being "mostly sedentary" (equal to 1 in the SGPALS) in 89 % of the included participants. The remaining 11 % reported level 2 (light physical activity). The reason for including individuals reporting level 2 was an initial difficulty in the recruitment of participants. However, there were no significant differences in aerobic capacity between participants reporting level 1 or 2 in SGPALS ($t = -1.281$, $p = 0.203$).

Baseline values of aerobic capacity differed between sex and age groups. For both women and men, the aerobic capacity was shown to be higher than expected given their reported level of physical activity. Instead of VO_2 peak values corresponding to levels for untrained individuals, mean values for each age group showed values representing normal, or higher than normal, levels in the general population.

At follow-up, 31 participants had dropped out (20 individuals in the IG and 11 in the CG) for different reasons (unwillingness to go through retesting (n = 17), injuries (n = 4), changed working conditions (n = 3), starting anti-depressant medication (n = 4), pregnancy (n = 2) and randomization to the CG but started to exercise (n = 1)). See flowchart in figure 4. No significant difference was seen between the drop-out rate of participants included in spring and those included in autumn; 23 % of the spring participants dropped out, and 28 % of the autumn participants dropped out ($p = 0.506$). The drop-out analysis of demographic data showed no significant differences in the group of participants that did not return for the follow-up compared to the group that completed the study.

Figure 4.

Flow diagram over the randomized control trial



Paper I

This paper includes the study protocol used for the RCT ASTI. It also presents some baseline results to highlight methodological issues regarding the performance of an intervention study.

At baseline, the HPA-axis response to the TSST showed a mean increase of 214 % in ACTH and 94 % in cortisol, but large intra-individual differences were seen. The main hypothesis for the RCT was that individuals performing regular exercise training for six months would show an attenuated physiological response compared to participants in the control group. The prerequisite for such a change was that a positive response (increase) in the outcome measures from pre-test to peak would occur at baseline. However, for 13 participants, negative responses (decreases) were observed for ACTH or cortisol, and one participant responded negatively in both ACTH and cortisol. Thus, it would not be possible to detect attenuated responses to acute stress in these individuals.

The intended target group for the study was untrained individuals with low aerobic capacity. Although the majority of the included participants reported themselves as untrained at screening, baseline levels of aerobic capacity were comparable to average levels in the general population. Thus, several individuals included in the RCT were not as sedentary as expected.

Paper II

Paper II presents cross-sectional analyses of the correlations between the physiological response to acute physical and psychosocial stress in women and men.

The time point for reaching peak values of ACTH and cortisol differed between the physical and the psychosocial stress tests. At the physical test, 56 % reached their peak ACTH immediately after the test. For cortisol, 56 % of the participants reached their peak after 20 minutes. At the psychosocial test, 97 % of the participants reached their peak ACTH level during or immediately after the test, and 65 % of participants peaked in cortisol 10 minutes post-test. SBP, DBP and HR all peaked during or immediately after the tests.

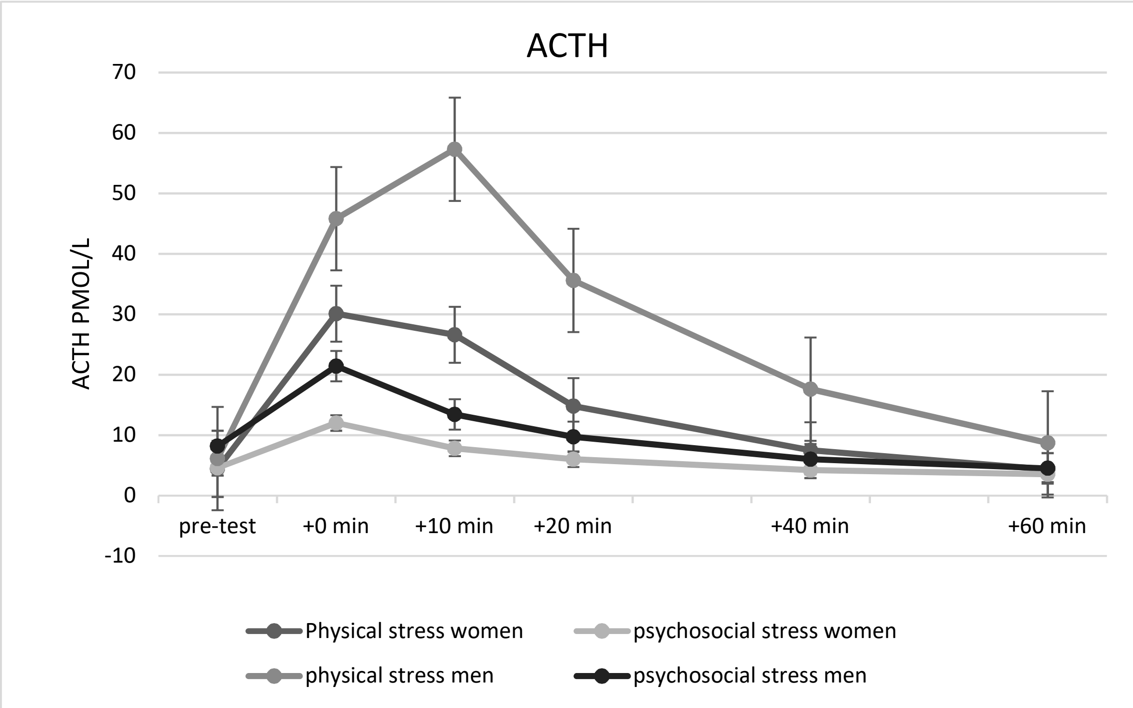
Significant main effects for ACTH AUC_i were seen for test and sex (see response curves in figure 5). That is, for both women and men, AUC_i was significantly lower at the psychosocial stress test than at the physical stress test, with men showing greater responses than women at both tests. Cortisol AUC_i did not differ between the tests, but significant main effects were seen for sex, showing greater response curves in men compared to women at both tests. There were also positive correlations between AUC_i at the physical and psychosocial stress tests for both ACTH and cortisol.

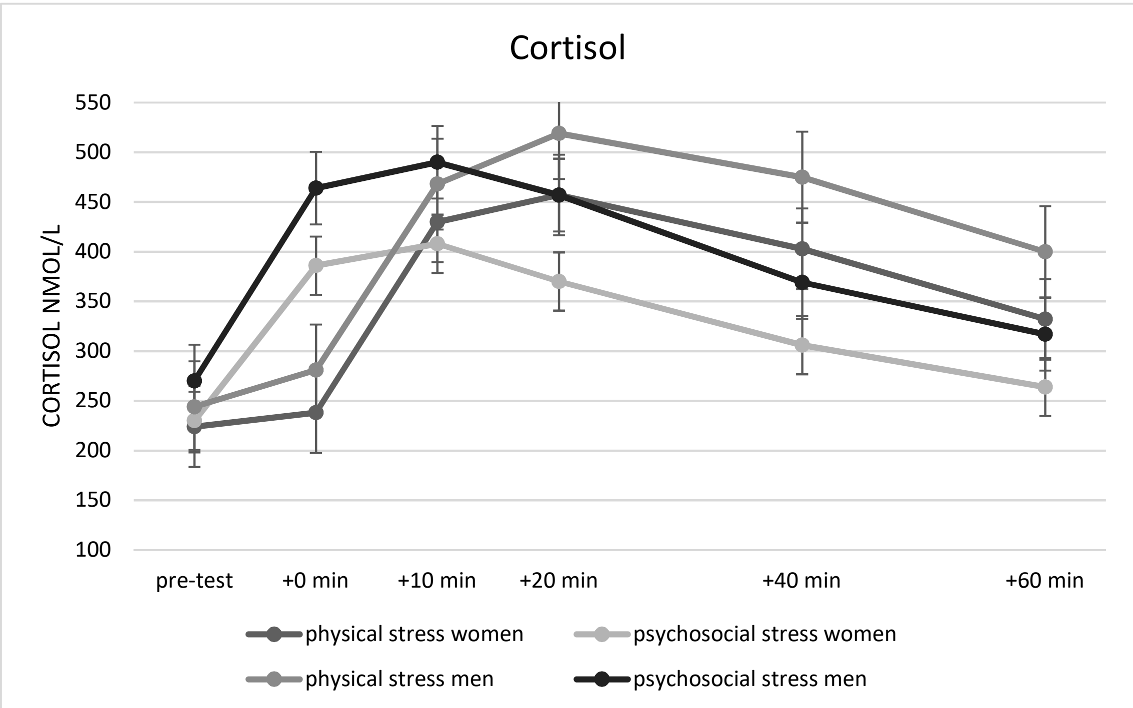
In the analyses of percental reactivity, autonomic variables showed significant main effects for test, with lower reactivity at the psychosocial stress test compared to the physical stress test for HR, and higher reactivity for SBP and DBP. Sex differences were seen in SBP, with women showing greater reactivity than men at the psychosocial stress test.

In the analyses of relationships between physiological response and perceived stress at the psychosocial stress test, no correlations were seen for any of the variables, regardless of whether high or low stress had been reported.

Figure 5.

Response curves for women and men at the physical and the psychosocial stress tests.





Paper III

The results from paper III present the main results from the RCT, on the effect of exercise training on physiological responses to acute psychosocial stress.

Compliance with the protocol varied considerably among participants in the intervention group. The lowest acceptable level was set to 26 exercise training sessions during the intervention period, which excluded 22 participants in the adherence-to-protocol analyses. Among the remaining participants, the mean number of sessions was 45 out of a possible 78.

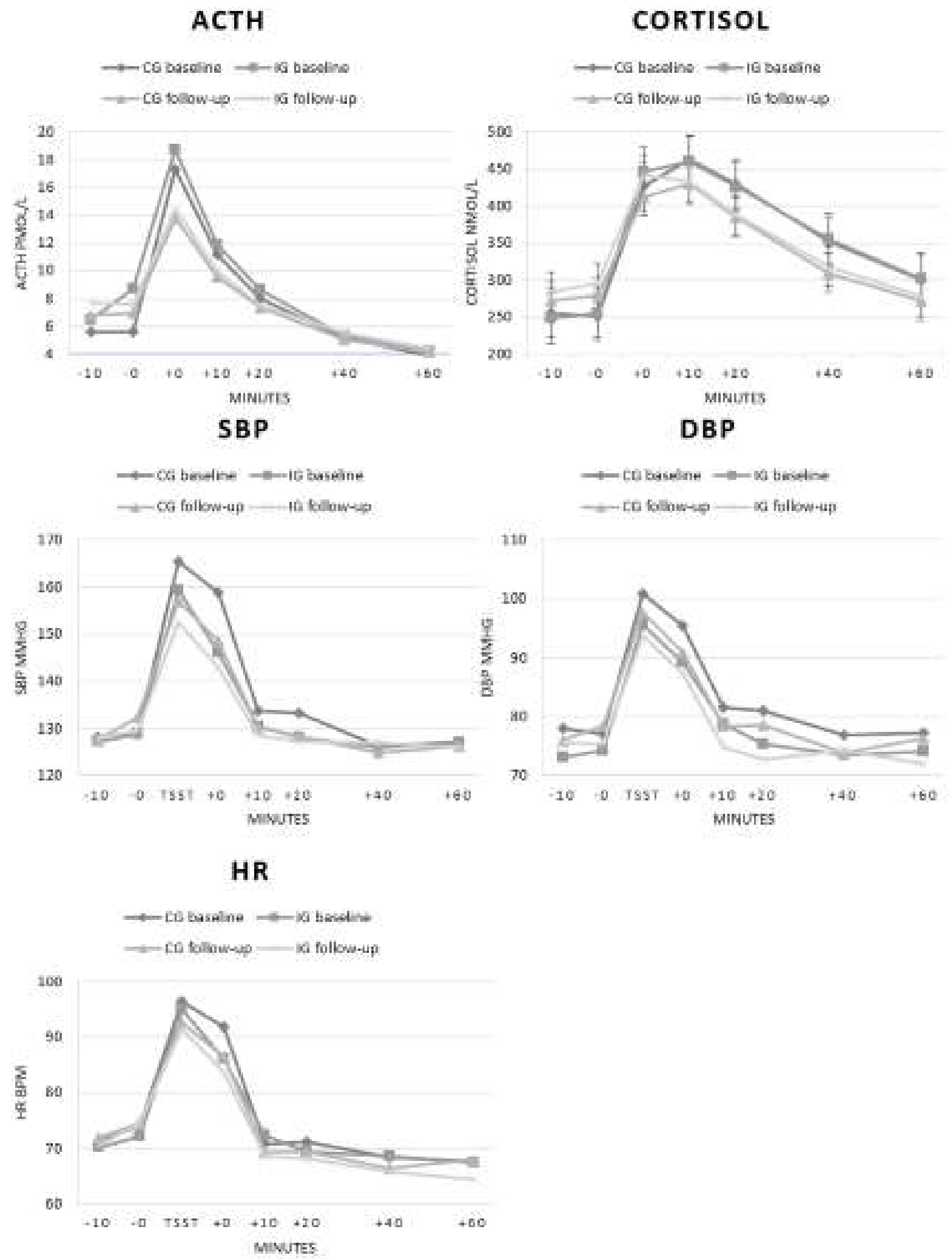
The mean level of aerobic capacity increased significantly in the intervention group (9.4 %) from baseline to follow-up. The increase did not change after exclusion of non-compliers. The mean time-to-exhaustion increased by 11 %, and when excluding non-compliers, the mean change was 9.5 %. Participants in the control group decreased their mean aerobic capacity and mean time-to-exhaustion (-3 % and -0.7 % respectively).

Both the intervention and the control group showed attenuated physiological responses to the stress test at follow-up, indicating habituations to the test or the test situation (see response curves in figure 6). ACTH and cortisol AUC_i showed decreased values for both groups, and the mixed between-within subjects ANOVA resulted in significant effects for time, but no group differences were seen at follow-up. For autonomic responses, no differences could be detected between the groups at follow-up.

There were no correlations between number of training sessions during the intervention and physiological response to the psychosocial stress test.

Figure 6.

Response curves for ACTH, cortisol, systolic and diastolic blood pressure and heart rate for the Trier Social Stress Test at baseline and the six-month follow-up



ACTH: adrenocorticotropic hormone, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate

Paper IV

In paper IV, the main focus was on DHEA and DHEA-S response to acute psychosocial stress and the potential relationship to aerobic capacity among women and men. Also, the catabolic/anabolic balance was analysed between cortisol and DHEA and DHEA-S, and the effect of age in the response to acute psychosocial stress.

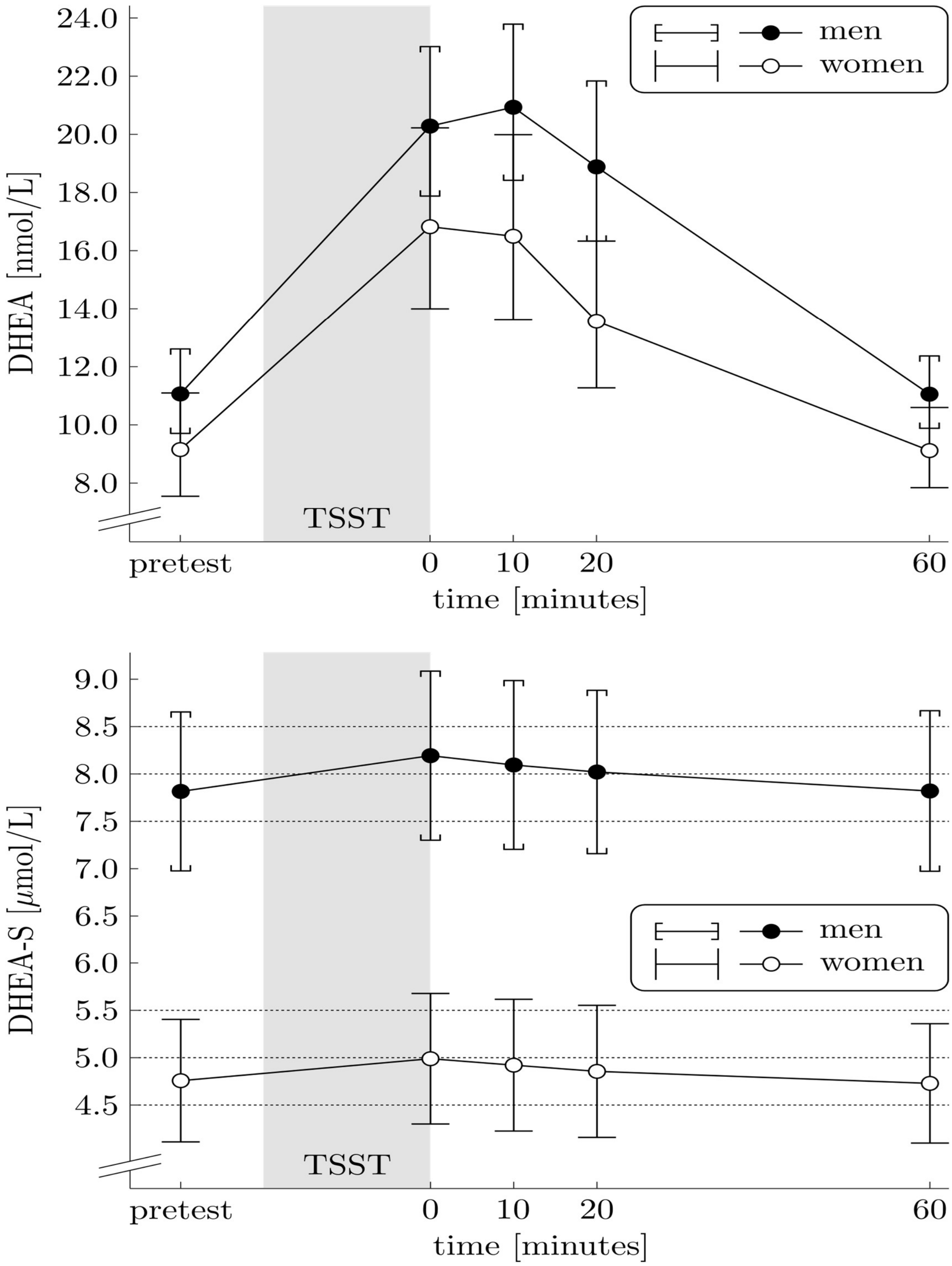
Both DHEA, DHEA-S and the cortisol/DHEA ratio increased significantly following the psychosocial stress test, with DHEA increasing on average 117 % for women and 120 % for men (see response curves in figure 7). Four individuals responded either negatively or not all in DHEA. The mean increase in DHEA-S was 10 % for women and 8 % for men. Negative responses were seen in DHEA-S for seven individuals, and six participants showed neither a positive nor a negative response to the stress test. The only variable that did not increase in response to the stress test was the cortisol/DHEA-S ratio.

Pre-test levels of DHEA and DHEA-S did not show any correlation to level of aerobic capacity. Nor did the magnitude in response of DHEA, DHEA-S, cortisol/DHEA ratio and cortisol/DHEA-S ratio to acute psychosocial stress show any significant correlations to aerobic capacity.

The response in DHEA was negatively correlated with age, but significant correlations were seen for DHEA-S, cortisol/DHEA ratio and cortisol/DHEA-S ratio and age.

Figure 7.

Response curves of DHEA and DHEA-S at the psychosocial stress test



DHEA: dehydroepiandrosterone, DHEA-S: Dehydroepiandrosterone sulphate,
TSST: Trier Social Stress Test

Additional analyses

No correlations were seen between aerobic capacity and HPA-axis responses to the psychosocial stress test (ACTH: t = 0.04, p = 0.682, cortisol: t = -0.13, p = 0.184). For autonomic variables, a negative correlation was found for HR (t = -0.21, p = 0.037), but no correlations were seen for blood pressure (SBP: p = -0.07, p = 0.502, DBP: t = 0.18, p = 0.067)

Discussion

This thesis presents results from an RCT evaluating effects of aerobic capacity and exercise training on responses to acute physical and psychosocial stress. The main findings were that the six-month exercise training intervention increased time-to-exhaustion and aerobic capacity, but no effect was found on the physiological response to acute psychosocial stress. Hormonal variables (ACTH, cortisol, DHEA and DHEA-S) as well as autonomic variables (systolic and diastolic blood pressure and heart rate) increased following both acute physical and psychosocial stress, showing that the physiological systems did react to the stressful situations. However, the correlations between the physiological responses to physical and psychosocial stress were weak. Unfortunately, methodological issues hampered the interpretation of results. These and other findings will be discussed below.

Cross-sectional findings

Three of the papers included in this thesis presented cross-sectional data (papers I, II and IV). Data from baseline assessments was used to study physiological responses to acute stress and to analyse relationships and also to examine methodological issues that arose in this study.

Physiological responses to acute stress

Acute physical and psychosocial stress resulted in significantly increased mean values from pre-test to peak in both HPA-axis, DHEA, DHEA-S and ANS variables. This was expected, given that the participants were healthy and that the physiological systems, which are meant to react to acute stress, operate satisfactorily (68). The reactivity in ACTH, cortisol and heart rate was greater at the physical stress test, whereas reactivity in systolic and diastolic blood pressure was higher at the psychosocial stress test, which was also expected. The differences may partly be due to the fact that the acute psychosocial stress test entails less muscular activity than the physical stress test and thus requires a lower grade of metabolic actions and puts a higher demand on the heart for circulation of blood (69). However, the peak values for ACTH at the psychosocial stress test might be incorrect. It was found that the true peak probably occurred during the test, when no samples were taken. Cortisol peaks with a 10-15 minute delay in relation to ACTH (70), and the post-test cortisol values indicated that peak in ACTH was plausibly reached before the first post-test sample. Because no blood samples were taken during the test, we do not know when the peak occurred and consequently not the actual magnitude of the peak value.

An interesting finding was that several individuals showed no or even negative responses to the psychosocial stress test in ACTH, cortisol, DHEA and/or DHEA-S. One plausible explanation is that these individuals were stressed already when they arrived at the lab. Only a short rest was applied before the test started. If the stress systems were activated before the test, there was no time to recover from that stressor before the TSST. However, the negative responses were also seen for ACTH and cortisol at the physical stress test, where the participants had at least one hour of rest before the test started. Another explanation could be that just the expectation of the tests as something unknown or unpleasant was enough to cause a stress reaction and start a process in the stress systems. In both cases, pre-test values would be heightened, and no or only small changes in the outcome measures would be detectable. However, an absence of cortisol response in some individuals has also been found in previous studies, showing 20-30 % non-responders in salivary cortisol, to the TSST (71). An earlier study found that cortisol non-responders were more likely to be smokers and/or females (72), but no such connections were seen in our study. Concerning DHEA and DHEA-S, not many studies can be found presenting data on responses to acute psychosocial stress, and only one shows that not all participants responded with an increase; in a study of young males, 30 % of the participants showed some variations in DHEA level after psychosocial stress, but there was no clear increase (73). Individual variations might be one explanation, but these variations may be caused by numerous underlying factors. The HPA-axis has been thoroughly studied primarily in terms of different aspects of responses to acute stress. Results from earlier studies have shown that experiences from childhood may impact the response to stress later in life (74). Also, an individual who has had a negative experience in a job interview will likely respond differently to a stress test that includes an interview-like situation compared to one who has had a good experience (19). Another study showed that one night of sleep deprivation leads to elevated pre-test cortisol levels and blunted cortisol reactivity in response to a TSST (75). These are factors that are not easy to control for and that might affect results markedly.

A positive correlation was seen between AUC_i at the physical and psychosocial stress tests for cortisol. That is, individuals showing a strong cortisol response to the physical stress test also responded strongly to the psychosocial stress test, although the time point for reaching peak differed between the tests. This might indicate that in a healthy individual with well-functioning stress systems, the body reacts similarly independent of the origin of the stressor. It also indicates that the large inter-individual differences in hormonal response pattern are not necessarily problematic, but just point towards individual variances. For autonomic responses, no correlations were seen in reactivity between physical and psychosocial stress. It might depend on the differences in the system's response times, with the HPA-axis reacting within minutes while the ANS responds in a few seconds.

Perceived stress

No correlations were seen between perceived stress and the physiological response to acute psychosocial stress. The mental aspect of the experience thus does not seem to be related to the actual response, which is in line with a recent study by Ali et al. (76). They induced a pharmacological suppression of physiological responses in healthy women and men before a psychosocial stress test. Despite the inhibited stress systems, the participants in the intervention and the control groups experienced the stress test as equally stressful.

Campbell and Ehlert reported in a review that only one-fourth of the included studies found associations between the physiological and emotional responses to the TSST (61). Many factors are involved and affect each system, influencing the outcomes and the results. As examples they mention aspects of assessment, where both timing of assessments and the tools used to assess might affect the outcome measure. Another important factor is the psychological part, affecting emotional regulation as well as motivational engagement with the test situation. Finally, they also mention the physiological aspects of the response, taking into account the individual characteristics of the stress systems.

Associations between aerobic capacity, DHEA and DHEA-S

In paper IV, correlations between aerobic capacity and pre-test levels of DHEA and DHEA-S, as well as reactivity of these hormones to acute psychosocial stress, were studied. The analysis did not show any correlations with neither baseline levels nor reactivity in DHEA or DHEA-S to the psychosocial stress test. It can be argued that participants included in the study reported themselves as untrained at screening and that the range of aerobic capacity would be too small. It turned out, however, that levels of aerobic capacity were higher than expected, with mean values corresponding to normal levels in the general population. The span ranged from 20.4 to 45.1 mL/kg/min for women and from 24.0 to 56.6 mL/kg/min for men, showing an acceptable range for analyses of correlation, although a larger range would have been preferable. Only two previous studies have been found that examine the relation between aerobic capacity and basal levels of DHEA and DHEA-S (77, 78). One of those studied premenopausal women (range in VO_2 peak: 23.8-50.1, mean 39.5) (78), while the other studied postmenopausal women (range in VO_2 peak: 33.3-56.4, mean 47 mL/kg/min) (77). Neither of the studies found any correlation between DHEA and aerobic capacity, although a greater range in VO_2 peak was observed compared to our study. The hypothesis in paper IV was that individuals with a higher aerobic capacity would also show higher pre-test levels and higher production capacity of DHEA and DHEA-S in response to acute psychosocial stress. Both are considered anabolic hormones and have been proposed to have a protective role in the body (79). Since aerobic capacity and exercise training are associated with overall health and well-being (1), it would seem reasonable to suggest that there could be a connection to the health-promoting hormones DHEA and DHEA-S.

Longitudinal findings

One of the four papers included in this thesis comprised both baseline and follow-up data, presenting the results from the exercise training intervention. Due to the number of drop-outs in the intervention group, the results must be carefully interpreted but may still give some useful information.

Effects of exercise training

Paper III presented the main results from the RCT, that is, the analyses of the effects of six months of regular exercise training on physiological responses to acute psychosocial stress. Compliance in the intervention group was lower than expected, yet the aerobic capacity and time-to-exhaustion increased from baseline to follow-up, showing effects of the intervention despite a relatively large number of non-compliers (compliance will be discussed in more detail in the Methodological considerations-section).

Participants in the control group showed lower values for aerobic capacity at follow-up compared to baseline. This might be a result of reduced motivation to perform to exhaustion at the second VO_2 peak test. However, time-to-exhaustion did not change, indicating that oxygen uptake was lower, despite similar duration of the test.

Both the intervention and the control group showed reduced HPA-axis responses to the psychosocial stress test at follow-up compared to baseline, and no significant differences were seen between the groups. An adaptation seems to have occurred for both groups, which makes it difficult to interpret the results from the intervention. Previous research has found that when at least four months pass between the psychosocial stress tests, the risk of habituation to the test is small (80). Obviously, this did not seem to be true in our study. The participants were not informed that the same test would be repeated. Pre-test levels of ACTH and cortisol were higher at follow-up, indicating higher stress before the test started, maybe as a result of not knowing what to expect. However, when they realized that the follow-up test was almost the same as the one they had performed the first time, most participants responded with a lower reactivity. A blunted reactivity to the TSST was observed also in the aforementioned RCT by Klaperski et al (57), although the decrease was not as large as in our study. This points at the difficulties of repeating a stressor, and the importance of novelty when eliciting stress in laboratory settings.

Cross-stressor adaptation hypothesis

It was not possible to evaluate data from the longitudinal analyses in paper III, on the effects of the exercise training intervention, due to the assumed adaptations to the psychosocial stress test. Thus, since there are methodological limitations, the hypothesis can neither be confirmed, nor be ruled out. In fact, in paper II an association was observed between cortisol AUC_i at the physical and psychosocial stress tests. It was shown that the response followed the same pattern, even though the physiological answer was not as strong at the psychosocial stress test as at the physical stress test. However, this association is based on cross-sectional data, which cannot be used to prove any reason to the association.

The role of aerobic capacity was also analysed, both in paper IV and in additional analyses. No correlations were seen between level of aerobic capacity and HPA-axis response. Moreover, the plausible effects of the anabolic hormones DHEA and DHEA-S did not contribute to any explanation for the hypothesis.

On the other hand, it could be observed that the adaptation to the acute psychosocial stress test resulted in attenuated responses, indicating less pressure on the responding systems. Both groups showed reduced responses to the TSST at follow-up, maybe depending on a more effective reaction, saving redundant expenses for the body. In healthy women and men, this is probably an adequate response, showing that the systems operate appropriately.

Methodological considerations

Conducting an RCT is complicated and requires extensive efforts. There are many pitfalls along the road, which are not always easy to take into account in advance. Despite rigorous planning, unexpected events often occur during a study.

One of the inclusion criteria in the ASTI study was based on decisions to include a working population of both women and men. Because the study entailed assessments of hormones, an age range of 20 to 50 years was set to diminish the risk that female participants had entered menopause. We also wanted to study a healthy population, and thus individuals with chronic diseases such as diabetes or cardiovascular disease were not included. The exclusion criteria were set to ensure that the primary the outcome measure would be correctly interpreted and due to prerequisites for the performance of a peak oxygen uptake test and an exercise training intervention. For example, individuals taking beta-blockers, psychopharmacological drugs, glucocorticoids or asthma medicine were excluded due to plausible effects on the HPA-axis- and ANS outcome measures.

Several critical methodological issues need to be highlighted. First, in this study, we used the SGPALS questionnaire to identify individuals with low aerobic capacity. The self-assessed response alternative 1 corresponds to a sedentary life-style, and an assumption that the individual is untrained. However, the results from the VO_2 peak test showed levels of aerobic capacity that were higher than expected, corresponding to normal levels in the general population (81). Generally, self-reported physical activity levels tend to be overestimated compared to true levels (82, 83). Here, the opposite effect was probably seen. Since the invitation was directed towards untrained individuals who wanted to start to exercise regularly, respondents might have considered it more beneficial to report lower levels of physical activity. There were therefore fewer "couch potatoes" included than desired. There are reasons to believe that the most inactive individuals would not respond to an advertisement for an exercise training intervention study, and more precisely targeted information is required to reach that group.

There is evidence that health benefits are greatest when the activity level is increased from "sedentary" to "moderately active" (84). Thus, it would have been preferable to perform the VO_2 peak test on individuals before participation to ensure that only those with low aerobic capacity were included. However, this would have demanded more resources. In addition, the VO_2 peak test was used as a physical stress test, and a large battery of physiological assessments were made in connection to the test. For ethical reasons, it would not be possible to conduct the test on individuals who had not already been included and had given their written informed consent.

Second, at both baseline and follow-up, a large number of tests and assessments were performed. For every test or assessment there were risks of errors that could potentially affect the test results. Each test was associated with specific difficulties that had to be handled separately. The VO_2 peak test included several critical moments, one of which was the performance of the test protocol. If unaccustomed to the efforts of a physical test performed to exhaustion, the strain and eventual pain might be perceived as frightening, and the participant may end the test before a true peak in aerobic capacity has been reached. However, in this study values of oxygen uptake, respiratory exchange ratio and breathing frequency were controlled during the test, and the test was terminated only after reaching certain criteria (a plateau and/ or decrease in oxygen uptake, a respiratory exchange ratio above 1.1, hyperventilation and an inability to maintain the required cadence on the bicycle).

Another critical part of the VO_2 peak testing was the equipment measuring oxygen uptake. The mask used to collect the expired gases during the test had to be very tightly fitted because a leak would have given false values of gas exchange. Also, the expired gases had to be assessed correctly, and all technical equipment had to work satisfactorily. Results from two of the tests in our study could not be used because of technical issues with the equipment.

At both the physical and the psychosocial stress tests, blood samples were collected at seven time points. For some individuals, the blood supply was not sufficient at all time points. Despite the inserted venous catheter, which in most cases facilitated the sampling, and the well experienced personnel taking the samples, it resulted in some missing data. For blood pressure and heart rate monitoring, an automatic blood pressure cuff was used, assessing continuously before, during and after the tests. However, the device was turned off during the physical test, given the technical problems with assessment during the test. Also, immediately after the test, the device was not giving accurate outcomes, resulting in many missing +0 values for autonomic responses.

After randomization, the intervention took place. The intended level of exercise training was based on frequency, time and the intensity needed to plausibly affect the physiological systems (85). Considering the relatively untrained population included in this study, the exercise level was set rather high. Although theoretically motivated to start regular exercise training, most of the participants who were randomized to intervention group found the practical implementation challenging. The compliance was lower than expected, resulting in a decreased limit for what was counted as compliance. From the initial level of three times per week, 45-60 minutes per session, we decided to approve participants who exercised for at least two sessions per week, at least 45 minutes per session, in at least half of the intervention period. This decision limited the number of drop-outs but also weakened the results from the adherence to protocol analyses.

The weak compliance illustrates the efforts required to bring about behavioural changes. Although participants were well aware of the positive effects of exercise training and were instructed on how to proceed, it was difficult for them to start and maintain the new life-style. They were encouraged to increase the activity level stepwise in order to adapt both physically and mentally, starting at two times per week with a 30-minute duration. However, three times per week was a tough goal for many participants. Despite having contact with a coach to discuss obstacles connected to exercise training, the participants struggled with compliance. Unfortunately, the coaching personnel changed several times during the implementation of the study, interrupting the continuity of the coaching. Additionally, to objectively measure frequency, duration and heart rate during the exercise training sessions, participants wore a pulse watch. As it turned out, there were some unexpected problems with handling the watches, which resulted in some missing data from the exercise interventions. In the control group, no problems with compliance were seen. Only one individual reported disrupted participation due to unwillingness to be a part of the control group.

The next critical point was associated with the follow-up assessments. It seems that among participants in the control group, the motivation to go through somewhat unpleasant testing, without having performed any activity to improve the results, was low. The compensation (one-year free access to a gym) was perhaps not sufficiently attractive. A second follow-up for the control group, to measure physiological improvements after increased exercise training, might have been more tempting for participants in this group.

Ethical considerations

All participation in the study was voluntary, and the participants were informed of their right to withdraw their participation at any time without stating a reason. However, some aspects need to be discussed in an ethical perspective.

It is primarily the two stress tests that need to be highlighted. The physical stress test, for example, was demanding and not only physically. For the included individuals, who were not accustomed to maximal testing and who, in addition, were required to wear an uncomfortable respiratory mask, it was common to experience the situation as unpleasant. Also, pushing the body to maximal effort is related to pain and discomfort when legs hurt and the breathing frequency is high, even for a well-trained athlete. Another ethical aspect is the exposure of participants to acute psychosocial stress. As part of the test, the participants were met with no form of response from the committee, which some of the participants experienced as unpleasant. Normally, participants are debriefed and informed about the false set-ups directly after the test. Since a similar test was to be used at follow-up, however, it was not possible to debrief the participants at baseline.

One important consideration of ethical aspects when conducting an exercise intervention study including a non-exercising control group, is the appropriateness of instructing untrained individuals, who were motivated to start exercise, to refrain from increasing their level of physical activity. However, taking into account the non-training period preceding inclusion in the study, which was several years for many of the participants, six months is not a very long time to delay the start of exercise training. After the follow-up, all participants in the control group were offered a motivational group meeting and received 12 months of access to a fitness establishment.

Implications for future research

Based on lessons learned from performing the RCT, several thoughts about future research emerge. One of the most important aspects concerns the need for greater control of the intervention, starting with the inclusion of supervised exercise training sessions. It will require considerable resources, but maybe it is worth the cost to potentially be able to secure more complete and reliable data. It is also important to include the intended target group, which requires more extensive testing before inclusion. However, ethical questions must be taken into consideration that could potentially prohibit such procedures. The type of psychosocial stress test must be carefully considered to avoid the risk of adaptations.

This research field has no shortage of studies. On the contrary, many researchers have tried to explore the various possible perspectives, yet no consensus has been reached. The great variability in study designs and methods is a problem. The field is in need of clear guidelines to control for differences and facilitate comparisons. For example, intervention studies should use equal methods to evaluate effects of the intervention, and similar protocols for assessments of outcome measures. On the other side, individual variances are not easy to adjust for even in well-designed studies. Additionally, in every-day life variations are even larger, and clear results from laboratory testing might not be generalizable to real life. Maybe it is time to ask whether it is meaningful to measure specific hormones in different situations without controlling for all aspects. The next question would be: what are the benefits from a strictly regulated and standardized study that is not anchored in reality? Other aspects may be more interesting, for example, the experience of stress, or finding the individuals who are at risk of developing long-term stress in the future. Also, more focus should be on health-promoting aspects such as the anabolic hormones, and on finding beneficial keys to health rather than continuing to focus on factors that are deleterious to us.

Conclusions

Exercise training is important for overall health and wellbeing. High levels of aerobic capacity are associated with a lower risk of several diseases, including stress-related diseases. This thesis has studied the cross-stressor adaptation hypothesis, which presents a theory of one possible mechanism behind the positive effects of exercise training in stress-related illness. Unfortunately, methodological shortcomings obstructed analyses of the effect of the exercise training intervention on physiological responses to acute psychosocial stress. However, cross-sectional analyses of the correlations between aerobic capacity and hormonal responses to acute stress found no associations and thus could not confirm the theory. Neither did the subjective perception of stress at the psychosocial stress test correlate with the actual physiological response.

A more unexpected finding from the included papers was the extent of inter-individual differences seen in response to acute stress. Many factors seem to affect the reaction, making it difficult to control for all of them. The hormonal system is highly complex. We know it is essential for survival and that daily life without a well-functioning system would be challenging. In healthy individuals, HPA-axis and ANS seem to react adequately, independent of level of aerobic capacity. However, methodological limitations require careful interpretation of the included studies.

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