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Karolinska Institutet, Stockholm, Sweden

# **PHYSIOLOGY OF ADVENTURE RACING**

**– WITH EMPHASIS ON  
CIRCULATORY RESPONSE AND  
CARDIAC FATIGUE**

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*“We'll go because it's Thursday, and we'll go to wish everybody a Very Happy Thursday.”*

Winnie-the-Pooh

## ABSTRACT

The overall aims of this thesis were to elucidate the circulatory responses to ultra-endurance exercise (Adventure Racing), and furthermore, to contribute to the clarification of the so called “exercise-induced cardiac fatigue” in relation to said exercise.

An Adventure race (AR) varies in duration from six hours to over six days, in which the participants have to navigate through a number of check-points over a pre-set course, using a combination of three or more endurance/outdoor sports, *e.g.*, cycling, running, and kayaking. This thesis is based on the results from four different protocols; 12- and 24-h ( $n = 8$  and  $9$ , respectively) in a controlled setting with fixed exercise intensity, and 53-h and 5-7-day ( $n = 15$  in each) in field setting under race conditions. The subjects in all protocols were experienced adventure racing athletes, competitive at elite level. Study I and II address the circulatory responses and cardiovascular drift, using methods for monitoring heart rate (HR), oxygen uptake ( $\text{VO}_2$ ), cardiac output (non-invasive re-breathing) and blood pressure, during ergometer cycling at fixed steady state work rate at periods before, during and after the ultra-endurance exercise. In Study III and IV we examined the possible presence of exercise-induced cardiac fatigue after a 5-7-day AR, from two different perspectives. In Study III analyses were performed with biochemical methods to determine circulating levels of cardiac specific biomarkers (*i.e.*, creatine kinase isoenzyme MB (CK-MB), troponin I, B-type natriuretic peptide (BNP) and N-terminal prohormonal B-type natriuretic peptide (NT-proBNP)). We also made an attempt to relate increases in biomarkers to rated relative performance. In Study IV we used tissue velocity imaging (TVI) (VIVID I, GE VingMed Ultrasound, Norway) to determine whether the high workload (extreme duration) would induce signs of functional cardiac fatigue similar to those that occur in skeletal muscle, *i.e.*, decreased peak systolic velocities. Using conventional echocardiography we also evaluated whether the hearts of experienced ultra-endurance athletes are larger than the normal upper limit.

The central circulation changed in several steps in response to ultra-endurance exercise. Compared to initial levels,  $\text{VO}_2$  was increased at every time-point measured. The increase was attributed to peripheral adaptations, confirmed by a close correlation between change in  $\text{VO}_2$  and change in arterio-venous oxygen difference. The first step of the circulatory response was typical of normal (early) cardiovascular drift, with increased HR and concomitantly decreased stroke volume (SV) and oxygen pulse ( $\text{VO}_2/\text{HR}$ ), occurring over the first 4-6 h. The second step, which continued until approximately 12h, included reversed HR-drift, with normalisation of SV and  $\text{VO}_2/\text{HR}$ . When exercise continued for 50 h a late cardiovascular drift was noted, characterised by increased  $\text{VO}_2/\text{HR}$ , (indicating more efficient energy distribution), decreased peripheral resistance, increased SV, and decreased work of the heart. Since cardiac output was maintained at all-time points we interpret the changes as physiologically appropriate adaptations.

Our findings in Study III point towards a distinction between the clinical/pathological and the physiological/exercise-induced release of cardiac biomarkers. The results imply that troponin and CK-MB lack relevance in the (healthy) exercise setting, but that BNP, or NT-proBNP adjusted for exercise duration, might be a relevant indicator for impairment of exercise performance. High levels of NT-proBNP, up to  $2500 \text{ ng} \cdot \text{l}^{-1}$ , can be present after ultra-endurance exercise in healthy athletes without any subjective signs or clinical symptoms of heart failure. However, these high levels of NT-proBNP seemed to be associated with decreased relative exercise performance, and might be an indicator of the cardiac fatigue that has previously been described after endurance exercise.

Study IV revealed that the sizes of the hearts (left ventricle) of all of our ultra-endurance athletes were within normal limits. The measurements of peak systolic velocities showed (for group average) no signs of cardiac fatigue even after 6 days of continuous exercise. This discrepancy between ours and other studies, involving *e.g.*, marathon or triathlon, might reflect the fact that this type of exercise is performed at relatively low average intensity, suggesting that the intensity, rather than the duration, of exercise is the primary determinant of cardiac fatigue.

## LIST OF PAPERS

This thesis is based on the four papers listed below, which will be referred to throughout this work by their Roman numerals.

- I. **Mattsson CM**, Enqvist JK, Brink-Elfegoun T, Johansson PH, Bakkman L, Ekblom B. “Reversed drift in heart rate but increased oxygen uptake at fixed work rate during 24 hours ultra-endurance exercise.” *Scand J Med Sci Sports* **20**(2):298-304, 2010.
- II. **Mattsson CM**, Ståhlberg M, Larsen FJ, Braunsweig F, Ekblom B. “Late cardiovascular drift observable during ultra endurance exercise.” *Med Sci Sports Exerc*, Epub 1 Dec, 2010.
- III. **Mattsson CM**, Berglund B, Ekblom B. “Extreme values of NT-proBNP after ultra-endurance exercise in healthy athletes – Related to impaired exercise performance?” *Submitted manuscript*.
- IV. **Mattsson CM**, Lind B, Enqvist JK, Mårtensson M, Ekblom B, Brodin L-Å. “No evidence of cardiac fatigue in tissue velocity curves at rest after 6 days of ultra-endurance exercise.” *Submitted manuscript*.

## RELATED PAPERS

1. Fernstrom M, Bakkman L, Tonkonogi M, Shabalina IG, Rozhdestvenskaya Z, **Mattsson CM**, Enqvist JK, Ekblom B and Sahlin K. "Reduced efficiency, but increased fat oxidation, in mitochondria from human skeletal muscle after 24-h ultra-endurance exercise." *J Appl Physiol* **102**(5):1844-9, 2007.
2. Berg U, Enqvist JK, **Mattsson CM**, Carlsson-Skwirut C, Sundberg CJ, Ekblom B and Bang P. "Lack of sex differences in the IGF-IGFBP response to ultra endurance exercise." *Scand J Med Sci Sports* **18**(6):706-14, 2008.
3. Sahlin K, Shabalina I, **Mattsson CM**, Bakkman L, Fernström M, Rozhdestvenskaya Z, Enqvist JK, Nedergaard J, Ekblom B, Tonkonogi M. "Ultra-endurance exercise increases the production of reactive oxygen species in isolated mitochondria from human skeletal muscle." *J Appl Physiol* **108**(4):780-787, 2010.
4. Enqvist JK, **Mattsson CM**, Johansson PH, Brink-Elfegoun T, Bakkman L, Ekblom B. "Energy turn-over during 24-hours and 6 days of Adventure Racing." *J Sports Sci* **28**(9):947-955, 2010.
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6. Wichardt E, **Mattsson CM**, Ekblom B, Henriksson-Larsén K. "Rhabdomyolysis/myoglobinemia and NSAID during 48-hours ultra-endurance exercise (adventure racing)" *Eur J Appl Physiol*, Epub Dec 22, 2010.
7. Borgenvik M, Nordin M, **Mattsson CM**, Enqvist JK, Ekblom B, Blomstrand E. "Alterations in amino acid concentrations in the plasma and muscle in human subjects during 24 hours or 6 days of ultra-endurance exercise." *Submitted manuscript*.
8. **Mattsson CM**, Enqvist JK, Ekblom B. "The Adventure Racing athlete: a physiological profile." *Submitted manuscript*.
9. **Mattsson CM**, Ståhlberg M, Ekblom OE, Braunschweig F, Ekblom B. "Reliability of inert gas rebreathing method for non-invasive determination of cardiac output at rest and different work rates." *In manuscript*.

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## LIST OF ABBREVIATIONS

2CH	Apical two-chamber view
2D	Two-dimensional
4CH	Apical four-chamber view
A	Late diastolic blood flow velocity
AO	Aortic root diameter
AR	Adventure race
ARWC	Adventure Racing World Championship
ASE	American Society of Echocardiography
AV	Atrioventricular
a-v O <sub>2</sub> diff	Arterio-venous oxygen difference
BMI	Body mass index
BNP	B-type natriuretic peptide; (Brain natriuretic peptide)
BP	Blood pressure
Bpm	Beats per minute
BSA	Body surface area
CBF	Cutaneous blood flow
CK-MB	Creatine kinase isoenzyme MB
CO	Cardiac output
CO <sub>RB</sub>	Cardiac output determined using non-invasive gas rebreathing
CV	Coefficient of variation
CW	Cardiac work
E	Early diastolic blood flow velocity
<i>e.g.</i>	exempli gratia [latin], for example
E/A	Ratio of early [E] to late [A] filling
ECLIA	Enhanced chemiluminiscence Immunoassay
EF	Ejection fraction
Epi	Epinephrine; adrenaline
FFM	Fat-free mass
Hb	Hemoglobin
Hct	Hematocrit; erythrocyte volume fraction
HR	Heart rate
HR <sub>max</sub>	Maximal heart rate
<i>i.e.</i>	id est [latin], that is; in other words
IRMA	Immunoradiometric assay
IVC	Isovolumic contraction
IVR	Isovolumic relaxation
IVSw	Thickness of the intraventricular septal wall
LoA	Limits of agreement
LV	Left ventricle
LVd	Left ventricular diameter (maximal during diastole)
LVpw	Thickness of the posterial wall of the left ventricle
MAP	Mean arterial pressure
n.d.	not detectable
NE	Norepinephrine; noradrenaline

NT-proBNP	N-terminal prohormonal B-type natriuretic peptide; (N-terminal pro-brain natriuretic peptide)
PLAX	Parasternal long axis view
PSV	Peak systolic velocity
$R$	Correlation coefficient
Rec	Recovery
RER	Respiratory exchange ratio
ROI	Region of interest
RPP	Rate pressure product
RV	Right ventricle
SD	Standard deviation
SV	Stroke volume
SW	Stroke work
TPR	Total peripheral resistance
TVI	Tissue velocity imaging
$V_E$	Pulmonary ventilation
$VO_2$	Oxygen uptake
$VO_2/HR$	Oxygen pulse
$VO_{2max}$	Maximal oxygen uptake at whole body work
$VO_{2peak}$	Peak oxygen uptake at specific exercise mode
W	Watt

# 1 INTRODUCTION

## 1.1 HISTORICAL ASPECTS OF ULTRA-ENDURANCE EXERCISE

The first thing that comes to peoples mind when you talk about endurance exercise is most often marathon running. The marathon allegedly dates back to ancient Greece where the messenger Pheidippides ran the approximately 40 km from the battlefield back to the capital Athens. He overcame the extreme physiological strain, delivered the good news of victory and when fell dead to the ground. However, in the eye of ultra-endurance exercise the marathon is a super-sprint!

Yet, ultra-endurance exercise is definitely not a new phenomenon. In 1762 Tomas Hauge sat the world record when he ran 100 miles in 23 h 15 min. A century later, pedestrianism (*i.e.*, endurance walking) had become popular. The longest races possible were 6-day races, that is, without competing on Sunday. The American Edward Payson Weston had a long-lasting professional walking career, holding numerous records for long-distance endurance events. For example, in 1879 he defeated the British champion "Blower" Brown, in a 550 mile (890 km) match that he completed in the impressive time 141 hours 44 minutes (less than 6 days)!



Still, people are always looking for new challenges. At the same rate as humans try to push boundaries and break the barriers of what the organism is capable of, new sports are created. One of these new extreme sports is adventure racing. This type of competitions first occurred in New Zealand in the 1980s as a combination of transferring Ironman Triathlon into the wild and away from strict courses, and to perform demanding expeditions in the form of a race. The Alpine Ironman, held in 1980, has been considered as the first adventure race (AR). Competitors ran, kayaked and skied individually for three days. A couple of years later, the

first edition of the now famous Coast to Coast race was held (243 km; 151 miles). In this race skiing was exchanged for cycling, setting the standard for which disciplines mostly involved in the modern adventure racing: trail running, cycling and paddling. Independently, a North American race, the Alaska Mountain Wilderness Classic debuted in 1982 and involved six days of unsupported wilderness racing (250-400 km; 150-250 miles). The race is still annual and the rules are simple: start to finish with no outside support, requiring that racers carry all food and equipment. In 1989, the modern era of adventure racing had clearly arrived with the launch of the Raid Gauloises. This race contained all the modern elements of adventure racing, including mixed-gender teams competing in a multi-day 400+ mile race. Over the years adventure racing has grown larger and spread all over the world. The major international competitions are becoming more and more prestigious and the financial incentives involved have substantially increased. The requirement for scientific structure for preparation and execution of the races naturally rises to the same degree.

### **1.1.1 Adventure racing**

The modern adventure racing is an ultra-endurance sport, in which a pre-set course is covered in the shortest possible time using a combination of three or more endurance/outdoor sports, *e.g.*, cycling, running, kayaking and other exercise modes. Other exercise modes that can be included are for example in-line skating, canyoneering, mountaineering, river rafting, cross-country skiing and they vary with the location of the race and the time of year. The duration of an AR varies from six hours to over six days depending on the type of competition. During these competitions participants have to navigate, through a number of check-points, from start to finish. A team normally consists of three males and one female, or four males. The teams must stay close together for the entire course. Each team member has to carry a backpack with compulsory gear, weighing approximately 5-10 kg. The races are most often of non-stop-character and it is at each team's discretion to decide when, and how much, to eat, rest and sleep, while the race clock keeps going. Teams arrange their own food, but can be allowed to receive assistance from a support team at transition areas (where athletes change exercise modes) approximately every 6-24 h. The races are held in a variety of weather and harsh environments where the team's speed is dictated by its weakest team member. To complete the race as fast as possible the athletes within the team help each other, *e.g.*, stronger racers carry more of the food and equipment and/or take a weaker team member in tow (*i.e.*, a faster runner tows a slower runner with an elastic cord attached between their waists). A race strategy that actually has been

scientifically evaluated with the conclusion that towing improved overall running performance considerably (Grabowski and Kram, 2008).

Thus, an AR varies in form and duration, but is no matter the nature of the specific competition still in many aspects an extreme sport, compared with more traditional endurance events. AR puts extreme physical and psychological demands on the participants due to its extreme duration and non-stop nature. The adventure racing athlete does not need to perform high maximum speed in each discipline, although this can occur. Instead, the demands of both endurance and technique are extremely high. Furthermore, we have estimated the total energy expenditure for a 24-h race for a fit male adventure racer to approximately 18-20 000 kcal, which is almost 10 times more than normal basal metabolism (Enqvist *et al.*, 2010). The effect of sleep deprivation on both mental and physiological functions during the races must be included in the total complex of problems during these extreme exercise durations. This makes the adventure racer to an extreme version concerning human performance, and from a human biological point of view the sport becomes very attractive to investigate. In consequence, the physiological adaptation to ultra-endurance exercise is versatile, with interesting research questions concerning for example energy balance, dietary intake and its consequences for choice of energy substrate during exercise, blood lipid changes, circulatory and muscular adaptations, hormonal status, immunological response, etc.

## **1.2 THE PROJECT “PHYSIOLOGY OF ADVENTURE RACING”**

The project “Physiology of Adventure Racing” was initiated in the spring of 2005 in a collaboration between Karolinska Institutet and The Swedish School of Sport and Health Sciences. Empirical observations claimed that athletes who have been engaged in this type of sport for several years have a large advantage compared to novices in the sport. It depends of course in part on the fact that they are more experienced, but even athletes from traditional endurance sports with a documented higher aerobic capacity (*i.e.*, higher  $\text{VO}_{2\text{max}}$ ) have difficulties to keep up with ultra-endurance specialists when exercise duration exceeds three or four hours. The research group was assembled with a combination of students that knew the sport and its problems (among others, the author of this thesis) and skilled experienced researchers. The initial approach was very broad and based on the vague hypothesis that the successful AR-athletes differed physiologically in some way from successful athletes in other (more traditional) endurance sports. In order to address as many aspects as possible and still

maintain a high scientific level several other research groups were invited to take part in the protocols and perform investigations in their field of specialty. So far, in addition to the four papers in this thesis, six papers have been published (Related papers 1-6, *i.e.*, Fernström et al., 2007; Berg et al., 2008; Sahlin et al., 2010; Enqvist et al., 2010; Wallberg et al., in press; Wichardt et al., in press) three are in manuscript (Related papers 7-9, *i.e.*, Borgenvik et al., submitted; Mattsson et al., submitted manuscript; Mattsson et al., in manuscript) and a handful more are under preparation.

### **1.3 PREVIOUS RESEARCH ON ADVENTURE RACING**

Since AR is a relatively new and rather small sport the scientific grounds surrounding it are still very limited. Like the main part of investigations on other ultra-endurance events the articles on AR are almost always based on data collected before and after the race. Measurements at any points during the exercise itself are non-existent. Another aggravating factor for this type of research is that normally there is no possibility to reliably standardise parameters such as work load, rest, sleep and diet during competition-based investigations.

When our project started in 2005, less than fifteen peer-reviewed studies could be found, and all of them were concerning medical aspects as injuries and infectious diseases. Fortunately, during the last five-six years a few other research groups have added information to the scientific knowledge base within this area. I will in the following paragraphs mention some key investigations concerning AR that have been conducted and published up till now (February, 2011).

#### **1.3.1 Outbreak of infectious diseases**

Several outbreaks of infectious diseases have been reported in conjunction with ARs. The first one was 13 cases of African tick-bite fever, caused by *Rickettsia africae*, in competitors after an AR in South Africa (Fournier et al., 1998). Other examples are outbreaks of leptospirosis following an "extreme-adventure" athletic event on the island of Guam (Haddock et al., 2002), during the race Eco-Challenge in Malaysia 2000 (Sejvar et al., 2003) and among participants in an AR in Florida, USA 2005 (Stern et al., 2010).

### 1.3.2 Injuries

Several studies have addressed the question of injuries during this extreme sport. In the beginning Kohler (2003) discussed the risk for injuries and the role of the sports chiropractor. Greenland (2004) also wrote an article along the same line of thought discussing the new roles of the medical staff involved in this type of races. After that, epidemiological studies of the injury spectra in AR athletes have been conducted. Fordham and co-workers (2004) studied the impact of the demographics and training characteristics of AR athletes on injury location and characteristics. The same year did Talbot and colleagues (2004) report that the prevalence of altitude illness was over 14% during the Primal Quest 2002 (a four-or-more-days non-stop race). During the same race the incidence and type of injury and illness occurring during an AR was examined, specifically identifying those resulting in withdrawal from the event (Townes et al., 2004). A similar study was also performed during the same race the following year (McLaughlin, 2006) reporting a comparable incidence of patient encounters with a high frequency of minor skin and soft tissue injuries, especially blisters. During the expedition-length (*i.e.*, >36 h) race Southern Traverse 2003 as many as 38 out of 48 investigated racers (79%) had musculoskeletal injuries after the race. All of the racers experienced pain during or after the race (Anglem et al., 2008). To conclude, musculoskeletal injury and complaint during ARs is commonly reported (Townes et al., 2004; Anglem et al., 2008; Newham-West et al., 2010; Wichardt et al., in press).

### 1.3.3 Exercise intensity and energy expenditure

Beside medical considerations exercise intensity and energy expenditure has been a subject of investigation. Ashley and colleagues (2006) reported that HR averaged  $>100 \text{ beats} \cdot \text{min}^{-1}$  in a competitor who finished a 300 km course in approximately 100 h. Helge and co-workers (2007) reported a heart rate (HR) of 80% of HR-reserve (peak – rest HR, *i.e.*, similar to exercise intensity measured as % of  $\text{VO}_{2\text{max}}$ ) in the beginning of a race, which was reduced to about 40% of HR-reserve after 15 h and remained on a similar level for the rest of the 115 h of racing. The same relative intensity and the decrease in intensity over time have later been verified (Lucas et al., 2008; Enqvist et al., 2010). Typically, the exercise intensity during an expedition-length AR is  $>60\%$  of HR-reserve during the first 12 h and progressively fall to approximately 40% of the HR-reserve at 24 h and remain at that level for the rest of the race. Concerning nutrition and energy turn-over Zimberg and co-workers (2008) estimated an energy expenditure (EE) of 24 500 kcal ( $\approx 365 \text{ kcal} \cdot \text{h}^{-1}$ ) and an energy intake (EI) of 14 700 kcal during a simulated 67 h AR. It is difficult to eat and drink enough during an AR, indicating that the typical race

competitors are unable to consume enough calories to offset their energy use. Our research group has found higher EE,  $\approx 750 \text{ kcal} \cdot \text{h}^{-1}$  during 24-h and  $\approx 365 \text{ kcal} \cdot \text{h}^{-1}$  during a 5-7-day race (Enqvist et al., 2010), but the simple explanation for our higher values could be that our subjects had higher body mass (BM). It should be recognised that AR can generate a significant negative energy balance, and that the adventure racers often present an inadequate nutritional profile both during racing and training (Zalcman et al., 2007; Zimberg et al., 2008; Enqvist et al., 2010).

### **1.3.4 Other investigations**

The objective of the present thesis is circulatory response and cardiac fatigue. Unfortunately, the existing scientific knowledge is meager and only one study has examined how the heart is affected during AR (Ashley et al., 2006), which is discussed more thoroughly in the following sections.

Apart from the papers listed above and our research group's scientific contribution (Related papers), there are a couple of diverse investigations. Lucas et al. (2009) investigated cognitive function and strength capacity after the Southern Traverse 2003 and found that only complex decision making was impaired by the race, and that strength was only modestly impacted ( $<20\%$ ), at least relative to the extent of decrease in pace that occurs in these races. Levada-Pires and colleagues (2010) examined the risk of post-exercise immunosuppression after the Ecomotion Pró 4-5-day race in Brazil, and they concluded that the race induced neutrophil and lymphocyte death.

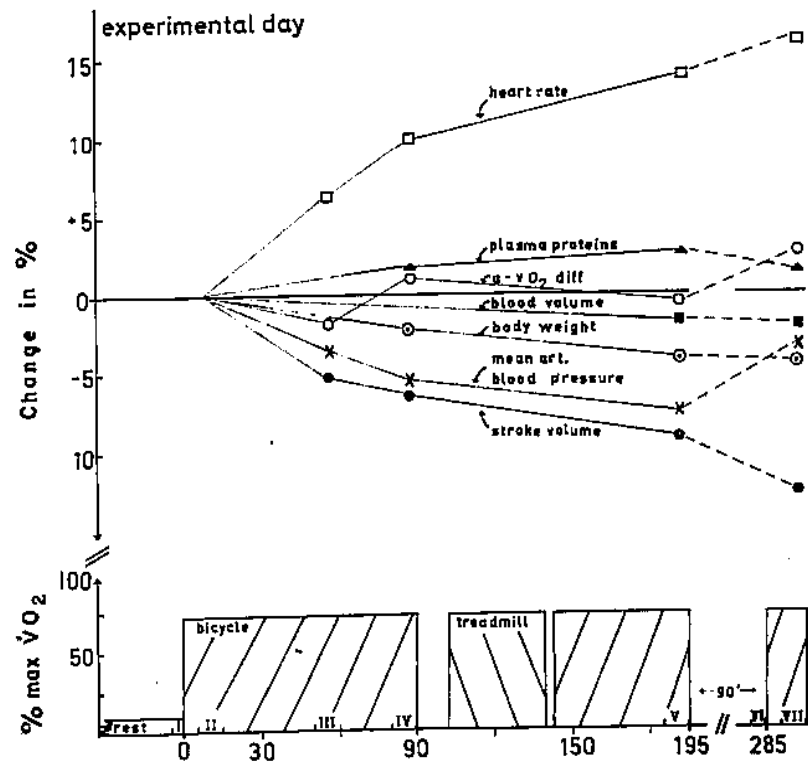
## **1.4 CIRCULATORY ADAPTATIONS**

### **1.4.1 Cardiovascular drift**

The central circulation changes during prolonged endurance exercise. The “cardiovascular drift” describes a number of well-established circulatory adaptations to prolonged endurance exercise at fixed work rate. Heart rate (HR) increases slowly during exercise at fixed work rate, with a concomitant decrease in stroke volume (SV) (Figure 1) (Åstrand et al., 1960; Ekelund, 1967; Saltin and Stenberg, 1964). In these articles the durations of exercise were six hours, three hours, and one hour, respectively. Even if physical training improves several circulatory parameters, for example increased maximal SV and decreased HR at a submaximal rate of work, the HR drift

with time remains (Ekblom, 1970). The cardiovascular drift is measurable already after 10 minutes of moderate exercise in both neutral and warm environments (Ekelund, 1967).

**Figure 1:** Circulatory adaptation to prolonged exercise at 75% of  $\dot{V}O_{2\max}$ .



Lower part: Experimental procedure for the experimental day. The effective work time during the prolonged period was 180 min (rest pauses included, 195 min). Upper part: the percentual changes in heart rate, plasma proteins, arterio-venous oxygen difference, blood volume, body weight, mean arterial blood pressure and stroke volume during exercise. (Saltin & Stenberg, 1964)

The decrease in SV during prolonged exercise is commonly suggested to be caused by a progressive increase in cutaneous blood flow (CBF) as core temperature rises, reducing central venous blood pressure and end-diastolic volume (Johnson and Rowell, 1975; Rowell, 1974). Consequently, dehydration during prolonged exercise also reduces total blood volume, which further contributes to the cardiovascular drift (González-Alonso et al., 1995; Saltin, 1964). However, the core temperature and the CBF remain stable after 20 min of exercise. It is therefore suggested that any further decline in SV is a consequence of an increase in HR (Coyle and González-Alonso, 2001; Fritzsche et al., 1999). This implies that the cardiovascular drift is initially driven by decreased SV but any change after approximately half an hour is instead driven by increased HR.

If this “classical” cardiovascular drift was to continuously progress during a 5-7-day AR it would implicate athletes with maximal HR when standing still. Since we know empirically that the participants are able to complete the races, and keeping a relatively high pace even during the later stages, this is highly unlikely. Some clarifying information was provided in an early study by Irma Åstrand (1960) where the subjects cycled and ran at fixed work rates corresponding to 50% of maximal oxygen uptake ( $VO_{2max}$ ) simulating a full physically demanding workday (7 bouts à 50 min during 8 h). HR increased on an average 11 beats  $\cdot$  min<sup>-1</sup> from the first two to the last two bouts. This is a similar increase after approximately six hours of exercise as after the three hours in the study by Saltin and Stenberg (1964), which indicates that the drift does not have a continuous progression. However, the cardiovascular drift during the full duration of an AR is unknown.

Besides the increase in HR, the oxygen uptake ( $VO_2$ ) at standardised submaximal work rate increased with on average 6% ( $0.09 \text{ l} \cdot \text{min}^{-1}$ ) in the study by Åstrand (1960). Saltin and Stenberg (1964) confirmed the magnitude, 5%, of the concomitant  $VO_2$  drift during 3 h exercise. The drift could be ascribed to decreased economy of movement, with general muscle fatigue leading to recruitment of alternative muscle fibres and agonist muscles (Dick and Cavanaugh, 1987; Westerlind et al., 1992). The increase in  $VO_2$  means that the work efficiency is decreased, and thus that more and more energy is required to maintain work rate or race pace. Since negative energy balance already is a problem for adventure racers the upward drift in  $VO_2$  is highly unwelcome, especially if the drift would continue along with exercise duration.

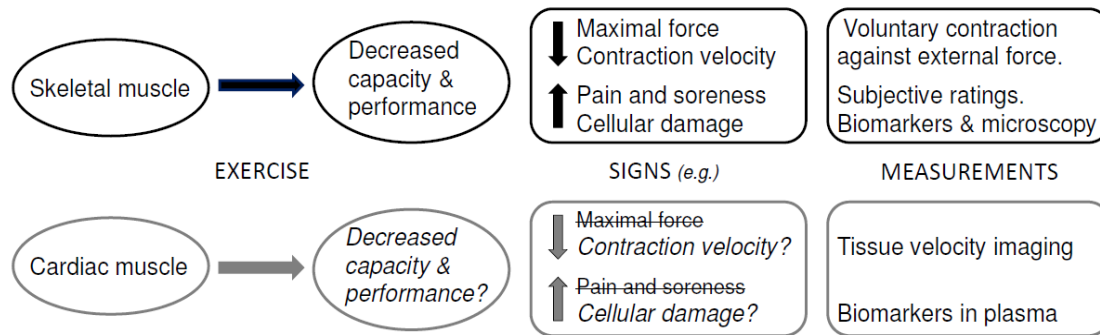
Even though the increased  $VO_2$  may be concomitant with the changes in HR, these drifts are not necessarily of the same origin.

## **1.5 CARDIAC DAMAGE AND “EXERCISE-INDUCED CARDIAC FATIGUE”**

The concept of exercise-induced cardiac fatigue was in modern time first proposed by Saltin and Stenberg in 1964 as an explanation for the reduced SV observed during prolonged exercise. However, the concept of cardiac fatigue is debated and so far there is no consensus. The general belief is that our hearts, if healthy, are unaffected by any exercise or strain we humans can expose it to, and that it simply continues to work until the day when it is all over. The major

obstacle to overcome is to find methods that actually can determine if there is such a thing as decreased cardiac capacity and performance. An overall hypothesis in Study III and IV was that cardiac muscle can be fatigued in the same way as skeletal muscle (Figure 2).

**Figure 2:** Schematic model of signs and measurements of exercise-induced fatigue in skeletal and cardiac muscle.



For investigations of skeletal muscle fatigue it is possible both to directly measure the maximal voluntary force and contraction velocity against external resistance, and to extract muscle biopsies and perform biochemical and microscopy analyses on the material. Unfortunately, these types of investigation are not available for evaluation of cardiac function in healthy humans. A remaining possibility, for both skeletal and cardiac muscle, is analyses of biomarkers in blood. Microscopy findings has proven damaged skeletal muscle cells after strenuous exercise, with a concurrent increase in plasma levels of biomarkers *e.g.*, creatine kinase (CK). The isoenzyme CK-MB is expressed in cardiac muscle and has therefore been used extensively as an indication for myocardial damage in heart attacks. Even though other, more specific biomarkers (further discussed in section “1.5.1 Cardiac biomarkers”) has replaced CK-MB in the clinical setting we report three different biomarkers in Study III. All of these biomarkers are a sign of muscle cell degradation and damage. However, both concerning skeletal and cardiac muscle, the exercise induced elevation in biomarkers is rapidly reversed, *i.e.*, within a few days. The same holds true for decrement maximal force and velocity of contraction in skeletal muscle. In a related manner, the measurable decrease in capacity and performance in skeletal muscle returns to initial levels after a period of rest. Thus, we interpret all rapidly reversed exercise-induced signs of cardiac damage as cardiac fatigue, and, hence, the expression “cardiac fatigue” is used throughout this text.

### 1.5.1 Cardiac biomarkers

Cardiac troponin T and I are considered to be highly sensitive and specific markers for detecting myocardial damage even in the presence of skeletal muscle injury. Several studies have demonstrated increases in troponin I or T after prolonged exercise, which would indicate that physical exertion may result in myocardial damage (Rifai et al., 1999; Neumayr et al., 2002; Scharhag et al., 2005; Neilan et al., 2006). A recent meta-analysis suggested that despite substantial methodological variation among studies, exercise-induced release of troponin T can be observed after exercise in almost half of the athletes participating in endurance exercise events (Shave et al., 2007). How these troponins are released is not entirely clear, but based on the transient nature of the increases, the mechanism has been hypothesised to be leakage of troponin from the cytosolic pool (Wu and Ford, 1999) due to membrane damage induced by oxidative stress or hypoxia (Michielsen et al., 2008). This notion is compatible with the increased production of reactive oxygen species in skeletal muscle that we have previously shown after 24-h of ultra-endurance exercise (Sahlin et al., 2010).

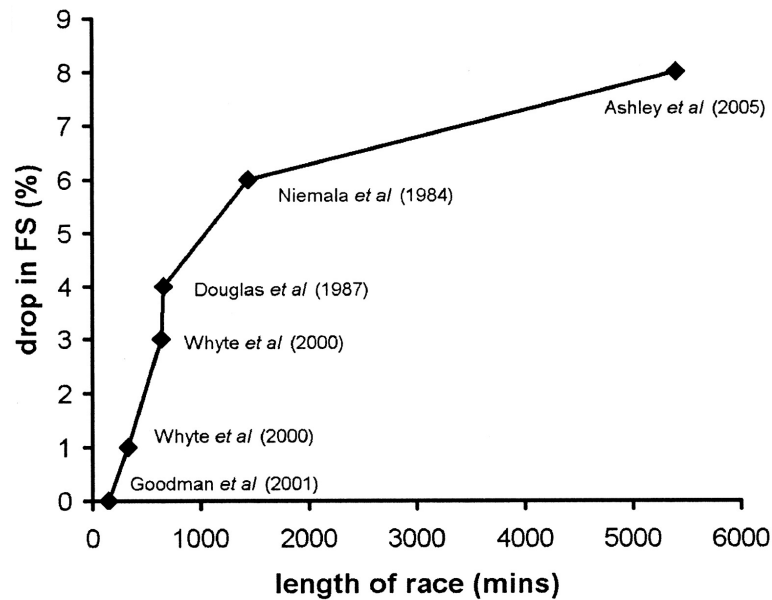
In clinical medicine, natriuretic peptides, including B-type natriuretic peptide (BNP) and the more stable N-terminal prohormonal B-type natriuretic peptide (NT-proBNP), are used to detect increased myocardial wall tension in conditions such as heart failure. Tachycardia, arrhythmias and physical exercise may also increase these peptides. Increases in BNP have been shown in patients with left ventricular dysfunction and BNP levels correlate both with heart failure stage according to the New York Heart Association classification, and with prognosis of cardiac dysfunction (Wei et al., 1993; Yoshimura et al., 1993; Yasue et al., 1994; Omland et al., 1996; Clerico et al., 1998). The suggested optimal cut-off point for BNP for making the diagnosis of congestive heart failure is  $>100 \text{ ng} \cdot \text{l}^{-1}$ , and the negative predictive value is  $<50 \text{ ng} \cdot \text{l}^{-1}$  (Maisel et al., 2002; 2004). The clinical reference value for NT-proBNP in healthy subjects is  $<100 \text{ ng} \cdot \text{l}^{-1}$ ; in clinical practice, values  $>300 \text{ ng} \cdot \text{l}^{-1}$  are strong indicators of heart failure and values  $>5000 \text{ ng} \cdot \text{l}^{-1}$  are highly significant predictors of mortality within three months (Januzzi et al., 2005; Januzzi, 2006). The highest values of NT-proBNP previously reported in healthy athletes are approximately  $600 \text{ ng} \cdot \text{l}^{-1}$  after a marathon (Niessner et al., 2003; Neilan et al., 2006) and after  $\approx 10 \text{ h}$  of running (Neumayr et al., 2005). Outliers in a study examining marathon, 100-km running and MTB-marathon (cycling) reached approximately  $900 \text{ ng} \cdot \text{l}^{-1}$  (Scharhag et al., 2005).

### 1.5.2 Cardiac function – Echocardiography

Employing two-dimensional echocardiography left ventricular function at rest, in the form of *e.g.*, decreased ejection fraction, has been shown to be reversibly impaired after marathon (George et al., 2004; Whyte et al., 2005; Middleton et al., 2007), ultra marathon (Niemelä et al., 1984; 1987), Ironman triathlon (Douglas et al., 1987; 1990a; 1990b; 1998; Whyte et al., 2000), and adventure racing (Ashley et al., 2006) (Figure 3). After this type of triathlon a significant reduction in atrial function (reflected in the ratio of early [E] to late [A], filling; E/A) has also been detected (Shave et al., 2004).

Although several other investigations have come to opposite conclusions, on the basis of their meta-analysis of findings on exercise for a maximum of 24 h Middleton and co-workers (2006) maintain that the ejection fraction and systolic blood pressure/end-systolic volume are, indeed, attenuated, indicating impaired systolic function, in part due to altered cardiac loading. In addition, this same meta-analysis revealed impairment of left ventricular relaxation, manifested by a decrease in E/A, in this case unrelated to changes in loading. The only published study concerning the effects of exercise of extreme duration (an 84-110-hour race) found reduction in both systolic and diastolic left ventricular function at rest following this exercise, with decreases in fractional shortening, ejection fraction and E/A (Ashley et al., 2006) (Figure 3).

**Figure 3:** Changes in fractional shortening.



Decline in fractional shortening plotted against length of race as reported for key studies. In the current study, the exercise challenge and drop in fractional shortening were greater than previously reported. FS = fractional shortening. (Ashley et al., 2006)

However, it should be noted that fractional shortening and ejection fraction does not give any information about SV, CO or if the heart is matching the metabolic demand.

In their recent review, Oxborough and colleagues (2010) arrived at conclusions similar to those reached by Middleton and co-workers (2006). In addition, they proposed that modern ultrasound techniques, which provide a more complete assessment of cardiac mechanistic function, should be applied to clarify the phenomenon of exercise-induced cardiac fatigue in great detail.

### **1.5.3 Cardiac function – Tissue velocity imaging**

Tissue velocity imaging (TVI), a well-established procedure for quantitative analysis of longitudinal myocardial velocities (Brodin, 2004), has been shown to be a powerful tool for quantifying regional ventricular function, and thereby a valuable aid in the diagnosis of patients with various types of heart disease (Palmes et al., 2000; Kiraly et al., 2003; Hayashi et al., 2006). By focusing on specific regions of interest (ROIs) in a sequence of TVI images, velocity parameters for both the left and right ventricles can be measured. The most clinically useful information obtained from such a myocardial velocity curve concerns the peak systolic velocity (PSV) and both maximal early (E', occurring during early diastolic filling) and late diastolic (A', occurring during atrial contraction) velocities. In addition to these main events of primary interest, information about the contractile and elastic function of the heart can be extracted from the periods of isovolumic contraction (IVC) and isovolumic relaxation (IVR), which contain short myocardial deformations involved in pre- and post-systolic reshaping of the ventricles (Edvardsen et al., 2002; Lind et al., 2004). One study involving assessment by colour TVI revealed that scarred segments of the myocardium in patients with coronary artery disease exhibit lower peak systolic velocities than the corresponding segments in healthy volunteers, both at rest and under stress, and, furthermore, that the ischemic segments demonstrate lower peak velocities and smaller increments in velocity (Pasquet et al., 1999).

## 2 AIMS

The overall aims of this thesis were to elucidate the circulatory responses to ultra-endurance exercise (Adventure Racing), and furthermore, to contribute to the clarification of the so called “Exercise-induced cardiac fatigue” in relation to said exercise.

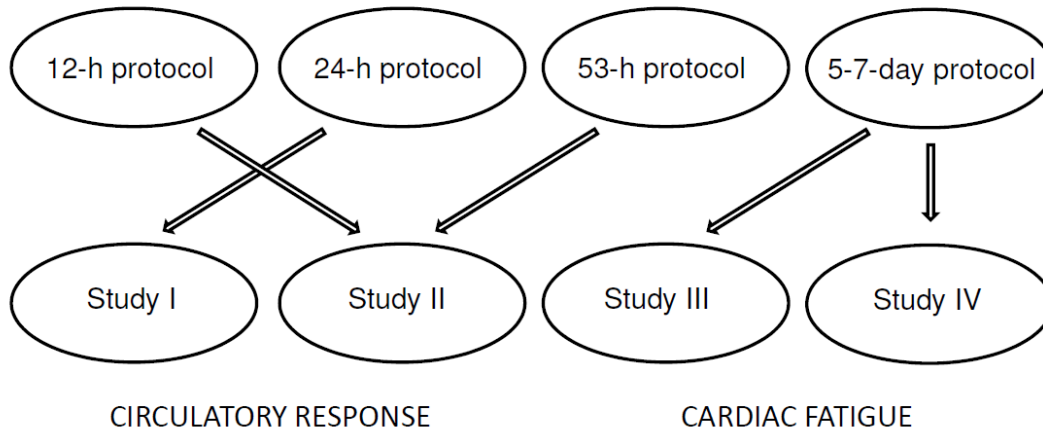
More specifically, the main aim of each of the individual papers was:

- I. To investigate the cardiovascular response at constant work rate during 24 h ultra-endurance exercise.
- II. To investigate the nature of the acute adaptation of the central circulation to ultra-endurance exercise seen in paper I, including the relative contributions of changes in SV and arterio-venous oxygen difference (a-v O<sub>2</sub> diff) to the increased oxygen pulse (VO<sub>2</sub>/HR), at different stages during prolonged endurance exercise. Furthermore, to relate our results to the work of the heart and the discussion of exercise-induced cardiac fatigue.
- III. To examine the levels of different cardiac biomarkers in response to ultra-endurance exercise, *i.e.*, 5-7 days of almost non-stop exercise in a competition with mixed endurance exercise events, so called adventure racing. In addition, we make an attempt to relate increases in biomarkers to rated relative performance.
- IV. To determine whether a high workload (extreme duration) induces signs of cardiac fatigue similar to those that occur in skeletal muscle, *i.e.*, decreased peak systolic velocities. A secondary aim was to evaluate whether the hearts of experienced ultra-endurance athletes are larger than the normal upper limit.

### 3 SUBJECTS AND METHODS

The four studies included in this thesis are based on data from four different exercise protocols (Figure 4). Overall, results and conclusions concerning circulatory response are derived from Study I and II, and those concerning cardiac fatigue from Study III and IV.

**Figure 4:** Schematic model of connections between exercise protocols, studies and primary area of research.



#### 3.1 SUBJECTS

The sport of Adventure racing imposes incredibly diverse physiological strains on the human body, and the participants have to balance demands such as sufficient endogenous storage of fat and high ability to carry, against sustained ability to run and perform other body mass related tasks. It seems desirable to simultaneously have a low body mass for running, strong thighs for cycling, and a dominant upper body for kayaking. Based on our findings the adventure racers display a distinct profile, in both anthropometric and physiological aspects, which differs from the specialist athletes' (*i.e.*, marathon runners, cyclists and kayakers, respectively). The athletes are relatively alike concerning body size and aerobic capacity, despite major differences in the training regime, hours of exercise each week, and years of experience. Compared to other endurance athletes the typical adventure racer is rather large and overall well-trained. However, we cannot distinguish if the similarities are due to a selection of individuals pre-disposed and suitable for this type of sport, or if the training for the sport itself sculpts a specific type of athlete (Mattsson et al., submitted manuscript).

Generally, the characterised adventure racers have aerobic capacity (*i.e.*, fractional utilisation; anaerobic threshold in relation to  $\text{VO}_{2\text{peak}}$ ) in the order: running > cycling > kayaking (*i.e.*, best trained in running), indicating that a shift in training regime in favour of kayak training could result in better overall performance. Furthermore, a comparison between top and bottom finishers in the Adventure Racing World Championship 2006 showed that the best ranked male adventure racers were taller, had higher body mass and BMI, but no anthropometric differences were found between the female top and bottom finishers (Mattsson et al., submitted manuscript). Physiological characteristics of the participants in Study I-IV (separated between protocols) are shown in Table 1.

**Table 1.** Subject characteristics

	Age (years)	Height (cm)	Body mass (kg)	$\text{VO}_{2\text{peak (cyc)}}$ ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )
<i>Men</i>				
12-h (n = 8)	31 ± 4	181 ± 5	81 ± 6	62.2 ± 2.7
24-h (n = 9)	27 ± 3	182 ± 4	80 ± 7	62.1 ± 5.3
53-h (n = 17)	31 ± 6	181 ± 5	79 ± 7	53 ± 8 <sup>a</sup>
5-7-day (n = 12)	27 ± 4	181 ± 5	81 ± 3	61.5 ± 3.8 <sup>b</sup>
<i>Women</i>				
53-h (n = 3)	27 ± 1	167 ± 3	66 ± 5	50 ± 6 <sup>a</sup>
5-7-day (n = 3)	30 ± 3	161 ± 7	56 ± 9	55.2 ± 2.6

Values are means ± SD. <sup>a</sup>  $\text{VO}_{2\text{peak}}$  values are estimated from submaximal cycling according to Åstrand and Ryhming (1954), <sup>b</sup> n = 11.

In all studies the subjects were fully informed about the procedure, possible discomfort involved, and their right to withdraw from the experiment at any point. All subjects were previously well acquainted with the test methods. Written informed consent was obtained from all subjects. The design of the study was approved by the Regional Ethics Committee in Stockholm, Sweden, in compliance with the Declaration of Helsinki.

### 3.1.1 Recruitment of subjects

The protocols were performed in the chronological order: 24-h (fall 2005), 5-7-day (fall 2006), 12-h (n = 4) (spring 2008), 53-h (fall 2008) and 12-h (n = 4) (spring 2009). The recruitment to

the first protocol was conducted through advertisements within the Swedish adventure racing community. All interested in participating sent their AR-specific *curriculum vitae* to the research group and the highest ranked athletes, based on experience and previous race results, were selected (nine plus two stand-ins). The 5-7-day protocol was the Adventure Racing World Championship which by itself indicates relatively high performance levels of the participants. All Swedish entrants were contacted during the month before the competition and informed about the research. The reason for only addressing the domestic teams was to ensure the possibility for tests before and after the race. All who volunteered were included in the investigations, and they belonged to teams that finished in 3<sup>rd</sup> to 24<sup>th</sup> place. The recruitment to the 53-h protocol was conducted in the same way as to the 5-7-day protocol. The essential difference (except exercise duration) was that the 53-h was a competition at a lower level, *i.e.*, only less experienced Scandinavian teams. To the 12-h protocols two Swedish top teams, of each four athletes, were recruited.

### **3.1.2 Gender perspective**

The female population in every endurance sport is consistently smaller than the male population. In adventure racing, women normally constitute 25%, or less, of the entire population. The main reason for this number is the gender mixed teams requiring at least one woman for every three men. In the 5-7-day protocol the rate of female volunteers is only one woman short of 25%. However, in the 53-h protocol all-male teams were allowed in a separate competition class which led to that only 15% of the subjects included in Study II were women. Due to the set-up with controlled individual relative intensity but exercise in coherent groups in the 24-h and 12-h protocols only men were recruited.

### **3.1.3 12-hour controlled intensity (laboratory setting)**

Eight male, Swedish ultra-endurance athletes, each with several years of international elite level training and competition, participated in the 12 hour protocol.

### **3.1.4 24-hour controlled intensity (laboratory setting)**

Nine male Swedish ultra-endurance athletes with experience from several years of training and competition at international elite level participated in the protocol. The subjects had been training for the last 3-9 years for ultra-endurance competitions. They had previously belonged to the Swedish elite in various sports, and had completed several ARs with durations of more than 48 h. Eight of the subjects had recent merits within top-10 in one or more of the world's major competitions for teams (*e.g.*, World Championship, "Primal Quest").

### **3.1.5 53-hour race situation (field setting)**

Seventeen male and three female Scandinavian ultra-endurance athletes of national level participated in the 53-h protocol. Compared to the other protocols these athletes were at a lower level of performance and had lower endurance capacity, which is evident in Table 1. The subjects also had a wider range of aerobic capacity, performance and training experience.

### **3.1.6 5-7-day race situation (field setting)**

The subjects were 12 men and 3 women who participated in the Adventure Racing World Championship (ARWC) in Hemavan, Sweden. They were all well-trained ultra-endurance athletes with several years' experience of training and competition at international top elite level, and eight of the participants belonged to one of the top-10 teams in the competition. An additional team (three men and one woman) of novice character without substantial experience was recruited to study III, but withdrew from the competition after just over 24 h due to general fatigue. They were therefore excluded from all analyses and their characteristics are not included in Table 1.

## **3.2 STUDY DESIGNS AND EXERCISE PROTOCOLS**

### **3.2.1 Characterisation tests**

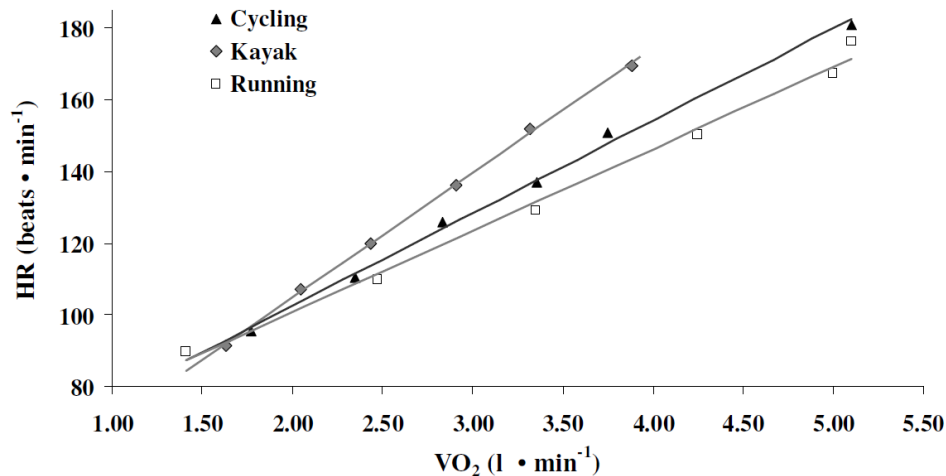
Two to four weeks before the 24-h and the 5-7-day protocols the athletes  $\text{VO}_{2\text{peaks}}$  during cycling (Monark Ergonomic 839E, Monark Exercise AB, Varberg, Sweden), kayaking (Dansprint aps, Hovide, Denmark) and treadmill running (Rodby Electronics, Vansbro, Sweden) were measured

by incremental all-out tests with raised work rate every minute. The tests for the three disciplines were performed with at least 24 h of rest in between.  $\text{VO}_{2\text{peak}}$  was reached according to Åstrand and Rodahl (1970) when: 1) total work time > 5 min, 2) levelling off of  $\text{VO}_2$  versus rate of work with  $\text{VO}_2$  on the highest work rate being within  $150 \text{ ml} \cdot \text{min}^{-1}$  from previous highest obtained value in the tests (Taylor et al., 1955) and 3) subjective rate of perceived exertion > 16 (Borg 1962). The inter-session CV for duplicate measurements on separate days according to this methodology in our laboratory is 2.6 %.

The same type of  $\text{VO}_{2\text{peak}}$ -test (cycling) was performed on the day before the 12-h protocol. Prior to the maximal test, on the same day, subjects performed incremental steady state tests (four steps of five min each) in order to establish the correlation between HR and  $\text{VO}_2$ .

In the 53-h protocol,  $\text{VO}_{2\text{peak}}$  was estimated from the initial submaximal cycling measurement (Pre) according to the method of Åstrand and Ryhming (1954). This method has been validated in several studies, for example Ekblom et al. (2007).

**Figure 5:** Correlations between heart rate (HR) and oxygen uptake ( $\text{VO}_2$ ) during different modes of exercise (kayaking, cycling, running) for a representative participant.



Each data point was retrieved in steady-state conditions during the last minute of a 5-min stage at a fixed work rate. (Enqvist et al., 2010)

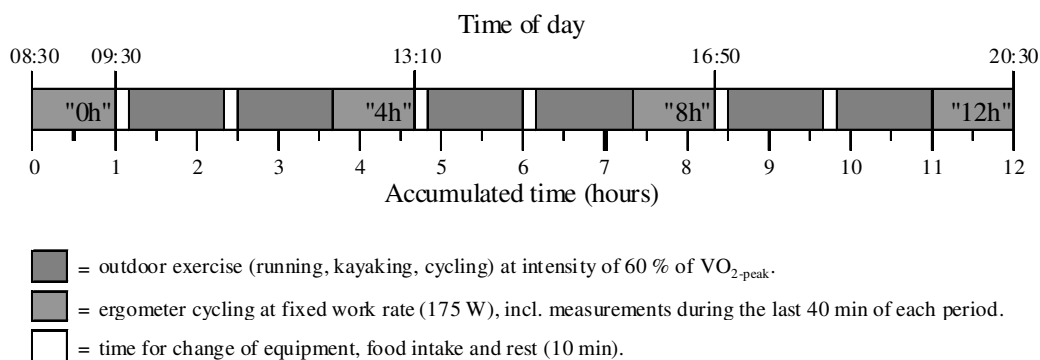
Prior to the all-out test subjects also performed incremental steady state tests (5 steps à 3 or 5 min) in each discipline in order to establish relation between HR and  $\text{VO}_2$ . The values used for

the correlation calculations were obtained lasting the final minute of each work rate. The mean coefficient of determination for the 12-h protocol ( $n = 8$ ) was  $R^2 = 0.985 \pm 0.007$  (cycling). Because of the different hemodynamic situations the HR:VO<sub>2</sub>-relation is specific to exercise mode (Figure 5). However, the mean coefficient of determination was reassuring for all disciplines in the 24-h protocol (cycling:  $R^2 = 0.995$ ; kayaking:  $R^2 = 0.975$ ; running:  $R^2 = 0.990$ ;  $n = 9$ ). The individual correlation in each discipline was used to control intensity and to determine the desired HR at the self-paced stages of the 12-h and the 24-h protocols. These correlations in combination with continuous HR-recordings were subsequently used for calculations of energy expenditure (Enqvist et al., 2010).

### 3.2.2 12-hour controlled intensity (laboratory setting)

The 12-h protocol began at 08:30 AM and consisted of almost continuous mixed exercise at controlled intensity. The subjects performed four blocks of ergometer cycling (Monark Ergonomic 828E, Monark Exercise AB, Varberg, Sweden) at a fixed work rate (175 W) for one hour each.

**Figure 6:** Schematic view of the 12-h protocol – controlled intensity.



Time points for conducted measurements at steady state ergometer cycling are marked "0h", "4h", "8h" and "12h". (Study II)

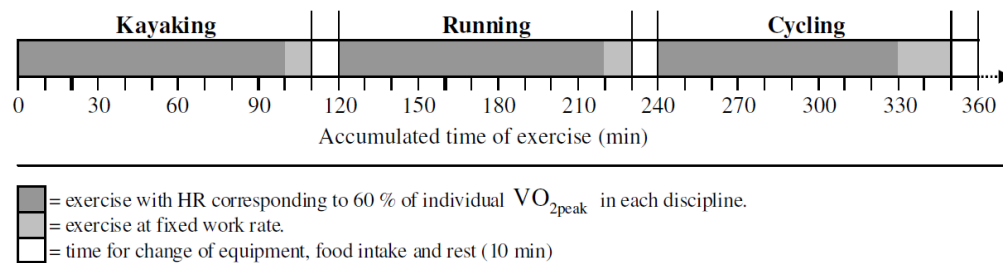
The assessment included measurements of cardiac output by a non-invasive gas rebreathing technique (CO<sub>RB</sub>), VO<sub>2</sub>, HR and systolic and diastolic blood pressure. From those values VO<sub>2</sub>/HR, SV, a-v O<sub>2</sub> diff, mean arterial pressure (MAP), stroke work (SW), cardiac work (CW), rate pressure product (RPP) and total peripheral resistance (TPR) were calculated. SW = SV x MAP, CW = SW x HR. RPP = HR x systolic blood pressure, is used as an indirect indicator of relative changes in the heart's oxygen consumption. Relative changes in TPR can be calculated by dividing MAP by CO. Measurements as described above were conducted during the last 40

min of these blocks, denoted “0 h”, “4 h”, “8 h” and “12 h”. In between these blocks of ergometer cycling the subjects performed different kinds of outdoor exercise (cycling, running, kayaking) at a work rate aimed at 60% of the individual  $VO_{2peak}$ . The intensity was continuously controlled using HR-monitors and corresponding HR. The subjects were allowed a total of 60 min (6 x 10 min) for rest, food intake and change of equipment in the course of the entire 12 hour exercise event (Figure 6). Intake of food and water was allowed *ad libitum*.

### 3.2.3 24-hour controlled intensity (laboratory setting)

The 24-h protocol was made up of almost continuous mixed exercise in a controlled setting. The study designs in both protocols in a laboratory setting allowed for repeated sampling and strictly controlled diet and work intensity. The athletes arrived in the laboratory following three days of standardised food intake of 4250 kcal/day (52% carbohydrates, 31% fat and 18% protein). A polyethylene catheter was inserted in an antecubital vein prior to exercise in order to facilitate repeated blood sampling. The subjects then, in groups of three, performed 12 blocks of exercise (4 x kayaking, 4 x running and 4 x cycling). Each block encompassed 110 min of exercise and 10 min of rest for food intake and change of equipment and clothes. (Figure 7)

**Figure 7:** Schematic view of the first 6 h of protocol 1: 24-h ultra-endurance exercise.



The schematic view above shows the components and outline of the first block, after which there were three identical 6-h blocks. HR = heart rate. (Enqvist et al., 2010)

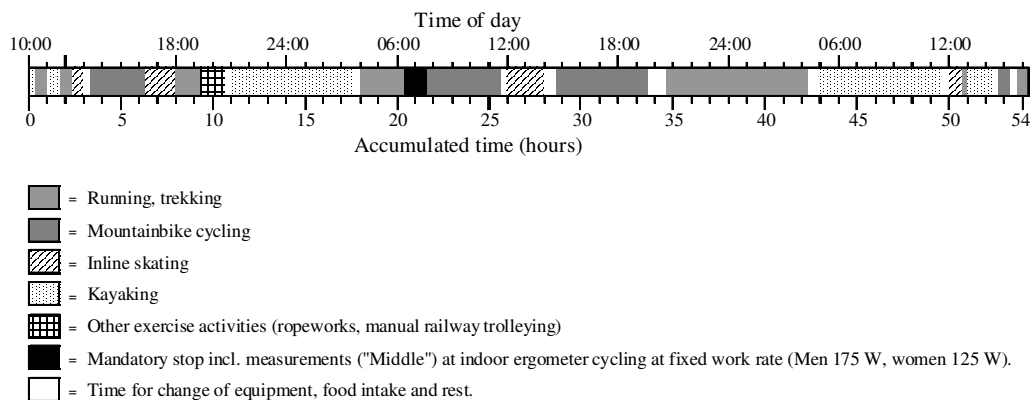
The three groups all performed the disciplines in the stated order, but due to logistics the first group started at 10:00, the second at 11:50, and the third at 13:40. The energy intake during the exercise (consisting of 59% carbohydrates, 29% fat and 12% protein) was aimed to give each person 50% of their estimated energy expenditure. Intake of water was allowed *ad libitum*. All tests and the kayaking exercise were held in-doors with temperature ranging between 18 and 22 °C. Running and cycling, except for the last 20 and 30 min of every exercise block, respectively, were performed outdoor. The temperature ranged between 2 °C at night and 22 °C during daytime. Work rate aimed at 60% of the individual  $VO_{2peak}$  in respective exercise mode. For the

last 20 min of every exercise block of cycling (*i.e.*, every 6<sup>th</sup> h) the work rate was standardised to 125, 150 or 175 W depending on the subject's  $\text{VO}_{2\text{peak}}$ . The initial values (0 h) for steady-state cycling at standardised work rates were collected before the main test. The last 10 min of each block of running and ergometer kayaking were at individual fixed standardised work rates, 9 or 10  $\text{km} \cdot \text{h}^{-1}$  with 1° incline and 65 to 85 W, respectively, depending on the subject's  $\text{VO}_{2\text{peak}}$ . HR-values for running and kayaking seen in Figure 12 are collected during the last 5 min of every exercise block.

### 3.2.4 53-hour race situation (field setting)

The experimental protocol studying 53 h of nearly continuous mixed exercise was performed as part of the TietoEnator Adventure Race held in Värmland, Sweden in May 2008. Subjects in teams of four (mixed gender or all male) completed a predetermined course of approximately 500 km, with an estimated winning time of 52 h. The race started at 10:00 AM, and consisted of running, mountain bicycling, kayaking, inline-skating, and rope activities (Figure 8). Time for rest as well as intake of food and water were allowed *ad libitum*. All measurements (same as described in the 12-hour protocol) were conducted indoors during steady-state ergometer cycling (Monark Ergonomic 828E, Monark Exercise AB, Varberg, Sweden) at a fixed work rate (175 W for men and 125 W for women, >5 min) before the start ("Pre"), half-way through ("Middle"), and within thirty min after finishing ("Post") the race. A compulsory stop approximately halfway through the race allowed the research team to administer the Middle measurements.

**Figure 8:** Schematic view of the 53-h protocol – adventure race.

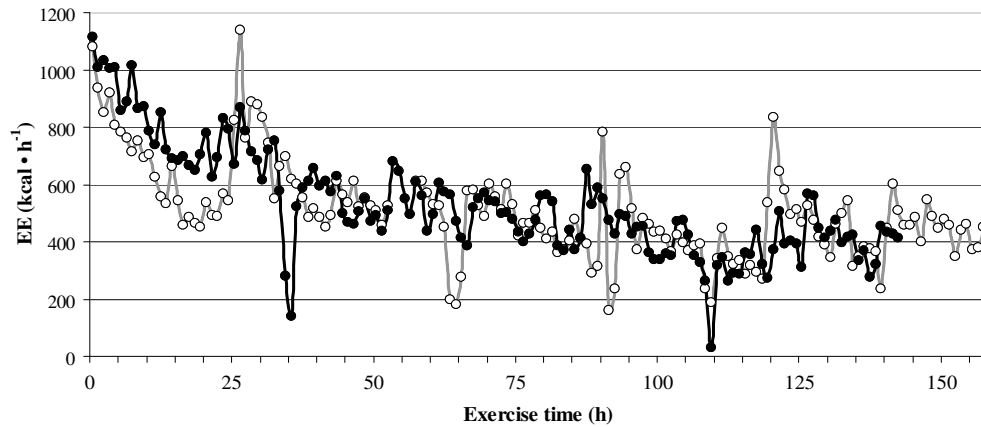


The timeline describes the duration of the race stages for the median team, with total race time of 54 h 20 min. The "Middle" measurement at steady state ergometer cycling is indicated within the timeline. The measurements "Pre" and "Post" were conducted before and within 30 min after the race. (Study II)

### 3.2.5 5-7-day race situation (field setting)

The race completed by our subjects involved non-stop of running, mountain biking, kayaking, in-line skating, climbing, caving and canyoneering on a predetermined course of more than 800km, which was to take approximately 5-6 days for the winning team as estimated by the organisers of this event. The subjects competed in teams consisting of three men and one woman. Sleeping, resting, eating and drinking were allowed *ad libitum*. The average intensity of exercise during this race has been reported elsewhere to be approximately 40% of  $\text{VO}_{2\text{peak}}$ , with a total energy expenditure of 80 000 kcal, or approximately  $525 \pm 100 \text{ kcal} \cdot \text{h}^{-1}$  (Figure 9) (Enqvist et al., 2010).

**Figure 9:** Energy expenditure per hour for two teams during the 5-7-day protocol.



Each point indicates mean energy expenditure (EE) during one hour ( $n = 6$  divided into two teams). Solid symbols are team 1 with a total race time of 142 h 14 min, and open symbols are team 2 with a total race time of 157 h 31 min. (Enqvist et al., 2010)

Since the measurements were carried out during an actual competition, participants prepared themselves individually prior to the race and did not adhere to a standardised pre-study diet with a following overnight fast.

## 3.3 METHODS

### 3.3.1 Heart rate measurements

HR was continuously recorded with a HR-monitor 610S (Polar Electro Oy, Kempele, Finland), validated against electrocardiogram (Moore et al., 1997; Porto and Junqueira, 2009).

### **3.3.2 Blood pressure measurements**

Blood pressure was measured using an aneroid sphygmomanometer (12 x 35 cm, Umedico AB, Rosersberg, Sweden) around upper arm. Presented data is an average of duplicates tests. All measurements were performed by two trained operators.

### **3.3.3 Blood samples and biochemical methods**

In the 12- and 24-hour protocol blood samples were drawn from a forearm vein at seated rest before exercise (Pre-Ex) and during the periods of steady state cycling.

In the 53-h and protocols blood samples were drawn with the subject at a seated rest position, in the morning the day before the race (Pre-Ex) and immediately after the end of the race (Post-Ex), and also in the 53-h after half of the race (Middle) and in the 5-7-day after 24 h of recovery (Rec-24h). Samples Post-Ex, Middle and Rec-24h were drawn at different times of day depending on the participants' performance and race time.

Catecholamines in plasma were determined after absorption to alumina at basic pH and desorption with perchloric acid. The catecholamines (Epi, NE) were separated using a high-pressure liquid chromatograph (HPLC) with a strong cation exchange resin and an electrochemical detector. Coefficients of variation (CV) were reported to be 7 and 8%, respectively (Hallman et al., 1978).

Hematocrit (Hct) and concentration of haemoglobin [Hb] in venous blood were determined using ADVIA™ 120 (Bayer Diagnostics, Leverkusen, Germany). CVs were 1.9 and 1.8%, respectively.

Plasma levels of creatine kinas isoenzyme MB (CK-MB), troponin I, and NT-proBNP were determined using sandwich enhanced chemiluminescence immunoassay (ECLIA) methodology analysed using Modular E170 (Roche Diagnostics Sweden AB, Bromma, Sweden). BNP in plasma was measured with a direct (without extraction) immunoradiometric assay (IRMA)

(Shionoria BNP, Cis Bio International, Elektrabox, Sweden). This is a sandwich-type IRMA, using two monoclonal antibodies prepared against two sterically remote epitopes of the human BNP molecule. The intra-assay coefficient of variation was 2% for CK-MB, 2% for troponin I, 1% for NT-proBNP and 6% for BNP. All samples were determined within the same assay to avoid inter-assay variation.

### **3.3.4 Oxygen uptake measurements**

Oxygen uptake ( $\text{VO}_2$ ) was measured using different methods in the different studies.

For the characterisation tests in the 24-hour protocol  $\text{VO}_2$  was measured with an online ergospirometry system (AMIS 2001, Innovision A/S, Odense, Denmark) based on mixed expired method with an inspiratory flowmeter. Before each test temperature, humidity and barometric pressure were measured, and gas analysers and inspiring flowmeter were calibrated. High precision gases ( $15.00 \pm 0.01\%$   $\text{O}_2$  and  $6.00 \pm 0.01\%$   $\text{CO}_2$ , Air Liquide, Kungsängen, Sweden) and indoor air were used for gas analyser calibration. The flowmeter was calibrated with a 3.0-liter syringe (Hans Rudolph Inc, Kansas City, MO, USA) at low, medium, and high flow velocity. The system's accuracy compared to the Douglas bag technique has a CV of 2.4% (Jensen et al., 2002).

At the 24-h exercise  $\text{VO}_2$  was measured during the last minutes of steady state periods using the Douglas bag technique. Expired air was collected and measured in duplicate bags. The volume of the expired air was measured with a Tissot spirometer (WE Collins, Braintree, MA, USA) and fractions of oxygen and carbon dioxide were determined using S-3A and LB2 gas analyser (Beckman Instruments, Fullerton, CA, USA), respectively.

In the 12-hour, 53-hour and 5-7-day protocols  $\text{VO}_2$  was measured with an online ergospirometry system (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) based on mixed expired method with an inspiratory flowmeter. The values presented in the results are means of duplicate tests. The system's accuracy compared to the Douglas bag technique has a CV of 1.2% (Foss and Hallén, 2005). Before each test, ambient temperature, humidity and barometric pressure

were measured, and gas analysers and inspiratory flowmeter were calibrated. High precision gases ( $15.00 \pm 0.01\%$  O<sub>2</sub> and  $6.00 \pm 0.01\%$  CO<sub>2</sub>, Air Liquid, Kungsängen, Sweden) and ambient indoor air were used for gas analyser calibration.

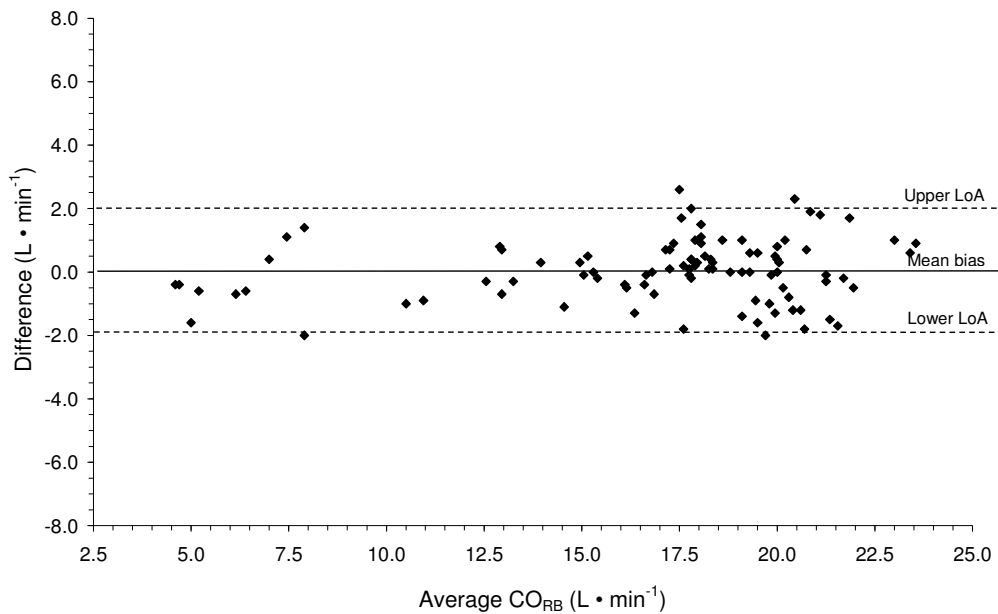
### 3.3.5 Cardiac output measurements

CO<sub>RB</sub> was measured as the mean of two tests at each point using a foreign gas rebreathing technique (Innocor<sup>®</sup>, Innovision A/S, Denmark), in which the subjects breathe a gas mixture in a closed system with a constant ventilation rate. The gas mixture consists of the test gas (1% SF<sub>6</sub> (inert blood insoluble gas), 5% N<sub>2</sub>O (inert blood soluble gas) and 50% O<sub>2</sub>, (Innovision A/S, Denmark)) diluted 1:4 with ambient indoor air. The logarithmically transformed disappearance curve of N<sub>2</sub>O is proportional to pulmonary capillary blood flow which, in the absence of significant pulmonary shunts, equals cardiac output. Duplicate measurements were performed, separated by at least three minutes to allow for complete inert gas clearance. This method has been described in detail elsewhere (Clemensen et al., 1994), and has been validated against both the direct Fick and thermodilution methods during resting conditions (Gabrielsen et al., 2002) as well as during exercise (Agostini et al., 2005). During the last couple of years, the use of this method has expanded and also been evaluated in healthy subjects in several different studies; validity compared to other rebreathing methods at rest and during exercise (Jakovljevic et al., 2008); validity compared to other non-invasive methods, Doppler ultra-sonic (Saur et al., 2009a) and cardiovascular magnetic resonance imaging (Saur et al., 2009b). In all these studies the method has been shown to as good as, or better, than other non-invasive measurements. In addition, the effects of variation in methodological variables such as breathing frequency and volume, intervals between rebreathing, and volume of dead space have been described (Damgaard and Norsk, 2005). Intra-session CV has previously reported to be 4.8% during resting conditions (Jakovljevic et al., 2008), and 4.3% during exercise at 130 W (Fontana et al., 2009).

To evaluate the reliability of this inert gas rebreathing method (Innocor<sup>®</sup>) during heavier exercise, and to clarify as to whether the technical error of measurement is of relative or absolute type we performed a reliability study (Mattsson et al., in manuscript). In total, 39 subjects (9 female and 30 male, age  $31 \pm 7$  years, height  $177 \pm 9$  cm, weight  $76 \pm 13$  kg) were recruited and they performed in total 95 duplicate tests, at rest ( $n = 10$ ) and over a large range of exercise work

rates ( $n = 85$ ). The inert gas rebreathing method appears to be a reliable assessment method for cardiac output in healthy subjects during exercise. A comparison of the difference in  $\text{CO}_{\text{RB}}$  between the measurements at rest ( $n = 10$ ,  $\text{CO}_{\text{RB}} 6.2 \pm 1.3 \text{ L} \cdot \text{min}^{-1}$ ) and the highest values during exercise ( $n = 10$ ,  $\text{CO}_{\text{RB}} 22.0 \pm 1.0 \text{ L} \cdot \text{min}^{-1}$ ) showed no significant difference ( $P = 0.97$ ), indicating that the error is not relative to the absolute value. The CV was 3.7%, ( $n = 95$ , 95% CI 2.9 to 4.5%), but we found a difference in averaged CV between 10.3% for resting measurements ( $n = 10$ , 95% CI 6.6 to 13.9%) and 2.9% during exercise ( $n = 85$ , 95% CI 2.4 to 3.4%). The absence of systematic difference between tests is important and indicates that no or only marginal learning effects are present, even though the assessment situation is a quite demanding task for the subject. The absolute measures of reliability (limits of agreement (LoA) - 1.9 to 2.0  $\text{L} \cdot \text{min}^{-1}$ ) indicate that any individual increase between two measurement less than 2.0  $\text{L} \cdot \text{min}^{-1}$ , or decrease less than 1.9  $\text{L} \cdot \text{min}^{-1}$  may be interpreted as within measurement error (due to instrument and/or biological variation) (Figure 10). Further, no systematic error seems to be present, indicated by the non-significant difference between average difference at low and high intensity.

**Figure 10:** Reliability of duplicate measurements of cardiac output using Innocor® (non-invasive re-breathing) (Mattsson et al., in manuscript).



To conclude, duplicate measurements of cardiac output using the Innocor® is not biased and has acceptable reliability, higher than other non-invasive alternatives. The technical error is of absolute type, and stable over the full range of measured values (Mattsson et al., in manuscript).

### 3.3.6 Rated relative performance

In the 5-7-day protocol each athlete rated his/her own performance during the last 12 h of the race on a scale from 1 to 3, where 1 = good, strong, carried extra weight, helped/pulled teammate, 2 = intermediate, took care of oneself, 3 = poor, weak, took help from teammate(s). In addition each athlete was rated according to the same scale by the other three members of his/her team. Thereafter the four ratings of each participant were pooled to give one value of relative performance.

It is important to mention that the rating is in relation to the rest of the team rather than race result, and also an indication of the participants' normal individual performance. Since a part of our hypothesis is that cardiac fatigue is induced by relative exercise intensity and load we consider it irrelevant to relate increase in cardiac biomarkers with race results. The study protocol, an ultra-endurance race situation, was suitable in part for the extreme duration and in part because the participants competed in teams of four. Regardless the team's ambition (*i.e.*, to finish top or bottom) each team is normally composed of participants with similar exercise capacity. Usually, each team has trained and competed together several times before and the teammates are therefore familiar with what should be considered "normal/intermediate performance" for him-/herself and the other team members. This rating scale has never been used before and is not validated; therefore we can at this point only provide an exploratory and descriptive value of performance. The scale is very simple with only three steps. The small number of scale steps limits the possibility of detecting small nuances but increases the consistency in rating. Accordingly, there is very good consistency in the evaluation of the performance for each participant. For twelve of the fifteen participants the assessments are unanimous, *i.e.*, the same value in all ratings.

If a subject's performance is rated high or low will very likely be affected by a vast number of factors other than cardiac fatigue, for instance skeletal muscle fatigue, central fatigue, energy deficiency and sleep deprivation. However, for the purpose of this thesis such confounding factors have not been taken into account, due to low numbers of participants. Residual confounding is therefore likely to be present.

### **3.3.7 Echocardiography and Tissue velocity imaging**

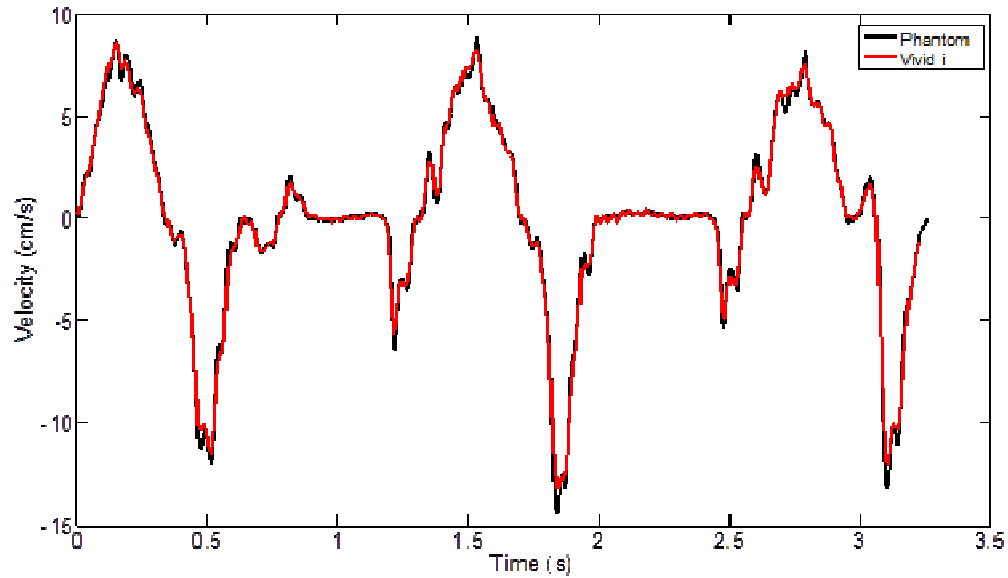
While resting in a left lateral recumbent position the subjects' hearts (sizes and myocardial velocities) were recorded at baseline, immediately after the race, and after one day of recovery by the same clinically experienced operator using both 2D-gray scale images and tissue velocity imaging (TVI) (VIVID I, GE VingMed Ultrasound, Hortens, Norway) with a standard 2D transducer (M3S). 2D recordings were obtained from the parasternal long axis (PLAX), and apical two-chamber (2CH) and four-chamber views (4CH) using both the 2D and TVI procedures. The colour TVI images with high temporal resolution were stored in cine-loops containing a minimum of three consecutive heartbeats. On the basis of the 4CH and 2CH views a circular region of interest (ROI) with a size of 4 mm was chosen at the basal segment of both the two left ventricular walls and the right ventricular free wall. No temporal filter was applied during the post-processing. All echocardiographic data were stored digitally (EchoPac workstation, GE VingMed Ultrasound) and subsequently analysed off-line utilising Matlab 7.0.1 to determine manually the isovolumic contraction velocity and duration (IVC v and t), peak systolic velocity (PSV), isovolumic relaxation velocity and duration (IVR v and t), and peak early (E') and late diastolic (A') velocities.

From the M-mode recordings the wall thickness and dimensions of the LV were calculated applying the leading edge-to-edge convention of the American Society of Echocardiography (ASE), in which a single representative cardiac cycle exhibiting the largest possible length of the LV cavity is used (Henry et al., 1980). LV mass was calculated in accordance with the formula recommended by the ASE (Lang et al., 2005) and this mass then related to body surface area (Mosteller, 1987), as well as to fat-free body mass, the latter being optimal according to Batterham and George (1998). All of these measurements were made during diastole.

The ejection fraction was calculated from the displacement of the plane of the atrioventricular valve (AV) according to a modification (Alam et al., 1990) of the approach developed by Höglund and colleagues (1988; 1989).

The specific example of the TVI machine VIVID I used in our study was tested for validity compared to a computer programmed phantom.

**Figure 11:** Validity and reliability of tissue velocity measurements using VIVID I.



The phantom was programmed after a healthy subject's displacement curve measured basal in septum. The readings were very satisfying with close agreement (Figure 11). The average error for measurements of velocity was  $-0.6 \text{ cm} \cdot \text{sek}^{-1}$  with SD  $0.2 \text{ cm} \cdot \text{sek}^{-1}$ , and for displacement even lower (close to 0) ( $n = 30$ ).

### 3.4 STATISTICS

The same types of statistical analyses were performed in study I-IV using Statistica for Windows, version 8 (StatSoft Inc., Tulsa, OK, USA). Data are generally presented as means  $\pm$  SD (in some cases min and max values are also presented) and were confirmed to be normally distributed using the Shapiro-Wilks W-test before performing parametric statistics. Repeated measures ANOVA were employed to analyse changes over time in all parameters. When an effect was detected a post-hoc analysis was performed with the Tukey HSD test. If the data did not conform to the assumption of sphericity, the  $P$ -value was subjected to Huynh-Feldt correction. A  $P$ -value of  $< 0.05$  was considered to be statistically significant. Trends were considered at  $0.05 < P < 0.1$ .

## 4 RESULTS

### 4.1 PHYSIOLOGICAL PARAMETERS AND MISSING DATA

#### 4.1.1 Study I

The total estimated average work intensity (in per cent of respective  $\text{VO}_{2\text{peak}}$ ) during the 24-h exercise was approximately 55% with the time for rest, food intake and change of equipment and clothes included. The calculation is adjusted according to the observed cardiovascular drift. There were no significant differences in work rate between the four different exercise blocks in the same discipline. There were no significant changes in Hb or Hct during the 24-h exercise, indicating maintained hydration and blood volume, according to formula by Dill and Costill (1974). The catecholamines, Epi and NE, both increased from Pre-Ex continuously during exercise, but decreased at rest at Rec to approximately halfway to Pre-Ex.

All subjects, but one, were able to perform all periods of standardised work rate. The unsuccessful participant failed to maintain his work rate during the last measurements due to general fatigue. He is therefore excluded from the results, even though his response patterns in all parameters until then were similar to the others.

#### 4.1.2 Study II

The average time of exercise for the 12-h and 53-h protocols was 11 h 44 min  $\pm$  5 min and 53 h 14 min  $\pm$  5 h 18 min, respectively. The “Middle” measurements were performed 20 h  $\pm$  2 h 30 min into the race. The outdoor temperature ranged between 3-5 °C during the 12 h, and between 9-16 °C during the 53-h protocol. All measurements in both protocols were conducted indoor at temperature ranged between 18-22 °C.

The estimated average work rate (in per cent of cycling  $\text{VO}_{2\text{peak}}$ ) during the 12 h exercise protocol was  $55 \pm 3\%$  of  $\text{VO}_{2\text{peak}}$ , including the periods for rest, food intake and change of equipment. The estimated average work intensity for the 53 h competition was  $37 \pm 5\%$  of  $\text{VO}_{2\text{peak}}$  (calculation made from ten subjects with complete HR-recordings). The  $\text{VO}_2$  during the

first bout of standardised ergometer cycling was  $51 \pm 2\%$  of  $\text{VO}_{2\text{peak}}$  in the 12-h protocol and  $49 \pm 6\%$  of  $\text{VO}_{2\text{peak}}$  in the 53-h protocol.

All subjects completed the assigned 12-h or 53-h protocols and all blocks of standardised ergometer cycling. However, in the Middle measurement during the 53 h event,  $\text{VO}_2$  data was missing from three subjects and HR and  $\text{CO}_{\text{RB}}$  data from one subject due to technical problems.

#### **4.1.3 Study III and IV**

The competition/exercise protocol took place under normal Nordic summer climate conditions with air temperatures during the day and night of 20-25 and 10-15°C, respectively. The average time for completion of the race was  $147 \pm 11$  h, and our subjects belonged to teams that finished in 3<sup>rd</sup> to 24<sup>th</sup> place.

One team recruited to study III ( $n = 3$  men and 1 woman) dropped out after only 24 h of racing due to general fatigue. They were therefore excluded from the analyses. The remaining participants were the same as recruited for study IV.

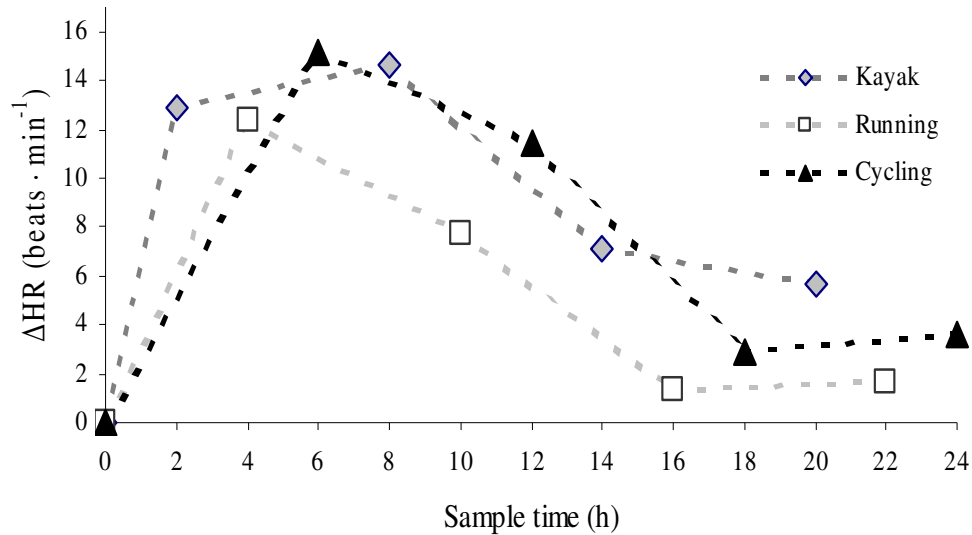
## **4.2 CIRCULATORY ADAPTATION**

### **4.2.1 Heart rate (HR)**

In the 24-h protocol HR at standard work rate in cycling was increased at six hours with  $13 \pm 6\%$  compared to 0 h, but thereafter there was a decrease towards initial values throughout the exercise. HR was fully recovered after 24 h of recovery. The initial increase and following decrease were similar in all exercise modes (Figure 12).

This kinetics of the drift was verified in the 12-h protocol where HR was increased at 4 h compared to 0 h, but thereafter drifted back towards initial values, although still elevated at 12 h compared to 0 h. HR had decreased below Pre at both the Middle and Post testing phases of the 53-h protocol.

**Figure 12:** Mean changes in heart rate (HR) at standardised work rate in different exercise modes during 24-h ultra-endurance exercise. (Study I)



#### 4.2.2 Oxygen uptake (VO<sub>2</sub>)

There were increases in VO<sub>2</sub> at standardised work rate at all points of measurement compared to Pre in all protocols. In the 24-h protocol VO<sub>2</sub> was increased at 6 h with  $10 \pm 6\%$  and at 12 h with  $17 \pm 8\%$  compared to 0 h, and thereafter VO<sub>2</sub> remained increased throughout the exercise. In difference to HR the VO<sub>2</sub> showed a trend towards maintained elevation at Rec ( $P = 0.07$ ). During the 12-h protocol, VO<sub>2</sub> was significantly increased at 8 and 12 h ( $10 \pm 5\%$ ) versus 0 h. Similarly, VO<sub>2</sub> increased during the 53 h event both at Middle ( $7 \pm 4\%$ ) and Post ( $4 \pm 5\%$ ) compared to Pre. However VO<sub>2</sub> at Post had slightly decreased ( $-2.4\%$ ,  $P < 0.05$ ) compared to Middle

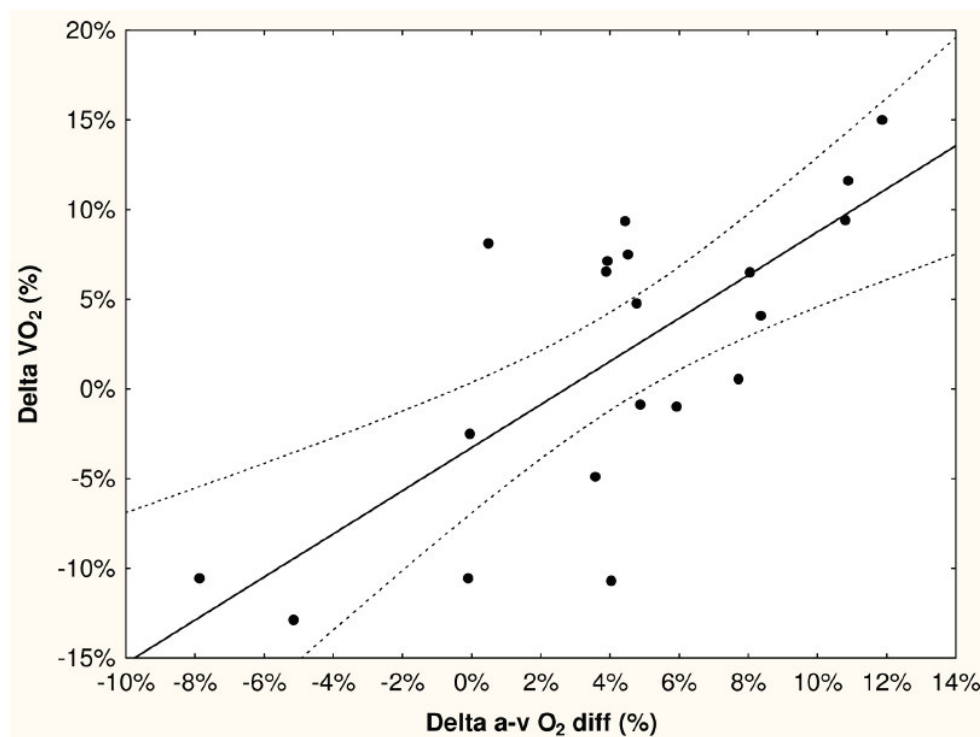
#### 4.2.3 Oxygen pulse (VO<sub>2</sub>/HR) – Work efficiency

The VO<sub>2</sub>/HR, expressed in ml O<sub>2</sub> · heartbeat<sup>-1</sup>, in the 24-h protocol the VO<sub>2</sub>/HR was not changed at 6 h compared to 0 h but increased with  $7 \pm 10$  ( $-3$ - $21$ ),  $13 \pm 11$  ( $0$ - $32$ ) and  $11 \pm 10$  ( $-3$ - $26$ ) % compared to 0 h at 12, 18 and 24 h, respectively. At Rec VO<sub>2</sub>/HR had decreased approximately halfway towards initial values. This circulatory adaptation was verified in both the 12-h protocol where VO<sub>2</sub>/HR was increased at 8 h and 12 h compared to 4 h, and in 53 h event, VO<sub>2</sub>/HR was increased both at Middle and Post compared to Pre.

#### 4.2.4 Cardiac output, stroke volume and work of the heart

During submaximal exercise  $\text{CO}_{\text{RB}}$  remained largely unchanged in the 12-h and 53-h protocols. However, statistically significant findings were a decreased  $\text{CO}_{\text{RB}}$  at 12 h compared to 8 h in the 12-h protocol and an increased  $\text{CO}_{\text{RB}}$  at Post compared to Middle in the 53-h protocol. The calculated SV during the 12-h protocol was decreased at 4 h compared to 0 h, but returned thereafter towards baseline levels. In the 53-h protocol SV was elevated at Post compared to Pre. In the 12-h protocol a-v  $\text{O}_2$  diff remained unchanged at 4 and 8 h but increased at 12 h compared to 0 h. In the 53-h protocol it was increased from Pre to Middle, but decreased from Middle to Post, but in that protocol there was no difference between Pre and Post. There was a correlation between change in  $\text{VO}_2$  and change in a-v  $\text{O}_2$  diff from Pre to Post in the 53-h protocol ( $P < 0.001$ ,  $r = 0.73$ ) (Figure 13). A trend towards the same correlation was noted between 0 h and 12 h in the 12-h protocol ( $P = 0.07$ ,  $r = 0.67$ ). Mean arterial pressure (MAP) was constant during the 12-h protocol, but decreased at both Middle and Post compared to Pre in the 53-h protocol.

**Figure 13:** Correlation of changes in oxygen uptake and arterio-venous oxygen difference after 53 h of adventure racing.



Per cent change in oxygen uptake ( $\text{VO}_2$ ) at steady state ergometer cycling versus per cent change in calculated arterio-venous oxygen difference (a-v  $\text{O}_2$  diff) comparing beginning and end of a 53-h adventure race ( $n = 20$ ). Dashed line represents 95 % CI. Correlation  $r = 0.73$ ,  $P < 0.001$ . (Study II)

Apart from a decrease at 4 h ( $P < 0.01$ ) in accordance with the traditionally defined early cardiovascular drift, SW was unchanged compared to Pre throughout both experiments. There were no differences in CW during the 12-h protocol. However, CW was decreased at Middle and Post compared to Pre in the 53-h protocol. In the 12-h protocol there were no changes in RPP, but at the 53-h protocol RPP was decreased at Middle by 15% and at Post by 11% compared to Pre (both with  $P < 0.001$ ). Similar to CW, RPP and MAP we found a decreased TPR at Middle and Post in the 53-h protocol of 7 and 13% ( $P < 0.05$  and  $P < 0.01$ ), respectively, but there were no changes in the 12-h protocol.

### 4.3 CARDIAC FATIGUE

#### 4.3.1 Cardiac biomarkers

The levels of CK-MB, BNP and NT-proBNP [mean  $\pm$  SD (min-max)] at Pre-Ex, Post-Ex and Rec-24 are shown in Table 2. The levels of all these biomarkers in plasma were elevated at Post-Ex compared to Pre-Ex. At Rec-24 levels were decreased, but still elevated compared to Pre-Ex. However, only 20% of the participants ( $n = 3$ ) had elevated levels after the exercise ( $0.05$ ,  $0.06$  and  $0.10 \mu\text{g} \cdot \text{l}^{-1}$ ). Two of them still displayed elevated levels at Rec-24 ( $0.05$  and  $0.08 \mu\text{g} \cdot \text{l}^{-1}$ ). The correlation between levels of NT-proBNP and BNP at Post-Ex was excellent ( $R^2 = 0.95$ ), but there was no correlation between NT-proBNP and CK-MB ( $R^2 = 0.09$ ).

**Table 2.** Cardiac biomarkers in plasma before (Pre-Ex) and immediately after (Post-Ex) a 6-day Adventure race, and 24 h into recovery (Rec-24h) ( $n = 15$ )

	Pre-Ex	Post-Ex	Rec-24h
CK-MB ( $\mu\text{g} \cdot \text{l}^{-1}$ )	$5 \pm 1$ (2-7)	$37 \pm 14$ (12-60)*	$17 \pm 7$ (6-30)* #
Troponin I ( $\mu\text{g} \cdot \text{l}^{-1}$ )	<i>n.d.</i>	<i>elevated in 3 subjects</i>	<i>elevated in 3 subjects</i>
BNP ( $\text{ng} \cdot \text{l}^{-1}$ )	$11 \pm 6$ (n.d.-23)	$41 \pm 46$ (n.d.-160)*	$22 \pm 18$ (n.d-63)*
NT-proBNP ( $\text{ng} \cdot \text{l}^{-1}$ )	$31 \pm 14$ (10-56)	$487 \pm 648$ (52-2480)*	$224 \pm 219$ (12-634)*

Values are means  $\pm$  SD (min-max). \*  $P < 0.05$  vs Pre-Ex, #  $P < 0.05$  vs Post-Ex. CK-MB, creatine kinase mb-fraction; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; n.d., not detectable ( $< 0.03 \mu\text{g} \cdot \text{l}^{-1}$ ).

### 4.3.2 Echocardiography – Cardiac size

The echocardiographic measurements revealed that the hearts (LV) of these adventure-racing athletes, although enlarged, were within normal limits. Compared to athletes participating in other endurance sports, such as cycling, cross-country skiing and canoeing both wall thickness and the diameter of the left ventricular cavity diameter were similar in our subjects, but not quite as large as those in rowers Pelliccia et al., 1991; Spirito et al., 1994). The LV mass measured in study IV (men  $223 \pm 27$  g and women  $114 \pm 20$  g; or related to fat-free mass: men  $3.3 \pm 0.4$  g · kg<sup>-1</sup> and women  $2.6 \pm 0.2$  g · kg<sup>-1</sup>) was slightly lower than that found for Swedish male cross-country skiers (250 g, or related to fat-free mass,  $3.9$  g · kg<sup>-1</sup>) (Carlsson et al., in press). The highest individual values for baseline cardiac size were demonstrated by a man; the thickness of the LV postural and intraventricular septal walls both 12 mm; the LV diameter, 60 mm; LV mass, 280 g; LV mass related to BSA,  $138$  g · m<sup>-2</sup>, and related to lean body mass,  $4.05$  g · kg<sup>-1</sup>. The highest corresponding values for a woman were 9 and 8 mm, respectively; 48 mm; 137 g;  $79$  g · m<sup>-2</sup> and  $2.73$  g · kg<sup>-1</sup>.

The functional measurements showed that HR increased from  $50 \pm 7$  to  $58 \pm 10$  bpm comparing baseline to after the 5-7-day protocol. Measurements of E/A at the same time points decreased from  $2.3 \pm 0.6$  to  $1.8 \pm 0.4$ , but EF increased from  $59 \pm 8$  to  $70 \pm 8\%$ . At all assessments, average LVd was stable at 53 mm.

### 4.3.3 Tissue velocity imaging

The values from tissue velocity assessments are shown in Table 3. Basically, all values are within normal limits. The contraction velocity in LV is increased HR after the exercise. The total duration of each cardiac cycle is decreased (*i.e.*, higher HR) and, consequently, contraction and relaxation times are decreased, except for the RVfree wall. Twenty per cent of the subjects displayed any IVRt at baseline, and the incidence of such relaxation duration increased to 60% at both of the later measurements.

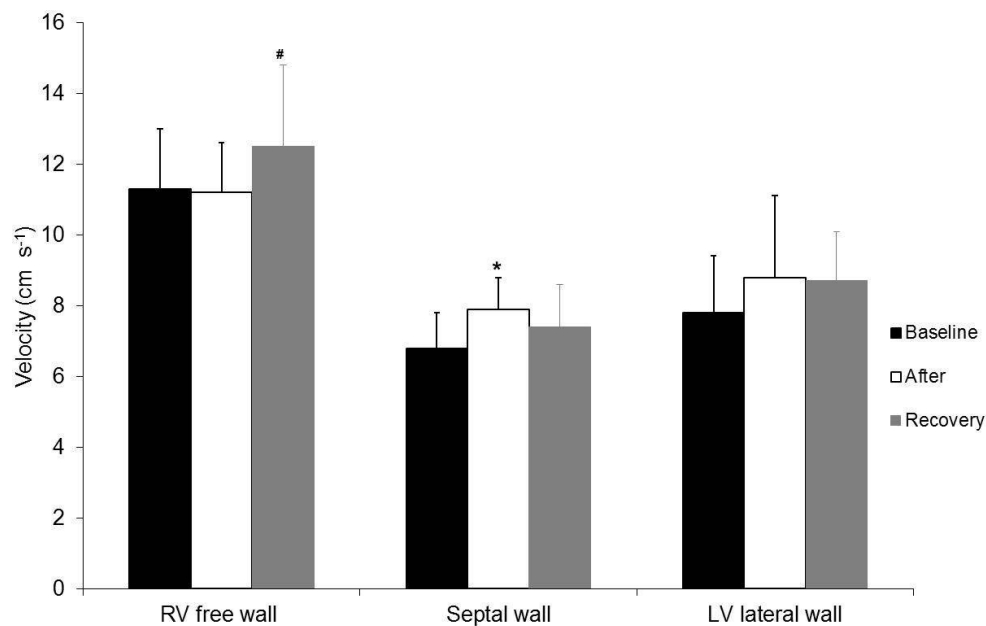
**Table 3.** Tissue velocity parameters at baseline, after a 6-day Adventure Race and following 2 hours of recovery (n = 15)

	Baseline	After exercise	Following recovery
IVCv LV ( $\text{cm} \cdot \text{s}^{-1}$ )	$3.36 \pm 1.26$	$4.97 \pm 2.53^*$	$4.60 \pm 2.36^*$
IVCt LV (ms)	$89 \pm 26$	$69 \pm 22^*$	$73 \pm 22^*$
IVRt LV lateral wall (ms)	$78 \pm 25$	$71 \pm 24^*$	$70 \pm 23$
IVCv RV ( $\text{cm} \cdot \text{s}^{-1}$ )	$8.42 \pm 2.13$	$7.60 \pm 2.98$	$8.24 \pm 3.11$
IVCt RV (ms)	$80 \pm 17$	$67 \pm 15^*$	$68 \pm 16^*$
IVRt RV <sup>a</sup> (%)	20	60	60

The values presented are means  $\pm$  SD. IVC, isovolumic contraction (v, velocity; t, duration; LV, left ventricle – a: mean of septal and lateral walls; RV, free wall of the right ventricle); IVRt, duration of the isovolumic relaxation. % of the subjects exhibiting IVRt RV of any duration. \*  $P < 0.05$  compared to the corresponding baseline value, #  $P < 0.05$  compared to the corresponding value after exercise.

The peak systolic velocities at baseline and immediately after exercise and after approximately 24 h of recovery were similar, except for significant increases in the septal wall velocity after exercise compared to baseline and in the velocity of the right ventricular free wall following recovery in comparison to immediately after exercise (Figure 14).

**Figure 14:** Peak systolic velocities ( $\text{cm} \cdot \text{s}^{-1}$ ) in different segments of the myocardium at rest at baseline, after approximately 150 h of continuous exercise (After), and following 24 h of recovery (Recovery) (n = 15).



LV, left ventricle; RV, right ventricle. \*  $P < 0.05$  compared to the corresponding baseline value, #  $P < 0.05$  compared to the corresponding value after exercise. (Study IV)

## 5 GENERAL DISCUSSION

### 5.1 CIRCULATORY ADAPTATION

This thesis confirms the well-established drift in HR during the first six hours of exercise, seen in earlier investigations (Åstrand 1960; Saltin and Stenberg 1964). After six hours there was an unexpected change. Instead of an increased or continuous rise in HR there was a decrease with time towards initial values at the end of exercise. Meanwhile,  $\text{VO}_2$  at the standardised work rate increased with approximately 10-15% at 12 h, and thereafter remained stable until the end of exercise. The magnitude of the increased  $\text{VO}_2$  at 12 h was approximately three times greater than previously reported. These two drifts combined gave an increased  $\text{VO}_2/\text{HR}$  which indicates that the distribution of blood, and thus oxygen, was changed towards a more efficient energy distribution.

#### 5.1.1 Heart rate (HR)

HR drifted in a similar way in both Studies I and II, with a continuous gradual increase during the first hours and thereafter a downward drift towards baseline. Interestingly, the inverted drift occurred even though Hb was decreased at the final measurements in the 12-h protocol, which in the literature is associated with incremental increases in both HR and CO (Ekblom et al., 1972; Woodson et al., 1978), since the decrease in Hb, with stable Hct, implies a decrement in oxygen-carrying capacity of 4.3% (Dill and Costill, 1974). This Hb decrease might be ascribed in part to the continuous mechanical trauma to the soles of the feet (Davidson, 1964). Such a haemolytic state is not without risk, and may in a worst case scenario induce kidney failure.

The reversed HR-drift could be associated with desensitisation of the heart's adrenergic receptors caused by high concentrations of catecholamines over an extended period of time (Eysmann et al., 1996; Friedman et al., 1987; Welsh et al., 2005; Hart et al., 2006). In agreement with this hypothesis, epinephrine and norepinephrine measured in Study I were continuously increased. Furthermore, another reasonable contributor to the reversed drift is a redistribution of blood flow from peripheral areas (*e.g.*, skin) to working muscles. In this case,

it would imply restored preload, which is in accordance with earlier findings (Ashley et al., 2006).

### 5.1.2 Oxygen uptake ( $\text{VO}_2$ )

The  $\text{VO}_2$  at standardised work rate was increased above baseline at every time point in all protocols.

The explanation might be found in a combination of adaptations. One parameter that would give increased oxygen cost during exercise is a change in metabolic substrate. For example, in Study I respiratory exchange ratio (RER) at the fixed work rate decreased from 0.88 to 0.83. This shift indicates a higher rate of fat utilisation, from 39 to 56%, according to the table by Zuntz (1901). Further calculations indicate an energy expenditure of  $11.03 \text{ kcal} \cdot \text{min}^{-1}$  during initial cycling ( $\text{VO}_2 2.25 \text{ l} \cdot \text{min}^{-1}$  and RER 0.88 corresponding to  $4.90 \text{ kcal} \cdot \text{l O}_2^{-1}$ ). If the work efficiency remained stable at the standardised work rate, the metabolic shift (decreased RER) would only give a minimal increase in  $\text{VO}_2$  to  $2.28 \text{ l} \cdot \text{min}^{-1}$  ( $+0.03 \text{ l} \cdot \text{min}^{-1}$ ).

Consequently, the metabolic shift is just a minor contributor to increased  $\text{VO}_2$ . Another possibility is that increased pulmonary ventilation ( $V_E$ ) would result in a higher  $\text{VO}_2$ . The oxygen cost of breathing becomes progressively greater with higher pulmonary ventilation, and may be up to 10% of total oxygen uptake during maximal exercise (Åstrand and Rodahl, 1970). This means for our subjects in Study I, with a  $\text{VO}_{2\text{max}}$  of  $\approx 5 \text{ l} \cdot \text{min}^{-1}$  and a maximal  $V_E$  of approximately  $200 \text{ l} \cdot \text{min}^{-1}$  the maximal oxygen cost of breathing will be 2.5 ml per litre of inspired air. Even if maximal values are used for calculations the oxygen cost for the increased  $V_E$  is less than  $+0.02 \text{ l} \cdot \text{min}^{-1}$ . These calculations lead to the suggestion that the central circulatory adaptation only explain less than 15 % of the increased  $\text{VO}_2$ . Instead, the changes in  $\text{VO}_2$  should mainly be attributed to peripheral (muscular) adaptations. A study on mitochondria from *musculus vastus lateralis* in the subjects that participated in the 24-h protocol showed a reduced mitochondrial efficiency, with both palmitioylcarnitine and pyruvate as substrates, by 9 and 6%, respectively (Fernström et al., 2007). This efficiency reduction could be a major contributor to the increased  $\text{VO}_2$ . Study II support this hypothesis, as shown by the direct correlation between change in  $\text{VO}_2$  and change in a-v  $\text{O}_2$  diff from Pre to Post in the 53-h protocol (Figure 13).

The remaining portion of the  $\text{VO}_2$ -drift could be ascribed to decreased economy of movement by recruiting alternate muscle fibres during prolonged exercise (Dick and Cavanaugh, 1987), without muscle damage (Westerlind et al., 1992), but still other causes are possible.

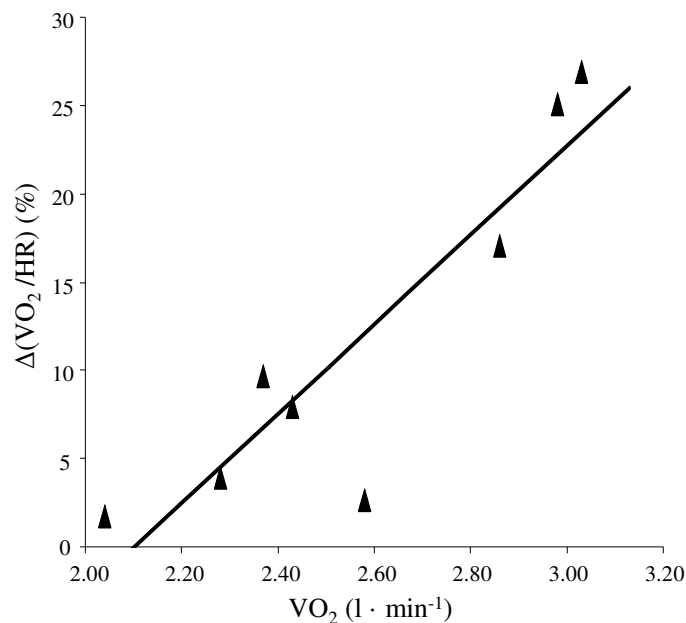
The magnitude of the increased  $\text{VO}_2$  was highest in the 24-h protocol, for measurements from 12-h and after. In the 53-h protocol the increase at Middle was approximately half compared to the highest observed drift in the 24-h protocol. There was a decrease in  $\text{VO}_2$  at Post compared to Middle. Even though all measurements were conducted at standardised work rate, the major part of the exercise performed in the 53-h protocol was in the form of a competition. During this type of race, the work intensity decreases over time (Lucas et al. 2008; Enqvist et al., 2010). This time-dependent decrease in external work intensity may explain the relative decrease of  $\text{VO}_2$ . The 53-h protocol was obviously longer but with lower average work intensity compared to the 12-h and 24-h protocols. In the study by Saltin and Stenberg (1964), subjects exercised at 75%  $\text{VO}_{2\text{peak}}$  for three hours, generating a 5% increase in  $\text{VO}_2$ . Therefore it could be suggested that the magnitude of the upward drift in  $\text{VO}_2$  is dependent on both exercise duration and relative work intensity.

### **5.1.3 Oxygen pulse ( $\text{VO}_2/\text{HR}$ ) – Work efficiency**

The combination of the two cardiovascular drifts described above also give an unexpected increase in  $\text{VO}_2/\text{HR}$  after the first six hours of exercise. In the 12-h protocol the lowest levels were found at 4 h. The values were then increased at 8 h and 12 h, and  $\text{VO}_2/\text{HR}$  was increased above initial level in all measurements during the 53-h protocol. Normally, an increased  $\text{VO}_2$  at submaximal work rate would be covered by an increased  $\text{CO}$ , usually by increased  $\text{HR}$ . In the present thesis  $\text{VO}_2/\text{HR}$  was increased despite the elevated  $\text{VO}_2$  and reduced mitochondrial efficiency (Fernström et al., 2007). This indicates that the distribution of blood, and thus oxygen, was changed towards a more efficient energy distribution. The increased  $\text{VO}_2/\text{HR}$  can be either a result of central circulatory adaptation (*i.e.*, increased  $\text{SV}$ ), or higher peripheral extraction, (*i.e.*, increases in a-v  $\text{O}_2$  diff, possibly by altered distribution of blood to, and within, working muscles). Study II revealed that a combination of the two might be the answer. During the first approximately 24 h of exercise the increased  $\text{VO}_2/\text{HR}$  is covered by increased a-v  $\text{O}_2$  diff, but at the last measurement in the 53-h protocol the late cardiovascular drift was occurring, including increased  $\text{SV}$  and normalised a-v  $\text{O}_2$  diff.

There are large differences in the response between subjects. These differences are not correlated to relative work rate, decreases in body weight, or concentration of catecholamines at the end of exercise. In the 24-h protocol the increased  $\text{VO}_2/\text{HR}$  during cycling at 18 and 24 h compared to 0 h is highly correlated to calculated mean  $\text{VO}_2$  ( $\text{l} \cdot \text{min}^{-1}$ ) during the whole 24-h ultra-endurance exercise session. The individual values are plotted in Figure 15, which indicate that the higher the total oxygen flux, the greater the increase in  $\text{VO}_2/\text{HR}$ . The explanation and the implications of this are not yet known.

**Figure 15:** Correlation between calculated mean oxygen uptake ( $\text{VO}_2$ ) during 24-h ultra-endurance exercise ( $\text{l} \cdot \text{min}^{-1}$ ) and increased oxygen pulse ( $\text{VO}_2/\text{HR}$ ) ( $\text{mL O}_2 \cdot \text{beat}^{-1}$ ) during cycling, at 18 and 24 h compared with 0 h (initial value) (%) in eight subjects ( $r = 0.90$ ;  $P < 0.01$ ). (Study I)



## 5.2 CARDIAC FATIGUE

Based on the results from this thesis we conclude that a late cardiovascular drift is present during this type of ultra-endurance exercise (Study I; Study II). This late drift involves increases in both SV and cardiac output (CO) at a standardised work rate, probably as a result of reduced peripheral resistance (decreased MAP). It is also noteworthy that, at these relatively low work intensities, we find no evidence of diminished ability to sustain the required CO or failure of oxygen delivery to the muscles. In Study II, we found that despite the extreme physiological load (*i.e.*, high total energy expenditure) of 53-h ultra-endurance exercise, both

the work performed by the heart (CW) and the oxygen consumption of this organ (as indicated by the rate pressure product – RPP) during standardised sub-maximal cycling were attenuated. This could be interpreted as cardiac fatigue (Saltin and Stenberg, 1964; Douglas et al., 1987; Whyte et al., 2000; Dawson et al., 2005; Ashley et al., 2006) but since CO was maintained, or slightly increased, we interpret this change as physiologically appropriate adjustment and a sign of higher work efficiency.

### **5.2.1 Cardiac size**

In short, in our investigations the hearts (LV) of adventure-racing athletes, although enlarged, were within normal limits.

However, the only other report on adventure racers presented a pooled value for the LV mass of men and women that was considerably larger (323 g) (Ashley et al., 2006). There is no reason to believe that such a large difference between these two similar study populations of intermediate-to-world class athletes actually exists. Rather, this discrepancy might reflect the calculation employed: here, we calculated as recommended in the ASE statement from 2005 (Lang et al., 2005), whereas in the investigation of Ashley et al. (2006) an older method of calculation that overestimates LV mass by approximately 20% may have been used (Devereux et al., 1986). With such an adjustment, the values from these two studies would be equivalent.

### **5.2.2 Cardiac biomarkers**

Concerning the different biomarkers, our results are in line with previous studies and knowledge. However, circulating levels of troponin increased to exceed the reference value only in three participants (20%). These participants did not rate their performance lower, and did not appear to be more affected by cardiac fatigue, than the other participants. This finding is in line with Scharhag and colleagues (2005) who found no evidence of a causal link between exercise-induced increases in cardiac troponins and myocardial damage, and suggested that the increases may represent a physiologic reaction under certain exercise conditions and lack pathological significance in healthy athletes. Consequently, we conclude that troponin release is not an appropriate indicator for evaluating exercise-induced cardiac fatigue. It has also been shown that the increases in troponin and NT-proBNP are not interrelated (Scharhag et al., 2005). The present study supports those findings; we could also demonstrate a lack of correlation between

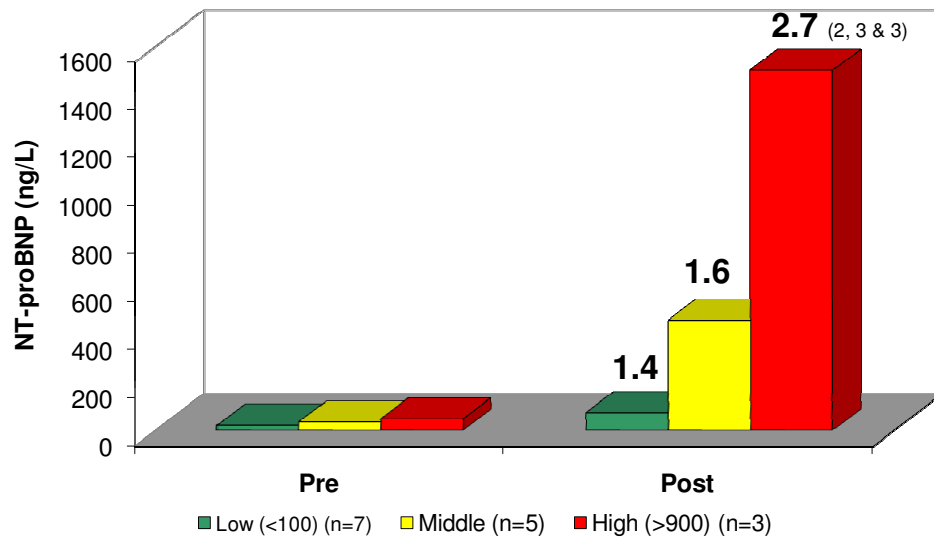
elevations in NT-proBNP and CK-MB. The increase in the CK-MB fraction during this type of exercise can probably be attributed to activity in skeletal muscle, as previously shown for marathon runners (Apple et al., 1985; 1987). Hence, our results suggest that neither troponins nor CK-MB can be used as an indicator for evaluation of cardiac strain or exercise-induced cardiac fatigue in healthy subjects.

However, measurements of BNP and NT-proBNP might still be valuable for detection and quantification of the level of cardiac strain, and possibly also cardiac fatigue. Despite differences in the half-life of BNP (20 min) versus NT-proBNP (120 min) the correlation between levels in plasma at Post-Ex is very strong ( $R^2 = 0.95$ ). It should be noted that the ultra-long duration of the exercise appears to make the clinical cut-off values for the biomarker with the longer half-life, NT-proBNP, inappropriate in the ultra-endurance exercise setting. Cut-off values should therefore be adjusted before plasma levels are interpreted in order to give relevant information of the actual cardiac strain and fatigue. After the ultra-endurance exercise (at Post-Ex) the cut-off point for BNP at  $100 \text{ ng} \cdot \text{l}^{-1}$  corresponds to  $1400 \text{ ng} \cdot \text{l}^{-1}$  NT-proBNP, which is almost five times the clinically used cut-off point ( $300 \text{ ng} \cdot \text{l}^{-1}$  for NT-proBNP). Note that the relation between circulating levels of BNP and NT-proBNP, and thus the relevant cut-off point for NT-proBNP, will most likely change depending on exercise duration. Because of its more stable nature, most exercise studies only give values for NT-proBNP, and might therefore overestimate the “negative effect” of endurance exercise on the heart. In the present study seven of fifteen participants had levels of NT-proBNP exceeding  $300 \text{ ng} \cdot \text{l}^{-1}$ , but only one subject had values of  $\text{BNP} > 100 \text{ ng} \cdot \text{l}^{-1}$  and  $\text{NT-proBNP} > 1400 \text{ ng} \cdot \text{l}^{-1}$ . In line with the hypothesis of a link between cardiac fatigue and high levels of the biomarkers, the only high responding subject’s performance was rated “3 – poor/weak”. It should be mentioned that we cannot determine if the increase in biomarkers and the decrease in performance are interdependent, or two independent processes occurring simultaneously.

### **5.2.3 Rated relative performance**

The aim was also to relate the increase in cardiac biomarkers to performance. At Post-Ex seven participants had NT-proBNP below the clinically used reference value ( $< 100 \text{ ng} \cdot \text{l}^{-1}$ ). The pooled rated performance for four of them was 1, and the remaining three were rated 2. At the other end of the spectrum, three participants had markedly higher levels than previously reported ( $> 900 \text{ ng} \cdot \text{l}^{-1}$ ) and their performance was rated 3, 3 and 2 (Figure 16).

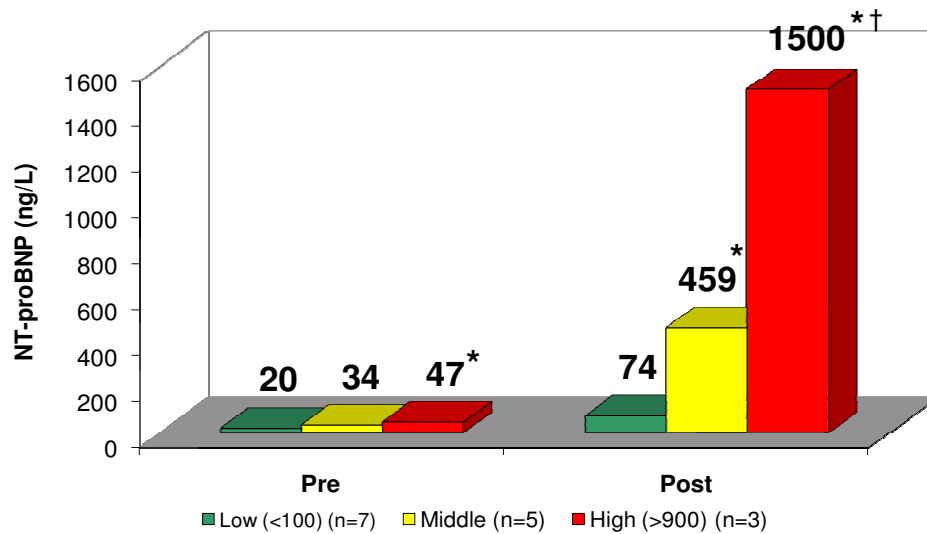
**Figure 16:** Comparison between NT-proBNP and rated relative performance.



The subjects were divided in three groups based on levels of NT-proBNP after a 5-7-day adventure race. Rating of relative exercise performance was conducted using a three-grade-scale where 1 = good, strong, carried extra weight, helped/pulled teammate, 2 = intermediate, took care of oneself, 3 = poor, weak, took help from teammate(s).

In congruence with previous observations by Sahlén and colleagues (2009) the baseline value of NT-proBNP was a good predictor of post-race value, even though the baseline value was well below the reference value ( $<100 \text{ ng} \cdot \text{l}^{-1}$ ) (Figure 17).

**Figure 17:** Comparison between NT-proBNP before and after a 5-7-day adventure race.



The subjects were divided in three groups based on levels of NT-proBNP after a 5-7-day adventure race.

#### 5.2.4 Echocardiography

As mentioned in the Introduction, several other investigations have documented reductions in EF following (ultra)endurance exercise and the clinical prognostic value of a low EF is well recognised (*e.g.*, the risk of death is elevated with an EF <35.5%) (Schwartz et al., 1984). In Study IV, however, compared to baseline value, EF was enhanced both after exercise and recovery. An early report (Douglas et al., 1987) showed decreased left ventricular diameter (LVd) following prolonged exercise, which could be interpreted as decreased preload, which correlates strongly with reduced EF. In studies where the preload has been kept stable, EF has also been maintained (Hassan et al., 2006). In the present case LVd was consistent at all time-points of measurement, which might have contributed to the increase in EF. Unfortunately, the limitations involved in calculating SV or CO from echocardiographic data do not allow us to present these values in the Results. Although no definitive conclusions can be drawn our findings do suggest that both SV and CO were maintained (and, indeed, that CO was slightly increased after both exercise and recovery). If CO is maintained, the metabolic demand is most likely being met, an obvious goal that should not be difficult to achieve when the body is at rest. CO is, of course, the product of HR times SV and in the present study HR was increased, possibly due to an elevated sympathetic drive, since high circulating levels of catecholamines after 24 h of continuous exercise have been observed (Study I). It has been proposed that increased HR is the primary change in the progression of normal cardiovascular drift (Fritzsche et al., 1999; Coyle and Gonzalez-Alonso, 2001), the decreases in SV and EF being secondary consequences and, thereby, doubtful indicators of cardiac fatigue.

In agreement with previous findings, the E/A of our subjects was reduced both after exercise and recovery in comparison to the baseline. This complex finding is difficult to interpret, but is considered to indicate impairment of myocardial relaxation. In our case E was maintained and the ratio decreased primarily due to an elevation in A. This preservation of E is in line with the sustained preload (LVd) discussed above. The elevation in A is, to some extent, a consequence of the shortened diastole associated with increased HR. Therefore, the decrease in E/A could be an adaptation to the increase in HR (from 50 bpm at baseline to 58 bpm after the exercise). However, despite this theoretical reasoning, a meta-analysis found no correlation between alterations in HR and E/A (Middleton et al., 2006).

### **5.2.5 Tissue velocity imaging**

The major focus in Study IV was on peak myocardial velocities. First of all, our participants exhibited normal values for all of the parameters concerning myocardial velocities assessed. Furthermore, there were no indications of any decrement in peak systolic velocities in any myocardial segment following ultra-endurance exercise. On the contrary, either after exercise and/or following recovery, these velocities were significantly elevated in two of the three walls measured (i.e., the left lateral, septal and right ventricular free walls). Although it might have been physiologically interesting to also make our measurements during the exercise itself, it has been shown previously that a damaged heart demonstrates impaired contraction velocities even at rest (Pasquet et al., 1999).

The decrements in IVR and IVC durations in both the left and right ventricles are primarily a consequence of the enhanced HR. Prolonged IVR would be indicative of an impairment of LV function (Betocchi et al., 1986; Leung et al., 1990). The only parameter in present study that could indicate decreased cardiac function is the increased incidence of measurable right ventricular IVR duration. The duration of right ventricular IVR is determined by systolic pulmonary artery pressure which when raised, delays the opening of tricuspid valves and proportionately prolongs the duration of IVR (Burstin, 1967; Lindqvist et al., 2006).

## **5.3 METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS**

### **5.3.1 Points of measurements during exercise**

A common limitation of cardiovascular research on ultra-endurance exercise is that it is almost exclusively performed during competitions. Even when HR is recorded continuously during competition, the drift in combination with the changes in work intensity throughout the races poses a problem. When reported, both HR and speed decrease from the start to the end of the race (Cottin et al., 2007; Neumayr et al., 2003). A major strength in present work is that Study I and II (first half) was performed as simulated races in a laboratory setting, enabling for controlled work intensity and multiple measurements of  $\text{VO}_2$ , blood sampling and other parameters during standardised work rates possible. In addition, the control of energy intake in the 24-h protocol was rigorous, with similar relative energy intake and composition for all subjects. Thanks to the kind and enduring athletes and the great support from the race organisers,

we could collect data for the second half of Study II as well as Study III and IV even during actual competitions.

### **5.3.2 Exercise intensity controlled using HR-monitor**

It can be considered somewhat of a paradox to use HR-values to control intensity in the 12- and 24-h protocols since one of the aims was to investigate cardiovascular drift. It would have been possible to ensure a fixed work rate throughout the protocol by setting the exercise ergometers to a resistance equivalent to 60%  $\text{VO}_{2\text{peak}}$  using data collected during the pre-tests. First of all, this was not entirely possible since the athletes partly exercised outdoor. Mainly, it should be noticed that no similar studies had been carried out before present ones, hence making it impossible to know if the participants would be able to keep a constant workload equivalent to 60%  $\text{VO}_{2\text{peak}}$  for 24 h. Heart rate curves, on the other hand, were retrieved from previous competitions.

### **5.3.3 Circadian rhythm**

Since study I was performed during a full day and night and study III and IV were carried out during the course of several days another aspect to consider is the normal circadian rhythm. Obviously, many bodily functions, such as hormonal status, are affected over the course of day and night. One parameter affected is HR. During continuous measurements of HR at bed rest minimum values were found between 4-5 am and maximum between 11-12 am. The difference between highest and lowest HR was approximately  $6 \text{ beats} \cdot \text{min}^{-1}$  (Kräuchi and Wirz-Justice, 1994). Wahlberg and Åstrand (1973) stated that HR during both submaximal and maximal exercise was  $3\text{-}5 \text{ beats} \cdot \text{min}^{-1}$  lower at night (3 am) compared to day (3 pm). The circadian rhythm is also preserved during a 24-h continuous walking race (Cottin et al., 2007). In study I the three groups of subjects began exercise at 10:00, 11:50 and 13:40. The starting times coincide with the maximum level of the circadian HR curve and the minimum should appear after 14 to 18 h of exercise, after which a re-increase is expected. As seen in Figure 12 this is evidently not the case. HR at 14 to 18 h is lower than at 2 to 8 h, but still higher than initial levels. No increase was then present during the last six hours. Even though circadian rhythm may partially explain changes in HR at fixed work rate the extent of the HR drift in Study I is at least double the amount suggested. In Study II the decrease in HR below initial values at the end of the 53 h event might be explained in part by normal circadian rhythm related changes in HR, since most of these measurements were conducted at night. However,

since CO was maintained at a stable level the decreased HR might be driven by an increase in SV, which is facilitated by decreased peripheral resistance (TPR and MAP).

Another aspect to address is the sleep deprivation that this set-up induces. Even though sleep-loss influences physiological effects, such as increased perceived exertion and reduced time to exhaustion during heavy exercise, the HR and metabolic parameters relevant for this study remain unchanged (Martin, 1981; Martin and Gadis, 1981).

#### **5.3.4 Race situation, climate and subjects**

There are several confounding factors in this type of field protocols in race settings (Study II-IV) that potentially could have an impact on circulatory parameters. For that reason comparisons in Study II between the 12-h protocol (controlled intensity) and the 53-h protocol (race) are limited by the fact that they are different in character and were performed by different individuals. An obvious limitation is the use of non-invasive methods, and furthermore the calculated estimates of cardiac parameters. Unfortunately, it is not possible to conduct more invasive measurements in a safe and ethical way during this type of exercise. A major limitation is that energy intake, electrolyte intake and status, and water intake was not controlled for. However, Hct levels were stable throughout both protocols indicating stable fluid status. Another factor that might influence the results is the actual race. It can be considered a limitation that both race-protocols were held in normal Nordic summer climate, and that all subjects were Scandinavian. We cannot with certainty claim whether a similar race, but with different set-up (stages duration and order), time for rest, and ambient temperature would not induce different results.

### **5.4 FUTURE PERSPECTIVES**

Study IV, as well as all previous studies concerning exercise-induced cardiac fatigue, involved monitoring subjects at rest prior to and after exercise. However, peak systolic velocities associated with maximal or near-maximal HR would provide a more accurate indicator of cardiac fatigue. Although a promising procedure for measuring TVI has recently been developed (Carlsson et al., in press), it is still difficult for subjects to achieve maximal HR following ultra-endurance exercise, due to general fatigue. Nonetheless, future studies concerning cardiac

fatigue would most likely benefit from measuring contraction velocities at higher (preferably maximal) work- and heart rate.

Since we suggest that intensity, and not duration, is the main determinant of cardiac fatigue an investigation designed to properly address this matter would be interesting and desirable. Such a study should ideally involve the same subjects performing exercise protocols of several different durations, *e.g.*, 3 h, 6 h, 12 h and 48 h, and each exercise duration protocol should be performed at several different relative intensities, *e.g.*, 40, 60 and 80 % of  $\text{VO}_{2\text{peak}}$ . Since it most likely will be impossible to exercise *e.g.*, at 80 % of  $\text{VO}_{2\text{peak}}$  for 48 h, some protocol might be performed until exhaustion.

We did not find a single athlete with pathologically increased cardiac size. However, a long term follow-up (5 and/or 10 years) of both myocardial size and function in the athletes examined in Study IV would be valuable. Furthermore, a cross-sectional study using MRI to examine possible damage and scarring in athletes who have been training and competing in ultra-endurance events for more than 10 or 15 years would illuminate long term risks.

The first studies we conducted were simply descriptive in its nature, and we then transcended to studies aiming for a more explanatory angle. An obvious and desirable progress for future investigations would be intervention studies.

## **5.5 PERSONAL REFLECTIONS**

As a (former) adventure racer and a scientific researcher I find it encouraging that we, during the five+ years of the project, have been able to scientifically prove many of the physiological reactions and adaptations that the athletes had noticed empirically. The fundamental idea leading up to the project was that adventure racing exposes the human organism to a unique situation, and that the successful adventure racer is a distinctive breed of athlete. If that should be true, traditional textbook knowledge of physiological adaptations and exercise training might not be fully applicable. Another concern was whether this type of exercise had serious detrimental

effects. This question that was unanswered and debated since research was sparse and empirical long-term follow-up not performed due to newness of the sport.

Summing up the project it feels safe to conclude that this exercise and athletes are somewhat unique. An example of something the athletes had felt was the difficulty to reach high HR after some hours of exercise. We showed that the reversed cardiovascular drift plays a part in this, associated with desensitisation of the heart's adrenergic receptors, shown by other investigators. There are apparently many aspects of the optimal training and competition strategies. We have illuminated that energy intake is important, and that eating and drinking (and be able to keep it) could be a required skill. The studies on mitochondria indicate specific adaptations, including increased maximal oxidation of fat. For this adaptation to occur, it is likely that athletes need to exercise continuously for many hours, which is not conventional in the training situation but is self-evident during adventure racing competition. Obviously, there are many reasons why experience is important for winning races. However, the physiological adaptations to repetitive ultra-endurance exercise may be crucial. For instance all athletes in the top-2 teams of ARWC 2006 were older than 30 years, and two out of four in the winning team was older than 40 years. The vast majority of the Swedish top-athletes of today have been competing for many years. Concurrently, the speed in the races has increased dramatically. For example, in the early races 450 km was completed in 5-6 days, but in the ARWC 2006 more than 800 km was covered in just over 5 days! Still, the athletes that have performed this evolving journey of the sport used to be specialists in one exercise discipline who then transcended to adventure racing. It will be interesting to see if the speed in the competitions will continue to increase with the next generation of athletes who specialize in adventure racing from an early age. An important observation from present thesis is that this type of exercise does not seem to have detrimental effects on the heart. Still, our present and future findings might be useful in clinical cardiology, especially concerning recovery. The athletes display rapid reversal in *e.g.*, biomarkers, whereas patients have a much slower kinetics. Even though the duration is extreme the risks does not seem to be higher than for other endurance events, such as marathon, pointing towards intensity as the primary determinant of cardiac fatigue and possible damage. This idea might be true also for other injury aspects. Several investigators have demonstrated high incidence of pain and injury during and after ARs. However, the athletes seem to be less injured during training than specialists in other endurance sports. A possible reason is that adventure racers, due to the many different exercise modes, can chose to put strain on different parts of the body, and consequently rest other parts, and still get full desired training effect.

## 6 CONCLUSIONS

- The sizes of the hearts (LV) of all of our ultra-endurance athletes were within normal limits.
- The central circulation changes in several steps in response to ultra-endurance exercise. Drifts over time occur in most of the measured parameters.
  - HR at fixed submaximal work rate increase only during the first six hours of exercise, after which HR gradually decreases towards initial values.
  - Compared to initial levels,  $\text{VO}_2$  is increased at every time-point (5-15%). The increase can be attributed to peripheral adaptations, confirmed by a close correlation between change in  $\text{VO}_2$  and change in a-v  $\text{O}_2$  diff.
  - Upward drift of HR and  $\text{VO}_2$  resulted in an increased oxygen pulse ( $\text{VO}_2/\text{HR}$ ) after the first six hours of exercise, and the calculated amount of oxygen extracted from each heart beat increase by approximately 10% (indicating a more efficient energy distribution).
  - When exercise continues for 50 h late cardiovascular drift can be observed, characterised by increased  $\text{VO}_2/\text{HR}$ , decreased peripheral resistance, increased stroke volume, and decreased work of the heart. Since cardiac output is maintained we interpret the changes as physiologically appropriate adaptations to ultra-endurance exercise.
- Increased levels of troponin and CK-MB seem to lack relevance in the (healthy) exercise setting, but that BNP, or NT-proBNP adjusted for exercise duration, might be a relevant indicator for impairment of exercise performance.
- Levels of NT-proBNP up to  $2500 \text{ ng} \cdot \text{l}^{-1}$  can be present after ultra-endurance exercise in healthy athletes without any subjective signs or clinical symptoms of heart failure. High levels of NT-proBNP seem to be associated with decreased relative exercise performance, and might be an indicator of the cardiac fatigue.
- Measurements of peak systolic velocities at rest showed no signs of cardiac fatigue in our ultra-endurance athletes even after 6 days of continuous exercise.

## 7 SAMMANFATTNING (SUMMARY IN SWEDISH)

Den övergripande målsättningen med denna avhandling är att redogöra för hur den centrala cirkulationen påverkas av ultra-uthållighetsarbete (multisport/Adventure Racing), samt bidra till kartläggningen av den så kallade "arbetsinducerade hjärtutmattningen".

Ett Adventure Race (AR) varierar i längd från 6 timmar till över 6 dagar. Deltagarna ska navigera via ett antal kontrollstationer över en förbestämd sträcka, och utföra en kombination av tre eller flera uthållighetsidrotter, t.ex. cykling, löpning och kajakpaddling. Denna avhandling är baserad på resultaten från fyra olika försöksprotokoll, 12- och 24-tim ( $n = 8$  och  $9$ , respektive) i en kontrollerad miljö och arbetsintensitet och 53-tim och 5-7-dagar ( $n = 15$  i vardera) i tävlingssituation. Försökspersonerna i samtliga delarbeten var erfarna elitaktiva multisportatleter. Studie I och II behandlar den cirkulatoriska responsen under ergometer cykling på fasta arbetsbelastningar under perioder före, under och efter ultra-uthållighetsarbete. Mätningarna innefattade hjärtfrekvens (HR), syreupptagning ( $VO_2$ ), blodtryck och hjärtminutvolym (icke-invasiv återandning). I studie III och IV undersökte vi eventuell förekomst av arbetsinducerad hjärtutmattning efter ett 5-7-dagars AR, från två olika perspektiv. I studie III användes biokemiska metoder för att bestämma plasmanivåer av hjärtspecifika biomarkörer (dvs. kreatinkinas isoenzym MB (CK-MB), troponin, B-typ natriuretisk peptid (BNP) och N-terminal prohormonal B-typ natriuretisk peptid (NT-proBNP)). Vi relaterade även öknings av biomarkörer med skattad relativ prestation. I studie IV användes mätningar av hjärtats kontraktionshastigheter (TVI) (VIVID I, GE VingMed Ultraljud, Norge) för se funktionella tecken på hjärtutmattning, dvs. sänkta topphastigheter på kontraktionen. Med konventionell ekokardiografi utvärderades om dessa atletes hjärtan var större än den normala övre gränsen.

Den centrala cirkulationen förändras i flera steg som svar på ultra-uthållighetsarbete. Jämfört med initialnivåerna var  $VO_2$  förhöjd vid samtliga mättillfällen. Ökningen kan tillskrivas perifera anpassningar, vilket bekräftas av ett nära samband mellan förändringar i  $VO_2$  och förändring i arterio-venös syreextraktion. Det första steget i det cirkulatoriska svaret var den typiska (tidiga) driften med ökning i HR och samtidigt minskande slagvolym (SV) och syrepuls ( $VO_2/HR$ ), som fortgår under de första 4-6 timmarna. Det andra steget, som fortgick till ca 12 tim, innebar omvänd HR-drift, med normalisering av SV och  $VO_2/HR$ . När arbetet pågick upp till 50 tim noterades en "sen" kardiovaskulär drift, som kännetecknades av ökad  $VO_2/HR$ , (vilket indikerar effektivare energidistribution), minskad perifer resistens, ökad SV, och minskat mekaniskt arbete i hjärtat. Eftersom hjärtminutvolymen hela tiden bibehölls så vi tolkar förändringarna som fysiologiskt adekvata anpassningar.

Resultaten från studie III pekar mot en åtskillnad mellan den patologiska och den fysiologiska frisättningen av hjärtspecifika biomarkörer. Resultaten tyder på att troponin och CK-MB saknar relevans, men att BNP, och NT-proBNP justerat för arbetstid, kan vara en relevant tecken för försämrad arbetsförmåga. Höga nivåer av NT-proBNP, upp till  $2500 \text{ ng} \cdot \text{L}^{-1}$ , kan förekomma efter ultra-uthållighetsarbete hos friska idrottare utan några kliniska symtom på hjärtsvikt. Dock verkade höga nivåer av NT-proBNP vara relaterat till försämrad relativ arbetsförmåga och en indikator på hjärtutmattning. Studie IV visade att storleken på ultra-uthållighetsidrottarnas hjärtan (vänster kammare) var inom normala gränser. Mätningarna av maximala kontraktionshastigheter visade (för gruppgenomsnitt) inga tecken på hjärtutmattning, ens efter 6 dygn av kontinuerligt arbete. Skillnaden mellan våra och andras fynd, skulle kunna bero på det faktum att denna typ av arbete utförs med relativt låg genomsnittlig intensitet, vilket tyder på att intensiteten, snarare än varaktighet, är den viktigaste faktorn för hjärtutmattning.

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