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Thesis for doctoral degree (Ph.D.) 2009

# *Cardiac Effects of Prolonged Exercise*

ANDERS SAHLÉN

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# Cardiac Effects of Prolonged Exercise

by

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**Karolinska  
Institutet**

Stockholm 2009

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Printed at Larserics Digital Print AB, Sundbyberg  
ISBN 978-91-7409-685-9



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# ABSTRACT

Long-distance running is growing in popularity. While moderate exercise unquestionably gives major health benefits, the effects of extreme physical exhaustion on cardiac function have been studied less. It has previously been observed that elevated cardiac biomarkers may be observed after exercise, the significance of which is not known. We **aimed** to study the cardiac impact of the 30-km endurance race *Lidingöloppet* using biomarkers N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) and troponin T (TnT) and characterised cardiac function by echocardiography and vectorcardiographic measures of ventricular repolarisation.

In **study I**, 22 healthy senior runners that participated twice in the *Lidingöloppet* race (in 2003 and 2006) were studied using cardiac biomarkers (TnT, NT-proBNP) at baseline and after the race. Echocardiography was performed at baseline. TnT increased reproducibly in a subset of participants. NT-proBNP increased in all subjects and individuals reached similar levels at the two races. Both biomarkers were associated with larger left atrium and NT-proBNP was associated with greater LV mass suggesting a link to cardiac filling pressures.

In **study II**, a group of 15 runners were studied longitudinally from baseline before the race, immediately post-race, on the next day and on day six. Vectorcardiography was used to determine measures of ventricular repolarisation and echocardiography was performed to examine cardiac functional changes. The race led to QT-interval prolongation mainly due to an increased  $T_{\text{peak-to-T}_{\text{end}}}$  interval, as well as an enlarged  $T_{\text{area}}$  which was sustained during the next 6 days. Runners with higher baseline NT-proBNP developed greater attenuation of myocardial velocities and a larger increase in  $T_{\text{area}}$ . Functional fatigue of the heart thus occurs in parallel with altered ventricular repolarisation.

In **study III**, predictors of biomarker release were analysed at baseline and after the race in 185 runners aged an average of 61 years. In multivariable regression, independent predictors of large biomarker release were established. NT-proBNP release was predicted by (1) a higher level present at baseline, (2) a larger increase in creatinine, (3) higher age and (4) longer race duration. Elevation of TnT was predicted by (1) higher age, (2) a larger increase in creatinine and (3) fewer previous race participations. In 15 cases (8.1%), NT-proBNP was already elevated at baseline. Clinical work-up showed that 4 of these runners (2.2% of whole study population) harboured serious cardiovascular disease of whom one suffered sudden cardiac death within a few months after the race.

In **study IV**, 23 experienced runners (median 11 previous race participations) were age-matched against 20 beginners (never raced previously in *Lidingöloppet*). Tissue Doppler imaging was used to analyse intra-left ventricular mechanical synchrony by computing the standard deviation of time-to-peak myocardial systolic velocity across 12 myocardial segments ( $T_s$ -SD). Furthermore, the I/D polymorphism of the Angiotensin-Converting Enzyme (ACE) gene was analysed. Post-race, beginners had higher levels of TnT in association with greater dyssynchrony. In multivariable analysis, post-race dyssynchrony was predicted by (1) lack of race experience, (2) more copies of the ACE D allele and (3) lower peak longitudinal systolic velocity.

In **study V**, 31 experienced participants were age-matched against 35 beginners and studied before and after *Lidingöloppet*. Blood tests drawn included NT-proBNP, TnT and C-reactive protein (CRP). Genetic analysis of ACE I/D status was also performed. Despite similar levels at baseline, post-race levels of all biomarkers were higher in beginners. Homozygous carriers of the D allele exhibited a larger release of NT-proBNP despite non-significant differences between allele groups at baseline, showing that genetic factors are important for the release of NT-proBNP in runners.

In **conclusion**, this study of predominantly senior long distance runners shows that prolonged exercise causes significant immediate and short-term effects on different aspects of cardiac function including biomarkers, myocardial velocities, ventricular repolarisation and intra-ventricular synchrony. Biomarkers rise especially in runners with less experience of endurance events. Levels of NT-proBNP are influenced by genetic factors and rise in the presence of cardiac dysfunction. Future studies need to address the potential clinical impact of these findings.

**Key Words:** Angiotensin-Converting Enzyme, Exercise, Natriuretic Peptides, Troponin

# LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
$A_m$	Late diastolic longitudinal lengthening velocity
Ang II	Angiotensin II
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
BMI	Body mass index
BNP	Brain natriuretic peptide
CRP	C-reactive protein
CRT	Cardiac resynchronisation therapy
CV	Coefficient of variation
eGFR	Estimated glomerular filtration rate
$E_m$	Early diastolic longitudinal lengthening velocity
IVSd	Inter-ventricular septal thickness at end-diastole
IQR	Inter-quartile range
LA	Left atrium
LAV	Left atrial volume
LV	Left ventricle
LVEDV	Left ventricular end-diastolic volume
LVEDVi	Left ventricular end-diastolic volume indexed for body surface area
LVEF	Left ventricular ejection fraction
LVM	Left ventricular mass
LVMi	Left ventricular mass indexed for body surface area
NT-proBNP	N-terminal pro-brain natriuretic peptide
PWDd	Posterior wall thickness at end-diastole
RyR	Ryanodine receptor
SD	Standard deviation
S-D delay	Difference in time-to-peak systolic velocity between septum and lateral wall
$S_m$	Peak systolic longitudinal shortening velocity
STI	Speckle tracking imaging
SV	Stroke volume
TDI	Tissue Doppler imaging
Tn	Troponin
TnT	Troponin T
TnI	Troponin I
TnC	Troponin C
$T_s$	Time-to-peak systolic velocity, from QRS onset
$T_s$ -Diff	Maximum difference in $T_s$ between any two segments
$T_s$ -SD	12-segment standard deviation for times-to-peak $T_s$ across 12 segments
VCG	Vectorcardiography
$Vo_2$ max	Maximum oxygen uptake

# LIST OF ORIGINAL PAPERS

This thesis is based on the following studies, which will be referred to by their Roman numerals.

## I

Anders Sahlén, Reidar Winter, Britta Lind, Per-Herman Jacobsen, Marcus Ståhlberg, Tony Marklund, Thomas Fux, Jan Svensson, Frieder Braunschweig.

Magnitude, Reproducibility, and Association with Baseline Cardiac Function of Cardiac Biomarker Release in Long-Distance Runners Aged  $\geq 55$  Years.

*Am J Cardiol* 2008;102:218-22.

## II

Anders Sahlén, Aigars Rubulis, Reidar Winter, Per-Herman Jacobsen, Marcus Ståhlberg, Per Tornvall, Lennart Bergfeldt, Frieder Braunschweig.

Cardiac Fatigue in Long-Distance Runners Is Associated with Ventricular Repolarization Abnormalities.

*Heart Rhythm* 2009;6:512-19.

## III

Anders Sahlén, Thomas P Gustafsson, Jan E Svensson, Tony Marklund, Reidar Winter, Cecilia Linde, Frieder Braunschweig.

Predisposing Factors and Consequences of Elevated Biomarker Levels in Long-Distance Runners Aged  $\geq 55$  Years.

*Am J Cardiol* 2009. *In press.*

## IV

Anders Sahlén, Kambiz Shahgaldi, Anna Aminoff, Philip Aagaard, Aristomenis Manouras, Reidar Winter, Ewa Ehrenborg, Frieder Braunschweig.

Effects of Prolonged Exercise on Left Ventricular Mechanical Synchrony in Experienced Long-Distance Runners and Beginners.

*Submitted.*

## V

Anders Sahlén, Philip Aagaard, Ewa Ehrenborg, Frieder Braunschweig.

Increased Cardiovascular Biomarkers in Long-Distance Runners: Importance of Previous Exposure to Endurance Events and the Angiotensin-Converting Enzyme I/D Polymorphism.

*In manuscript.*

# INTRODUCTION

## Historical aspects of endurance running

The origin of the modern marathon race is based on the myth of the Greek messenger Pheidippides. Historical sources claim that he ran approximately 40 km from the battlefield at Marathon to the capital of Athens. After delivering the news of the victory of the Greek army over the Persians, he died of sudden heart death. The Victorian poet Robert Browning elaborated on the story in his 1879 poem entitled *Pheidippides*, by writing: ‘there was joy in his blood bursting his heart’ which conveys the idea that the runner may in fact have succumbed to cardiac rupture. It is noteworthy that Pheidippides was a professional runner who regularly would have delivered messages over great distances. Sadly, the original myth has little support from credible historical sources. Many historians have therefore questioned what actually happened and whether Pheidippides did indeed ever exist.<sup>1</sup> (Figure 1)

When the Olympic Marathon was introduced in 1896, the death of Pheidippides contributed to expectations amongst the general public that race participants were risking their lives. Reports of cases of collapsed endurance participants reached an ever growing audience which further fuelled this new fascination with the perceived danger of exercise-induced lethal exhaustion.

As endurance sports became gradually more popular on a large scale during the latter half of the 20<sup>th</sup> century, the view of marathon races started changing: while running a marathon may



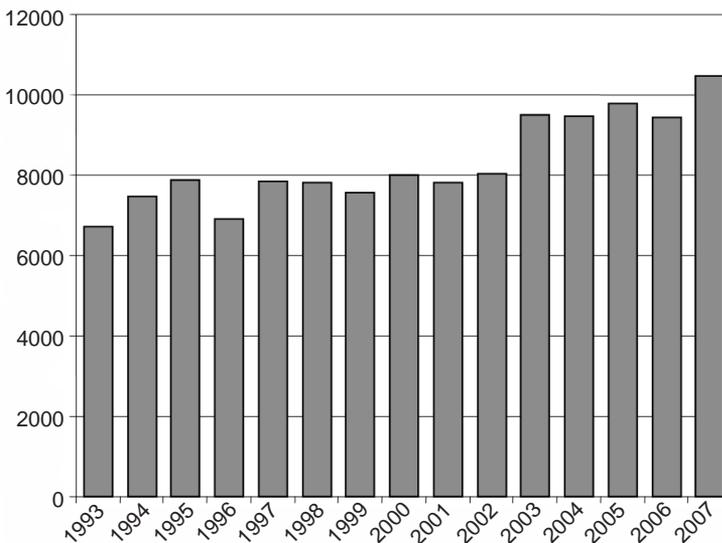
**Figure 1.** Pheidippides, the runner who allegedly suffered sudden death after completing the historical race between the battlefield at Marathon and Athens in 490 BC.

initially have been thought of as something of a near-death experience, the participants were increasingly viewed as not only highly trained but as having extremely good overall health. In the 1970s, this translated into the marathon race itself being regarded as a ‘health event’ and marathon running was even proposed as the ‘graduation ceremony’ after a myocardial infarction, marking the ultimate success of cardiac rehabilitation and the elimination of future cardiac risk.<sup>2</sup> This trend is likely to have culminated with a famous claim made by Bassler in 1974 that marathon runners are protected from coronary heart disease: the so-called *Bassler hypothesis*.<sup>3,4</sup>

## Endurance running – an extreme form of exercise

Long-distance running has since then matured as a sport and has become a lifestyle for millions of runners around the world. The number of long-distance races held every year is growing rapidly and presently exceeds 500 marathons in the U.S.A. alone, attracting close to half a million entrants. This trend has also been seen in Sweden. (Figure 2)

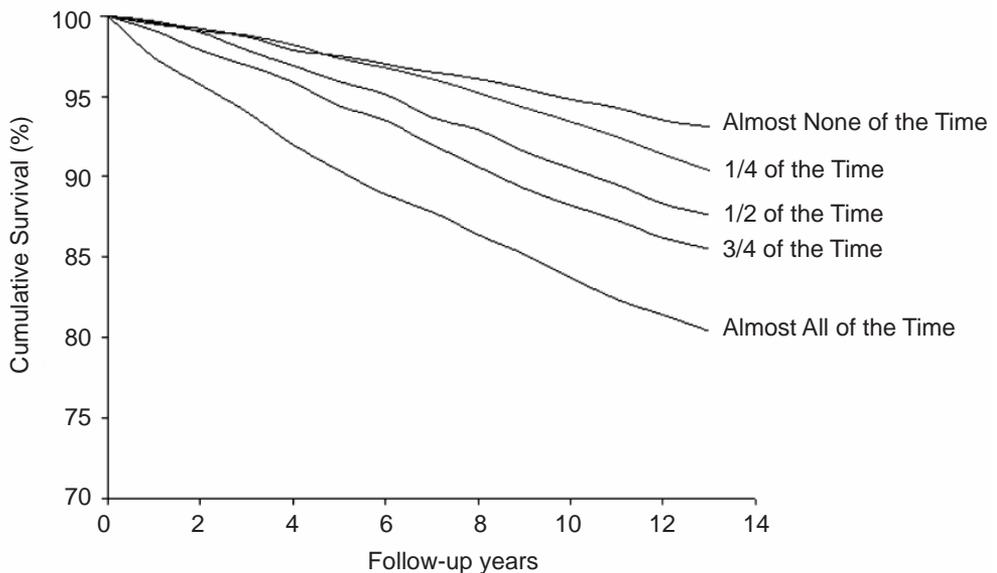
While the benign effects of moderate-intensity exercise are well established, less attention has been directed towards studying the impact of extreme physical exertion during high endurance events. Concerns have been expressed both in the scientific community<sup>5</sup> and lay press<sup>6,7</sup> that athletes that over-exert their cardiovascular system may in fact be putting their health at risk. Most of the long-term follow-up studies of athletes, however, have shown them to remain healthy and actually live longer than their sedentary peers.<sup>8</sup>



**Figure 2.** Number of participants in the Lidingöloppet 30-km race between 1993 – 2007. Adapted from reference 44.

## Health benefits of moderate-intensity exercise

There is a trend in our modern society that a sedentary lifestyle is becoming more prevalent. This has contributed to an increasing prevalence of several chronic medical disorders like diabetes, cardiovascular disease and cancer.<sup>9</sup> This places a heavy burden on healthcare resources and leads to millions of deaths every year. To counter this development, the general population is often advised to engage in moderate-intensity exercise on a regular basis. One commonly made recommendation includes 30 minutes of brisk walking, five days per week.<sup>10</sup> Prospective observational studies have shown associations between regular physical activity and favourable disease outcomes in a range of important medical conditions such as obesity, hypertension, diabetes mellitus, cardiovascular disease, thromboembolic stroke, osteoporosis, malignancies like breast and colon cancer, cognitive dysfunction and psychiatric disorders such as anxiety and depression (reviewed by Kesaniemi<sup>11</sup>; Figure 3) While less evidence has come from adequately designed, randomised controlled trials in this field, it is widely considered that regular, moderate exercise lowers the risk of many of these disorders.<sup>12,13</sup>



**Figure 3.** Risk of all-cause mortality associated with the time spent sitting in a cohort of 17,013 Canadians aged 18 – 90 years followed for an average of 12 years in a longitudinal cohort study (Canada Fitness Survey). The Kaplan-Meier survival curve shows a progressively higher risk with increasing time spent sitting: Log-rank  $\chi^2 = 174.4$ ,  $df = 4$ ,  $p < 0.0001$ .

Reprinted with permission from reference 13.

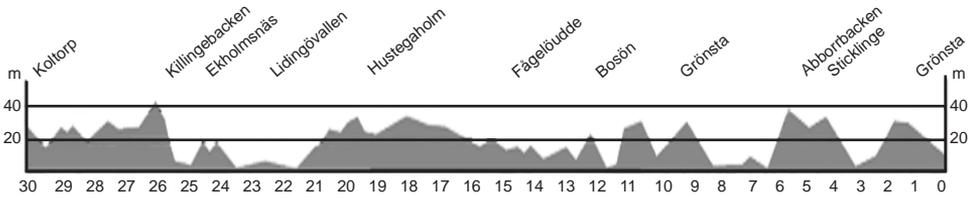
## Cardiac physiology at rest and during moderate exercise

The underlying cellular and molecular events underpinning muscle cell contraction both under normal resting conditions as well as moderate exercise have been relatively well characterised. Cell membrane depolarisation activates voltage-gated (L-type) calcium channels. Calcium influx causes activation of the Ryanodine receptor (RyR) at the sarcoplasmic reticulum which, especially in cardiomyocytes, leads to an even greater increase in intracellular calcium by means of a positive feedback mechanism (calcium-induced calcium release). The conversion of the spreading depolarisation into force production (excitation-contraction coupling) relies on the binding of intracellular calcium to troponin C on actin filaments which causes cross-bridging between actin and myosin and, as actin is pulled towards the centre of the sarcomere, the cell contracts. The energy required for this process is obtained through hydrolysis of ATP produced in mitochondriae mainly clustered around the RyRs. Relaxation of the cell occurs once intracellular calcium has been cleared from the cytosol by one of several ion channels which is also powered by ATP, leading to the reversal of the aforementioned process and the termination of contraction.<sup>14</sup>

During exercise, cardiac output needs to increase to meet a larger demand. Stroke volume and heart rate are the two key determinants of cardiac output and both rise early in the course of a bout of exercise. In theory, stroke volume is determined by 4 factors: (1) volume of venous blood returned to the heart, (2) distensibility of the ventricles, (3) contractility of the ventricles and (4) aortic or pulmonary artery pressure (the pressure against which the ventricles must contract). In practice, factors 1 and 2 determine how much end-diastolic volume increases, and factors 3 and 4 determine how much end-systolic volume decreases. In healthy subjects, stroke volume gradually increases up to an intensity of approximately 40 – 60% of maximal capacity, after which it plateaus. After that point, heart rate alone is responsible for further augmentation of cardiac output.<sup>15</sup> At heart rates above 60 min<sup>-1</sup>, systolic levels of intracellular calcium increase in the cardiomyocytes, leading to positive inotropic effects on the heart. This rate-related increase in contractility<sup>16</sup> has been proposed to explain as much as 40% of exertional augmentation of cardiac output and is commonly referred to as the force-frequency relationship.<sup>17</sup> Parasympathetic withdrawal is responsible for most of the early elevation of heart rate which occurs on initiation of physical activity. With more intense exertion, a relatively more prominent role is played by increased sympathetic tone. This leads to increased myocardial inotropy and lusitropy as well as redistribution of blood from the gut, spleen, liver and kidneys. Humoral feedback from blood-borne metabolites and release of adrenaline and noradrenaline from the adrenal medulla also contribute to such systemic circulatory changes.<sup>18</sup>

## The physiology of fatiguing exercise

Prolonged and heavy physical exercise is known to lead to fatigue, a phenomenon studied especially in skeletal muscle cells and can be characterised as reduced force of contraction, rate of relaxation and velocity of contraction. Several different explanations have been proposed for fatigue, which can be broadly characterised as *peripheral* or *central*. The simplest model is the peripheral explanation, which proposes that fatigue sets in when mechanisms fail that are directly related to muscle force production in general and calcium handling in particular. Such peripheral aspects include depletion of energy substrates or accumulation of energy turnover metabolites, leading to limited calcium availability or decreased myofilament sensitivity to calcium. Lack of glycogen or ATP, alternatively



**Figure 4.** Race course profile of the Lidingöloppet 30-km cross-country race which has over 60 hills and is considered by many runners to be the toughest long-distance race in Sweden.

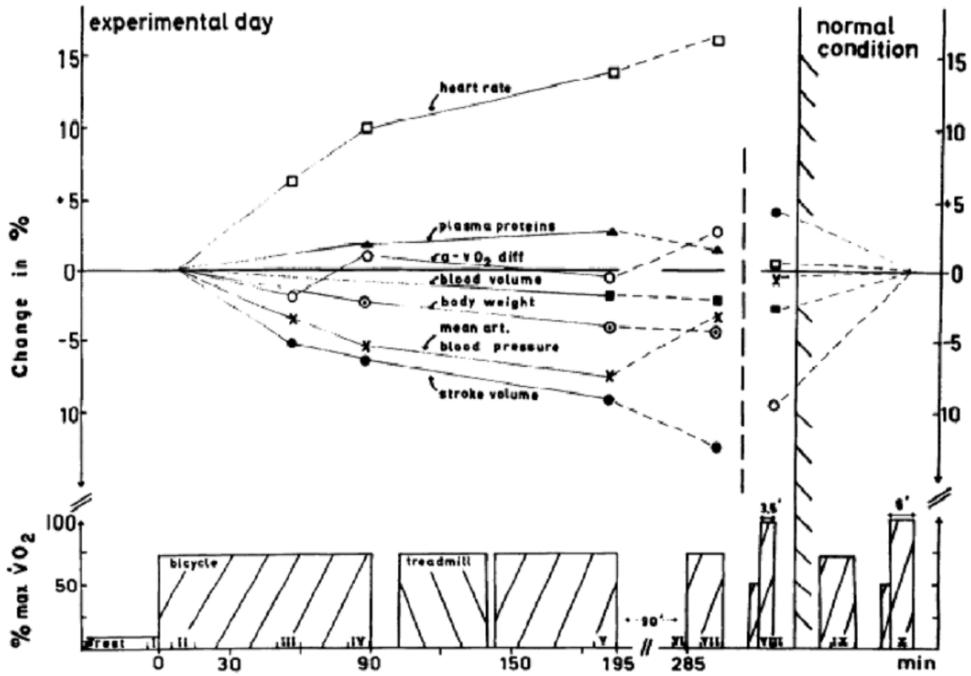
accumulation of e.g. lactic acid from glycogen breakdown or ADP have been proposed.<sup>19–21</sup> Rising concentrations of inorganic ions (e.g. magnesium or phosphate)<sup>22,23</sup> and ammonia<sup>24</sup> have also been suggested.

Critics of the peripheral fatigue model have argued that this view is reductionistic and simplistic. Instead, a more complex perspective has been advocated, which suggests that fatigue is a sensation which arises in the central nervous system following integration of afferent information available from a range of sources: peripheral muscle chemoreceptors and mechanoreceptors as well as e.g. core temperature (*the central governor model*).<sup>25,26</sup> As this model implies that fatigue is a sensory perception rather than a physical phenomenon it is suggested that anticipation based on prior experience (‘forecasting’) may improve the ability to withstand fatigue owing to less perceived exertion.

Previous studies in endurance athletes have suggested that prolonged exercise may cause depression of the function of the heart, too, a phenomenon which is referred to as *cardiac fatigue* in this thesis.<sup>27</sup> While most of the published data regarding skeletal muscle fatigue were obtained from experiments *in vitro*, we used the 30-km cross-country race *Lidingöloppet* as an *in vivo* model for cardiac fatigue. (Figure 4) The background of this phenomenon is reviewed below.

## Cardiovascular drift and cardiac fatigue

The effects of fatiguing exercise on the function of the heart were first studied by Bengt Saltin and Jesper Stenberg<sup>28</sup> in four subjects who exercised for 180 minutes. They used dye dilution methodology to measure cardiac output which allowed them to calculate stroke volume, and found that a downward trend in stroke volume (*cardiac fatigue*) was paralleled by an upwards trend in heart rate (*cardiovascular drift*). (Figure 5) While cardiovascular drift and cardiac fatigue were both documented in several subsequent articles,<sup>29,30</sup> however, the possibility that these phenomena related primarily to a reduction of intravascular volume was raised early.<sup>31,32</sup> Advances made in imaging methodology in later years has enabled cardiac function to be characterised with a lower sensitivity to loading. This has confirmed the presence of attenuated cardiac function after exercise, including reduced tissue velocities on tissue Doppler imaging (TDI)<sup>33,34</sup> and reduced myocardial strain on both TDI<sup>35</sup> and speckle tracking imaging (STI, please refer to *Methods*).<sup>36,37</sup> Interestingly, previous studies in this field have not clarified whether cardiac fatigue is associated with altered ventricular repolarisation. Early studies suggested this may be so<sup>38</sup> but at least two subsequent reports were not able to confirm this finding.<sup>39,40</sup> The techniques employed in this thesis therefore included echocardiography (both ‘track-side’ and in a dedicated laboratory, including TDI



**Figure 5.** Cardiac fatigue documented in an experimental setting by Bengt Saltin and Jesper Stenberg in 1964. An upward drift in heart rate is shown by the top line (cardiovascular drift) and a gradual decline in stroke volume is shown by the bottom line (cardiac fatigue). Reprinted with permission from reference 28.

and STI) and vectorcardiography, as well as serum analyses of cardiac biomarkers at baseline and after prolonged exercise. All of these modalities allow alterations of cardiac function to be characterised with high sensitivity.

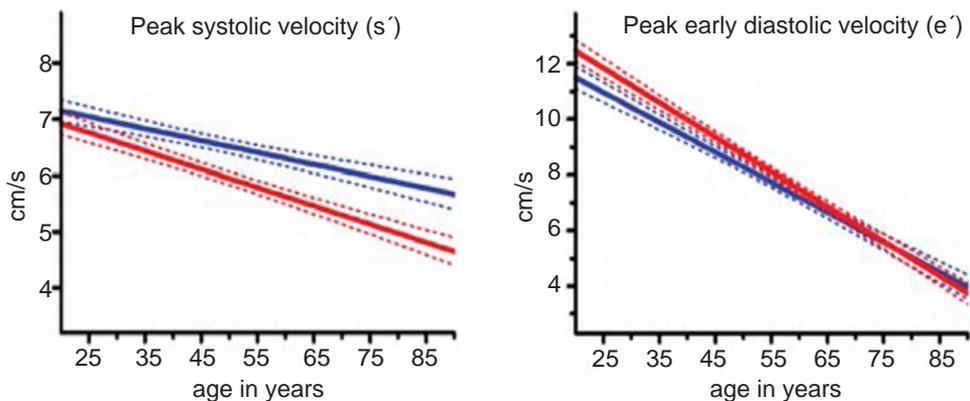
### Trends in endurance exercise and trends in cardiac fatigue research

Much of the important early work in the field of cardiac fatigue was performed in very highly trained athletes undergoing extremely demanding events, such as an uninterrupted 24-hour foot race or the Hawaii Ironman.<sup>27,41</sup> In contrast, several recent studies in this field have focussed on the growing segment of relatively less experienced participants that make up a large proportion of more ‘moderately’ exhaustive events, typically marathon races. In a landmark study by Neilan and co-workers, the authors stratified the sample population post-hoc based on pre-race training. In the least-trained group (training mileage <45 miles/week i.e. <72 km/week), runners exhibited a larger increase in cardiac biomarkers post-race as well as greater cardiac fatigue evidenced by depressed tissue velocities.<sup>35</sup> In the present thesis, the data obtained in paper III indicated that experience plays an important role for biomarker release. Subjects were therefore recruited into studies 4 and 5 according to prior exposure to endurance events.

Recent years have seen a surge in the proportion of runners of senior age, so-called master athletes which has loosely been defined as aged > 40 – 45 years. This is the fastest-growing age segment in many long-distance events.<sup>42</sup> Ageing is associated with decreased fitness and exercise performance. This is due to natural changes occurring with ageing (reviewed below), injury, disease and lifestyle changes.<sup>43</sup> Senior athletes can thus be expected to somewhat struggle to achieve similar race times as their younger peers. Furthermore, runners in this group appear to be less trained as evidenced by their gradually longer race times documented in the *Lidingöloppet* over the past 15 years.<sup>44</sup> Previous epidemiological data and published case series also indicate that senior athletes actually run a considerably higher cardiovascular risk than their younger peers.<sup>45,46</sup> As previous studies in this field have unfortunately not included senior participants, we chose to exclusively focus on this group in papers I – III of this thesis (please refer to *Methods* below).

### Impact of ageing on cardiovascular physiology and exercise performance

Multiple important changes occur in ageing which have a profound impact on cardiovascular physiology. Studies evaluating this have commonly quantified physical functional capacity using the variable  $\dot{V}O_{2\max}$  (maximum oxygen uptake) which is widely considered to be the best measure of overall cardiopulmonary function and may be calculated using the Fick equation as the product of maximum heart rate multiplied by maximum stroke volume (i.e. maximum cardiac output) multiplied by maximum arteriovenous  $O_2$  difference (i.e. the greatest possible extraction of oxygen in peripheral tissues). Ageing has been very clearly documented to lead to a progressive reduction of physical performance and  $\dot{V}O_{2\max}$  as outlined by Lakatta.<sup>47</sup> The Fick equation is influenced in several ways by age: (1) maximum heart rate is reduced by approximately 1 beat/min per year, partly due to decreased sensitivity of the sinoatrial node to  $\beta$ -adrenergic stimulation.<sup>48</sup> (2) The ageing heart cannot deliver the same stroke volume during exercise. This is due in part



**Figure 6.** Longitudinal cardiac function expressed as tissue velocities in persons without hypertension, diabetes and ischaemic heart disease. Red and blue curves refer to women and men, respectively. Stippled curves indicate 95% confidence intervals. Reprinted with permission from reference 51.

to limited augmentation of ejection fraction<sup>49</sup> and also to a decline in cardiac longitudinal function which occurs as part of ‘normal ageing’,<sup>50,51</sup> (Figure 6) This in turn predisposes the heart to exacerbated dysfunction of diastolic relaxation during exercise. This is likely to be compounded by unfavourable loading<sup>52</sup> which partly reflects higher systemic blood pressure<sup>53</sup> and greater total peripheral resistance<sup>54</sup> during exercise. (3) Reduced tissue oxygen extraction also occurs with higher age which is likely to reflect several underlying processes of the oxygen carrying capacity of the blood (e.g. a lower haemoglobin) as well as declining function of peripheral tissues including senile sarcopenia and disuse atrophy<sup>55</sup> as well as reductions of muscle vascularity and deterioration of mitochondrial function.<sup>56</sup>

## The role of cardiovascular biomarkers in health and disease

A growing number of assays are becoming commercially available which analyse levels of biomarkers. This relatively loosely defined term refers to enzymes, hormones or biological substances that define cardiac stress, malfunction or myocyte injury. Biomarkers are increasingly used for diagnostic and prognostic purposes in a range of cardiovascular disorders and are also, to some extent, used to screen for disease in healthy individuals. Morrow and de Lemos have set out three fundamental questions which may be used to assess the clinical value of a biomarker: (1) *Can the clinician measure it* (Are there accurate, repeatable, cost-effective assays with short turnaround times)? (2) *Does it add new information* (which is not already available from a careful clinical assessment)? (3) *Does it help the clinician to manage patients?*<sup>57</sup>

### *Natriuretic Peptides*

The finding that rats injected with an extract of atrial myocardial tissue developed copious diuresis and natriuresis led to the discovery of Atrial Natriuretic Peptide (ANP) by Adolfo J de Bold in 1981.<sup>58</sup> This opened up an entirely new field of cardiovascular research by showing that the heart has an endocrine function. Further work led to identification of a family of natriuretic peptides which included not only ANP but also Brain Natriuretic Peptide (BNP). This hormone was first encountered in porcine brain. In humans it is mainly produced in the cardiac ventricles. Natriuretic peptides are liberated from the atrial (ANP) or ventricular (BNP) wall in response to elevation of wall stress, as this causes mechanical stretch of cardiomyocytes leading to the release of storage granules close to the cell membrane. Their actions include natriuresis and diuresis as well as decreased smooth muscle tone which reduces systemic resistance and blood pressure, and increases cardiac output.<sup>59</sup> The main clinical use for this group of peptides is in diagnostic testing for left ventricular dysfunction and heart failure. BNP has received more interest owing to its longer biological half-life. In particular, the 76 amino acid N-terminal fragment pro-Brain Natriuretic Peptide (NT-proBNP) is co-secreted with BNP and has a half-life of 1 – 2 hours. This makes it a better target for blood testing than both BNP and ANP.<sup>60</sup> In clinical practice, the negative predictive value of low NT-proBNP levels is stronger than the positive predictive value of elevated levels due, in part, to the impact of age, gender and renal function on plasma levels.<sup>61</sup> In addition to its diagnostic importance, NT-proBNP is also characterised by a very strong ability to prognosticate. In fact, in this respect it is so powerful that, even at levels that are within the normal range for diagnostic purposes, NT-proBNP is a strong predictor of adverse clinical outcomes such as cardiovascular events and death.<sup>62</sup>

*Cardiac troponins*

Troponins (Tn) are structural proteins found in both skeletal and cardiac muscle cells and are made up of three subunits: TnT, TnI and TnC. The troponin complex is bound to the contractile apparatus of the myocytes and plays an important role in mediating its interaction with calcium during normal physiological circumstances. In addition to the complex-bound TnT and TnI, approximately 6 – 8% of TnT and 2 – 8% of TnI are present as free, non-complexed molecules in the cytosol. Muscle cell damage leads to release of troponins into the blood stream; an early peak occurs which reflects transmembrane leakage of the cytosolic pool, followed by a sustained release of complex-bound troponin which appears within a few hours of the onset of ischaemia. In the case of cardiac troponins, serum levels are normally extremely low. Elevated cardiac troponins in serum are therefore indicative of cardiac myocyte damage and elevation above a pre-defined threshold is diagnostic for myocardial infarction.<sup>63</sup> Cardiospecific troponin assays have become essential to clinical cardiology and they have revolutionised the way myocardial infarctions are diagnosed.

The presence of cardiac troponin in serum also has negative prognostic importance both in acute myocardial infarction<sup>64</sup> and in e.g. renal failure, which is characterised by an inability to clear troponin from the circulation and therefore often causes significant elevation even in the absence of symptoms.<sup>65</sup> As with NT-proBNP, sub-*diagnostic* levels of circulating cardiac troponin may also carry important negative *prognostic* value in healthy volunteers as was recently shown using a high-sensitivity troponin assay.<sup>66</sup>

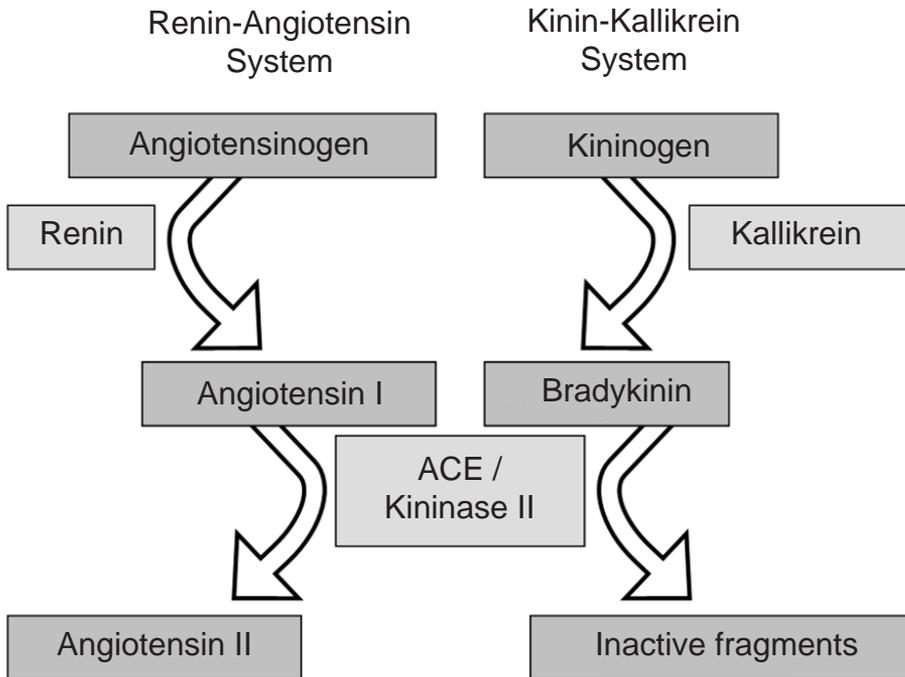
In the case of TnI there are 10 – 20 different commercially available assays. Each of these uses its own antibody (which has its own epitope on the troponin I subunit) and requires its own analytical reagents. This has led to considerable difficulties in making comparisons between studies that use TnI. On the contrary, there has always only been one commercially available analysis for TnT, manufactured by Roche Diagnostics. The earliest assays for cardiac troponin were limited by cross-reactivity between troponin of skeletal muscle vs. cardiac origin. This is not the case with the 3<sup>rd</sup> generation TnT assay which is directed against a unique epitope found only on the cardiac TnT isoform, eliminating all cross-reactivity with skeletal muscle TnT even in the presence of rhabdomyolysis.<sup>67</sup>

*C-reactive protein*

Inflammation leads to production of IL-6 by macrophages and other cells. This acts on the liver cells to produce C-reactive protein (CRP) which is believed to be important for the immune system by assisting in complement binding to foreign and damaged cells. Levels of CRP rise with inflammatory activity. It may therefore reach detectable levels with the arterial inflammation that occurs in atherosclerosis.<sup>68</sup> Several studies have shown that healthy volunteers with minor elevations of baseline CRP have an increased risk of cardiovascular events.<sup>69</sup>

**Angiotensin-Converting Enzyme I/D Polymorphism**

Angiotensin-Converting Enzyme (ACE) is the enzyme that converts Angiotensin I to Angiotensin II (Ang II) in the body, a key component of the renin-angiotensin system which is of great importance for the regulation of blood pressure and circulating volume. ACE is also known as *kininase II* and plays a role in the kinin-kallikrein system by degrading bradykinin into inactive fragments.<sup>70</sup> (Figure 7) Bradykinin is a vasoactive polypeptide which acts mainly via the bradykinin  $\beta$ 2-receptor to cause a variety of effects including vasodilatation, hypotension and production of nitric oxide and prostaglandins (reviewed by Regoli<sup>71</sup>).



**Figure 7.** Role of Angiotensin-Converting Enzyme in the metabolic pathways of the Renin-Angiotensin as well as Kinin-Kallikrein Systems.

Cambien and co-workers discovered an insertion/deletion polymorphism of the Angiotensin-Converting Enzyme (ACE) gene in 1990 which determines the carrier's ACE activity.<sup>72,73</sup> The ACE D allele is associated with greater activity of ACE which leads to higher levels of Ang II and lower levels of bradykinin. Carriers of the D allele have been shown to develop unfavourable cardiac remodelling especially under conditions characterised by activation of the renin-angiotensin system. As this occurs commonly in cardiac disease, carrying the D allele is not surprisingly predictive of a poor prognosis in the presence of coronary artery disease<sup>74,75</sup> and also in heart failure due to ischaemic heart disease or dilated cardiomyopathy.<sup>76</sup> Renin-angiotensin system activation also occurs with physical exercise and Ang II is known to be a growth factor for muscle cells. Sports physiologists therefore developed an early interest in the ACE I/D polymorphism and noted that carriers of the D allele exhibit rapid strength gains on initiation of physical training. This led to early reports that a 'gene for human athletic performance' had been discovered.<sup>77</sup>

## Cardiac synchrony

### *Electrical synchrony*

In patients with severe heart failure compounded by cardiac conduction disorders, delayed activation commonly occurs in some parts of the heart, especially the lateral wall. Such patients benefit from implantation of a biventricular pacemaker which enables simultaneous

electrical stimulation of both ventricles, a treatment intended to synchronise the otherwise asynchronous (or 'dyssynchronous') mechanical activity of the right and left ventricles and is referred to as cardiac resynchronisation therapy (CRT).

From the outset in the 1990s, CRT trials enrolled patients with a QRS width > 120 ms. As 25 – 30% of patients did not respond to this costly and invasive therapy, it was suggested that modalities such as echocardiography may better predict response by identifying lack of ventricular mechanical synchrony.<sup>78</sup> Various ways to identify mechanical dyssynchrony were proposed and several studies found these to correlate better to treatment benefit than QRS width, i.e. the original CRT criterion.<sup>79,80</sup> In the recently published Predictors of Response to CRT (Prospect) study, 498 patients who had standard CRT indications were recruited in 53 centres. Subjects also underwent echocardiography to assess its incremental predictive value over standard QRS criteria. The results were largely disappointing: echocardiographic parameters were modestly accurate in predicting CRT response and did not improve patient selection beyond existing QRS width-based guidelines. Importantly, there was considerable inhomogeneity between centres: echocardiographic technique differed (in terms of image loop frame rates, as regards the vendor of the echocardiographs used etc.) and study quality varied between centres (TDI data was non-assessable in a large proportion of studies from many centres). Agreement between readers was accordingly a problem. It is likely that measurements of dyssynchrony require more training and are more complex than previously appreciated.<sup>81</sup> For the purpose of clinical selection of patients for CRT, the available evidence today does not support the use of echocardiographic techniques. Instead patient management should be based on QRS criteria.<sup>82</sup>

### *Mechanical synchrony*

Recent clinical interest in heart failure and CRT response has thus led to considerable attention being directed to the mechanical activation pattern of the left ventricle in health as well as disease. In this context, it is important to recognise that several of the echocardiographic indices proposed for quantification of dyssynchrony actually provide a measure of the heterogeneity of ventricular mechanical activation between LV segments. It is known that the left ventricle is not activated in an entirely synchronous fashion under normal resting circumstances.<sup>83,84</sup> This is not something new: observations of physiological dyssynchrony were first made more than 80 years ago and were described in the absence of cardiac dysfunction.<sup>85,86</sup> As complete synchrony is uncommon in the normal heart, it has actually been suggested that diminished dyssynchrony (increased synchrony, 'hypersynchronisation') may be abnormal.<sup>87</sup> Nevertheless, increased dyssynchrony is a more common finding in cardiac disease and may result from several different disorders of the left ventricle of which one is conduction disease and bundle branch block (reviewed by Kass<sup>84</sup>). One of the best known criteria for quantifying intra-left mechanical dyssynchrony was pioneered by Yu and co-workers and is based on TDI-derived measurements of the time from Q-wave onset to peak longitudinal velocity. The standard deviation across 12 segments of the left ventricle (LV; 6 mid-ventricular, 6 basal) is calculated, providing a measure of inter-segmental heterogeneity i.e. mechanical dyssynchrony.<sup>88</sup> In paper IV we studied whether prolonged exercise leads to increased dyssynchrony, using this 12-segment standard deviation technique.

## **Ventricular repolarisation**

The membrane potential of a myocyte is -60 to -90 mV during diastole and is maintained by the sodium-potassium pump. When the cell is activated, it goes through four phases each characterised by ion channels interacting to create an action potential as follows.

During the initial phase (0), there is rapid inflow of sodium ions across the membrane which causes the cell to depolarise. Phase 1 consists of brief repolarisation caused by transient outward potassium flow. Phase 2 is characterised by a balanced outward flow of potassium ions and inward flow of calcium and sodium ions, which causes the membrane potential to temporarily plateau. It is this calcium influx that initiates contraction of the cell as described under *Cardiac physiology at rest and during moderate exercise* above. Inward flux decreases during phase 3 which leads to repolarisation and restoration of normal resting transmembrane potential. Diastole occurs during phase 4.

Disorders of impulse formation can predispose the heart to arrhythmias. These include increased *automaticity* which may arise when the diastolic potentials of cells change, as well as *triggered activity* initiated by afterdepolarisations (i.e. depolarising oscillations in membrane voltage induced by one or more preceding action potentials). Early afterdepolarisations occur before full repolarisation of the cell membrane has occurred and leads to prolongation of repolarisation time manifested in the ECG as lengthening of the QT-interval, an important marker of increased propensity to ventricular arrhythmias.<sup>89</sup> In contrast, delayed afterdepolarisations occur once cell membranes have fully repolarised and may arise e.g. secondary to digitalis intoxication or in the setting of a recent myocardial infarction.<sup>90</sup>

Ventricular repolarisation is an important aspect of cardiac function which, as mentioned above, had not been examined fully in previous reports describing cardiac fatigue in athletes. In paper II, we used vectorcardiography to characterise cardiac electrophysiology and specifically studied ventricular repolarisation.

# AIMS

The general aim of this study was:

- To evaluate the immediate (hours) and short-term (days) effects of prolonged exercise on cardiac function

The specific aims were:

- To assess cardiac biomarker increase in participants of the long-distance race *Lidingöloppet* aged  $\geq 55$  years.
- To investigate whether such biomarker increase is reproducible in individual runners
- To examine the role of prior running experience on biomarker elevation after an endurance race
- To investigate the underlying factors that predict release of troponin T and NT-proBNP in senior participants in endurance exercise
- To investigate whether runners may have underlying, clinically important disorders which lead to biomarker elevation
- To examine echocardiographic attenuation of cardiac function after prolonged exercise in senior athletes
- To examine changes in ventricular repolarisation after prolonged exercise
- To assess the effects of prolonged exercise on left ventricular mechanical synchrony
- To investigate the importance of the ACE I/D polymorphism for biomarker levels and left ventricular synchrony in the setting of endurance exercise

# MATERIAL AND METHODS

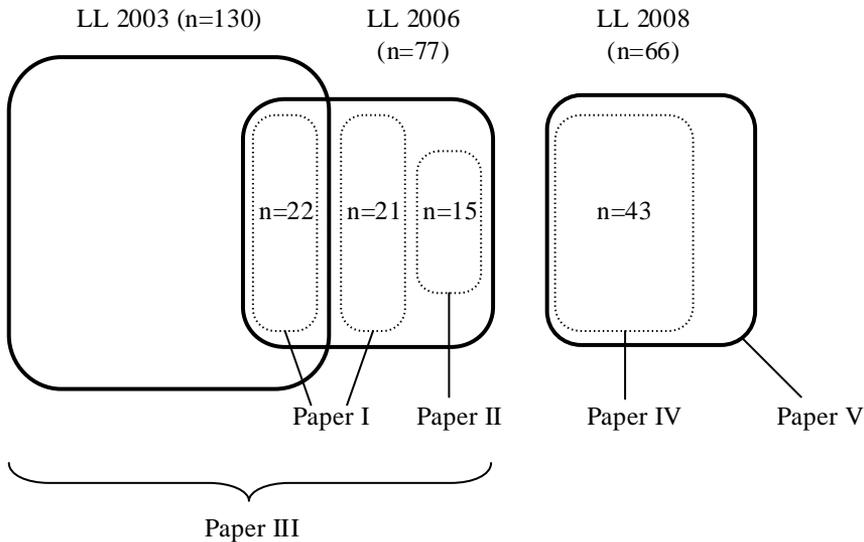
## Recruitment of subjects

An overview of the recruitment of subjects is shown in Figure 8.

### Study I – III

Studies I – III included participants aged  $\geq 55$  years at the Lidingöloppet 30-km foot race. Recruitment was based on lists of participants scheduled to participate as provided by the race administration. A written questionnaire was sent out to those runners who appeared to likely be eligible based on age and address (study II). The questionnaire covered aspects of prior training as measured e.g. in hours and kilometres per week, and also the physical health of athletes including past and present medical disorders (e.g. atrial fibrillation, hypertension, asthma, diabetes, “known cardiac murmur” etc.) and any on-going medical treatment be it on prescription or over the counter.

For article I, we identified entrants at the race in 2006 that had already participated in 2003. In order to enhance the statistical power of the echocardiographic analyses, the study population was doubled by including an equally large group of runners registered to partake in 2006. Article II was performed as a sub-analysis of the group participating exclusively in the race in 2006 and included detailed serial echocardiographic and vectorcardiographic evaluations (please see below). As this study was designed to take place over the course of 6 days, runners living in the area around Stockholm were given preference.



**Figure 8.** Overview of Lidingöloppet races and of the subjects enrolled into the studies of this thesis.

All subjects were carefully instructed not to engage in any strenuous physical activity 2 weeks before the study as well as for the duration of the study. Article III was based on pooled data obtained in either 2003 or 2006. For runners participating in both years, results from 2006 were analysed. Sample sizes for the different studies were determined by logistic and practical aspects. This included factors such as the number of staff involved in taking blood tests at the race and their estimated maximum capacity so as to avoid queuing.

#### *Study IV – V*

Subjects in studies IV and V were enrolled from lists of entrants into the Lidingöloppet race in 2008 using the aforementioned questionnaire. These were stratified into two groups: *Beginners* at the *Lidingöloppet* who had never previously participated in this event, and *Experienced* runners who by definition had raced on  $\geq 8$  previous occasions. Echocardiographic assessment (study IV) was conducted in a sub-group consisting of  $n=43$  subjects of which 23 were experienced and 20 beginners. Study V was performed in  $n=66$  runners of which 31 were experienced and 35 beginners. Groups were matched for age and gender as described below. In the recruitment process no age cut-off was used, partly as this would have made matching very difficult: few young runners have raced 8 times previously and few senior runners have never participated previously.

#### *Male vs. female runners*

Studies I and III included a mixed sample comprising male as well as female runners. In 2003, subjects were recruited into study III irrespective of sex. In 2006, however, all eligible female subjects were invited to participate.

In contrast, only male participants were recruited into studies II, IV and V. In the case of study II, this was purely coincidental and reflects the small number of eligible female participants. In studies IV and V, however, this was a deliberate choice made for several reasons: female participants in the setting of endurance sports differ from male participants in several important ways (fewer previous participations, lower body weight, longer race duration). Female participants may differ in terms of pre-race training (e.g. less running, more cross-training exercise). As pre-race exposure to running was the principal variable used to prospectively stratify subjects in studies 4 and 5, it was decided to avoid criticism on this point by exclusively including male runners. It was also anticipated that the very small number of female runners eligible for the *experienced* group would lead to problems with the matched design.

## **Blood tests**

To collect blood specimens before and immediately after the *Lidingöloppet* race, subjects were asked to come to a designated blood testing area in close proximity to the changing rooms near the start and finishing lines of the race. Runners were requested to show up no more than 2 hours before the race, and again as soon as possible afterwards. On each occasion body weight was recorded (Exclusive, EKS International, Wittisheim, France) and used to calculate Body Mass Index ( $BMI = \text{weight} / \text{height}^2$ ). Blood pressure was measured once at baseline (studies I – II, IV – V). Blood was drawn from an antecubital vein. Haematological tests such as haemoglobin, haematocrit and white cell count were run on whole blood using standard laboratory methodology. For all biochemical analyses, EDTA-containing bottles were used which were kept on ice and centrifuged within 4 hours. To ensure analyses were

performed without delay, a shuttle service was in operation on the race day which transferred specimens between the track-side blood testing facility and the biochemical laboratory. The supernatant plasma was analysed prior to freezing in all studies. The blood tests presented in study I and III were obtained at two different races: 2003 and 2006, but were performed using identical methodology and reagents. Cystatin C was analysed in paper V, which is an alternative marker of renal function which is produced at a constant rate and eliminated exclusively by glomerular filtration.<sup>91</sup> It has been suggested that cystatin C constitutes a more reliable measure of renal function than creatinine in endurance sports participants.<sup>92</sup> The estimated glomerular filtration rate (eGFR) was calculated using the formula  $eGFR = 99.43 \times [\text{cystatin C}]^{-1.5837}$  (in mL)<sup>93</sup> as this method is accurate in subjects with preserved renal function.<sup>94</sup>

#### *NT-proBNP*

In studies I – III and V we analysed NT-proBNP using an assay which has a coefficient of variation (CV) of < 6% (Roche Diagnostics Scandinavia, Bromma, Sweden). As per the manufacturer's advice, the normal ranges used were as follows: male subjects aged > 50 years (studies I – III): < 194 ng/L; female subjects aged > 50 years (studies I, III): < 220 ng/L; male subjects aged < 50 years (study V): < 84 ng/L.

#### *Troponin T*

Troponin T was analysed in all studies using a 3<sup>rd</sup> generation assay which has a CV of < 7% (Roche Diagnostics Scandinavia, Bromma, Sweden). The manufacturer advises that a decision limit is used for myocardial necrosis of  $\geq 0.03 \mu\text{g/L}$  in clinical practice, which was adopted in all our studies as the upper limit of the normal range. The Roche Diagnostics 3<sup>rd</sup> generation TnT assay has a limit of detection of  $0.01 \mu\text{g/L}$  which posed a particular problem in data handling by making TnT a non-continuous variable. In all 5 papers we transferred the less-than value into that number for both pre- and post-race TnT (i.e.  $<0.01 \mu\text{g/L}$  was replaced by  $0.01 \mu\text{g/L}$ ). This was done based on the assumption that the elevated concentrations of TnT seen mainly post-race were unlikely to be significantly affected by minor, undetectable deviations downward from  $0.01 \mu\text{g/L}$ . This approach was also advocated by senior experts in the field who were consulted in this matter.<sup>95,96</sup> In paper III we explored the predictors of TnT release after dichotomising the post-race TnT as  $\geq$  or  $< 0.03 \mu\text{g/L}$  (i.e. elevated vs. non-elevated) in contrast to NT-proBNP which was log transformed to normality and analysed using linear analysis.

#### *C-reactive protein*

A high-sensitivity assay was used to measure CRP which allows its detection within the range 0.20 – 380 mg/L with a CV of 4% (Beckman Coulter, Bromma, Sweden).

## **Genetic analysis of Angiotensin-Converting Enzyme I/D polymorphism**

In studies IV and V, DNA was isolated from whole blood using the Qiagen Blood and Cell Culture DNA Midi kit (Qiagen GmbH, Hilden, Germany). The polymorphism consists of an insertion of a 287-bp Alu repeat (EMBL-SVA X62855; NC\_000017.10) and all individuals were genotyped using polymerase chain reaction as described elsewhere.<sup>97</sup>

## Echocardiography

Transthoracic echocardiography was performed using a Vivid 7 system in studies I – IV. “Track-side” examinations performed immediately post-race were carried out in a changing room near the race finishing line which had been temporarily converted into an echocardiographic laboratory. For these examinations (study II) a portable echocardiograph (Vivid i) was used (both from Vingmed, Horten, Norway). A 2.5 MHz transducer was used in all studies. All studies were recorded onto digital media and stored for subsequent off-line analysis.

### *Conventional echocardiography*

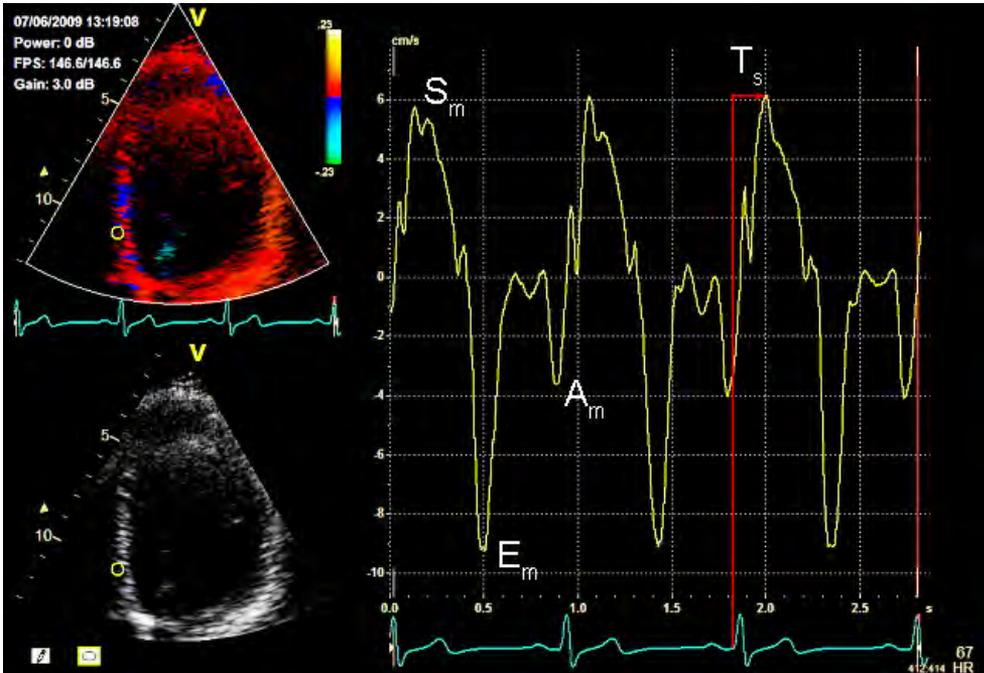
Left atrial (LA) size was evaluated by planimetry of the end-systolic LA area in the apical 4-chamber view in studies I and II, and by LA volume (LAV) using the prolate ellipse method in study IV. The left ventricular (LV) cavity size was measured using LV end-diastolic diameter in the parasternal long-axis view in study I. The LV size and systolic function was described by Teicholtz formula in study II and by Simpson’s biplane formula in studies I and IV (expressed as e.g. LV end-diastolic volume (LVEDV), LV stroke volume (SV) and LV ejection fraction (LVEF)). Wall thickness in studies I and IV was measured using end-diastolic measurements of septal (IVSd) and posterior wall thickness (PWDd) in the parasternal long-axis view. In study I – II, LV mass was calculated based on the ASE convention ( $LV\ mass = 1.04 \times [(LVEDD + PWDd + IVSd)^3 - LVEDD^3] \times 0.8 + 0.6\ g/L$ ) and in study IV based on the Penn convention ( $LV\ mass = 1.04 \times [(LVEDD + IVSd + PWDd)^3 - (LVEDD)^3] - 13.6\ g/L$ ).<sup>98</sup> All cardiac dimensions were indexed for body surface area where applicable (LAVi, LVEDVi, LVMi etc) Pulsed-wave Doppler was used to interrogate trans-mitral early (E) and late (A) diastolic filling.

### *Tissue Doppler imaging and speckle tracking 2-D strain*

Three-beat loops of colour-coded tissue Doppler were used for TDI analyses, analysed at a dedicated EchoPAC workstation (General Electrics, Horten, Norway). Longitudinal cardiac velocities were interrogated using a 5-mm sample volume and systolic ( $S_m$ ), early ( $E_m$ ) and late diastolic lengthening ( $A_m$ ) were recorded as the median of three beats as shown in Figure 9. The ratio  $E/E_m$  was based on the average  $E_m$  from the septal and lateral walls.

In study IV, analyses were performed of intra-left ventricular mechanical synchrony. The three techniques were based on the time from telemetric Q-wave onset to peak  $S_m$  (this interval is referred to as  $T_s$  (Figure 9) Measures of intra-left ventricular synchrony used included (1) the standard deviation across 6 mid-ventricular and 6 basal segments (septal, antero-septal, anterior, lateral, posterior and inferior) referred to as  $T_s$ -SD.<sup>88</sup> (2) The difference in  $T_s$  between septum and lateral wall (S-D delay; measured relative to septal  $T_s$  i.e. positive when the lateral  $T_s$  is longer than the septal and vice versa)<sup>99</sup> and (3) the maximum difference in  $T_s$  between any two of the aforementioned 12 segments ( $T_s$ -Diff).<sup>100</sup>

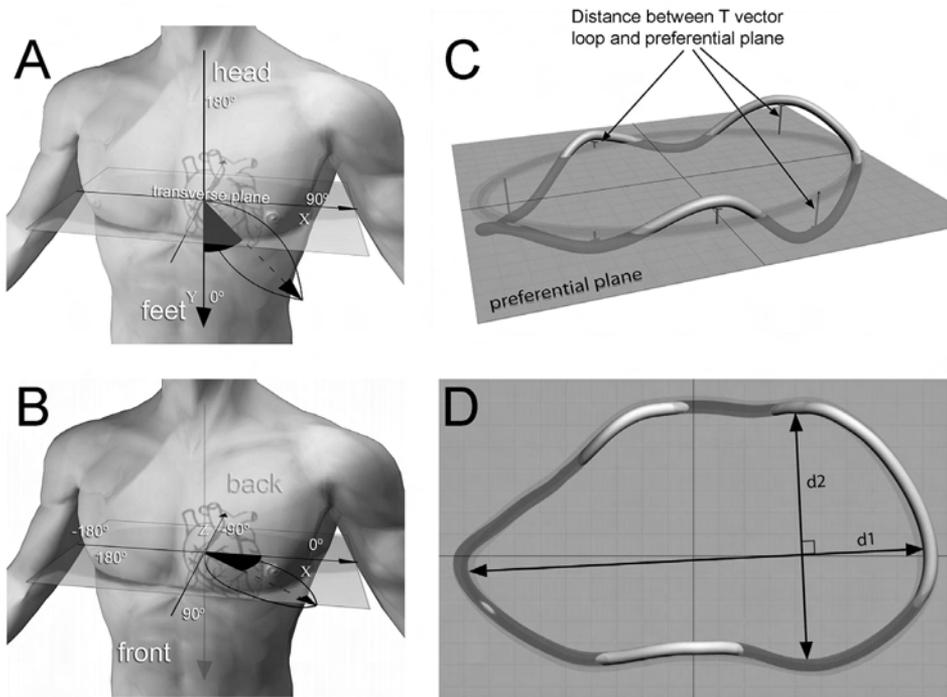
Global peak systolic longitudinal strain was analysed in study II using speckle tracking imaging, a method which utilises the presence of natural acoustic speckles in the recording and tracks them through the image loop to calculate tissue deformation as measured in percent.<sup>101</sup> This method has been shown to correlate well to strain in animals measured by sonomicrometry as well as using magnetic resonance imaging in humans.<sup>102</sup>



**Figure 9.** Myocardial velocity curve by colour tissue Doppler imaging. An apical 4-chamber view is shown with the sample volume placed at the basal septum (yellow circle). Y-axis represents myocardial velocity (cm/s) and the X-axis represents time. The velocity curve shows a positive peak during systole ( $S_m$ ) and two negative peaks during diastole (early,  $E_m$  and late,  $A_m$ ). The time from Q-wave onset to peak  $S_m$  ( $T_s$ ) is shown in red.

## Vectorcardiography

A detailed description of vectorcardiography is available elsewhere.<sup>103</sup> In brief, vectorcardiograms were recorded using a MIDA 1000 system (Myocardial Infarction Dynamic Analysis, Ortivus AB, Danderyd, Sweden) which uses 8 electrodes positioned according to the Frank orthogonal lead system (X, Y and Z). In addition to the conventional measures also available from a standard surface electrocardiogram (ECG; QRS and QT intervals) it enables a detailed characterisation of cardiac electrical activity in three dimensions plus time as reflected by the T vector and T vector loop (referred to as T loop). Vectorcardiograms were recorded from all cardiac cycles averaged over a 5-minute period with subjects at supine rest. T vector and T loop morphology were analysed off-line using customised software,<sup>104</sup> (Figure 10) Based on the three-dimensional PQRST complex, the QRS and QT intervals (corrected for heart rate using Bazett's formula<sup>105</sup> and the  $T_{\text{peak}}\text{-to-}T_{\text{end}}$  ( $T_{\text{peak-end}}$ ) were measured. The CVs for the QT and  $T_{\text{peak-end}}$  intervals, previously analysed in 10 healthy individuals, were 2.8% and 6.2%, respectively. The maximum spatial amplitudes of the QRS and T vectors were measured and are referred to as  $QRS_{\text{amplitude}}$  and  $T_{\text{amplitude}}$ , respectively. The ST segment deviation from the iso-electric line 60 ms after the J point, was measured and is referred to as the ST vector magnitude (ST-VM). The angle between the QRS and the T vectors in space was calculated in degrees ranging from  $0^\circ$  to  $180^\circ$  and is commonly considered to be more abnormal the wider it is.<sup>106</sup> The T vector was described using the terms  $T_{\text{elevation}}$  and



**Figure 10.** Vectorcardiographic parameters.

A:  $T_{\text{elevation}}$ : angle between maximum T vector and a cranio-caudal axis perpendicular to the transverse (horizontal) plane depicted by the rectangle.

B:  $T_{\text{azimuth}}$ : angle between maximum T vector projected onto the transverse plane and left extreme of the X-axis.

C:  $T_{\text{avplan}}$ : distortion of the T loop from the preferential plane, computed as mean distance between the periphery of the loop and this plane. (Small  $T_{\text{avplan}}$ : 'healthy' loop)

D:  $T_{\text{eigenvalue}}$ : shape of the T loop, calculated as ratio between the 2 highest diameters ( $d1/d2$ );  $d1 > d2$ . (High  $T_{\text{eigenvalue}}$ : 'healthy' loop)

Figures in panels A, C and D are reproduced from reference 103 with the permission of the publisher. The figure in panel B is reproduced with permission from Dr. Liliane Wecke, MD, PhD; Cardiac Memory in Two Human Models, doctoral thesis, Karolinska Institutet, Stockholm 2006.

$T_{\text{azimuth}}$  which are explained in Figure 10. The T loop was characterised by its  $T_{\text{avplan}}$  (in  $\mu\text{V}$ ) and  $T_{\text{eigenvalue}}$  (dimensionless) which both appear in Figure 10, as well as the  $T_{\text{area}}$  (in  $\mu\text{Vs}$ ) calculated as the area between the T wave and the iso-electric line from the J point to the end of the T wave in the X, Y and Z leads ( $T_{\text{area}} = (T_x^2 + T_y^2 + T_z^2)^{1/2}$ ).

## Statistical analyses

All data are presented as mean  $\pm$  standard deviation (SD) or as median (inter-quartile range) as appropriate. The statistical analyses in all papers were performed using Statistical Package for Social Sciences (SPSS) version 15 (Chicago, Illinois, USA). In all analyses, a two-tailed p-value  $< 0.05$  was considered statistically significant.

Normality was assessed using Kolomogorov-Smirnov's test (papers III – V) or the Shapiro-Wilk test (paper I). Log transformation was applied when appropriate, especially in the case of NT-proBNP which was routinely log transformed to normality. Differences between groups were assessed using a paired or unpaired T-test and Levine's test for homogeneity of variances, or alternatively a Mann-Whitney U-test, as appropriate. Correlations were tested by computing Pearson's correlation coefficient  $r$  or Spearman's  $\rho$ . Analysis of variance (ANOVA) was used to analyse data divided into three or more subgroups (e.g. subgroups defined by prior experience in paper III or by ACE I/D allele status in papers IV – V). Coefficients of variation were calculated as the standard deviation of the differences divided by the mean.

#### *Repeated measures analyses*

Changes over time (pre-post measurements performed in papers I, III – V and serial data in paper II) were analysed by calculating a delta score by subtracting the value recorded at baseline from those obtained later. The term *release* refers to this delta score and denotes differences between baseline and post-race (please refer to *Blood tests* above).

Serial data in paper II was analysed using the non-parametric Friedman's ANOVA for repeated measures. The pre-post measurements taken in papers IV and V constitute a special case as the study design included two subgroups (beginners vs. experienced runners). While it would have been desirable to analyse pre-post data as a repeated measures ANOVA and enter the dichotomous variable *experience* as a fixed factor, this was unfortunately not possible as most variables studied were non-normal, leading to a violation of the assumptions for ANOVA. Furthermore, Friedman's non-parametric ANOVA does not allow a fixed factor. Therefore, pre-post data in studies IV and V were analysed as delta scores, dichotomised based on *experience*, and between-subgroup differences were subsequently tested using a Mann-Whitney U-test.

#### *Regression analyses*

In paper III, regression analyses were performed to determine which factors independently predict high post-race levels of TnT or a large release of NT-proBNP. The predictors of NT-proBNP release were analysed using log delta NT-proBNP as dependent variable in a model incorporating those variables that correlated in univariate analysis as independent variables. Linear regression was also used to analyse predictors of post-race dyssynchrony in paper IV. In contrast, the predictors of TnT elevation were analysed using  $TnT \geq 0.03 \mu\text{g/L}$  as dependent variable in logistic regression analysis (please refer to *Blood tests* above). Model fit was tested by applying the Hosmer-Lemeshow test. Residuals were examined for normality, homoscedasticity and to identify outliers. Influence statistics included Cook's distances and leverage values. Collinearity diagnostics included analysis of tolerance and V.I.F. Interactions were evaluated by entering centred interaction variables into hierarchical regression, using an F test to assess model improvement. The dedicated software Italassi v1.1 which is available at [www.provalisresearch.com](http://www.provalisresearch.com) was used to plot interactions. In order to facilitate independent variables to be compared, coefficients were presented standardised as  $\beta$ -weight ( $\beta$ ) in linear regression and as the Wald statistic in logistic regression.

# RESULTS

## Subjects

Basic characteristics of subjects in papers I – III were relatively similar as these race participants were recruited using similar inclusion criteria. The mean age of subjects was as follows. Paper I:  $61 \pm 4$  years (range 55 – 72); paper II:  $62 \pm 5$  years (range 55 – 71); paper III:  $61 \pm 5$  years (range 55 – 79); paper IV:  $46 \pm 7$  years (range 34 – 63); paper V:  $43 \pm 8$  years (range 29 – 63). In papers I and III the study population included both men and women (paper I: Male:Female n (%): 37:6 (86:14); paper III: 132:53 (71:29))

For study I, a group of 22 runners raced in both years and were studied using cardiac biomarkers on both occasions. The analysis was expanded by adding 22 more runners participating in 2006, out of whom 1 runner quit, leaving a total of 43 runners who underwent echocardiography (please see below).

For study II, there were 16 eligible male runners who underwent serial measurements. One subject was excluded from all analyses due to right bundle branch block, leaving  $n=15$  runners in the final dataset. This study population engaged in exercise an average of  $4.6 \pm 1.6$  hours per week, but their median number of previous *Lidingöloppet* participations was only 1 (0 – 21) and almost one-half of this study population had never participated previously.

The pooled dataset in study III comprised 132 eligible subjects studied in 2003, and 80 subjects in 2006. Two runners quit the race in 2003 (leaving  $n=130$ ), and 3 runners quit in 2006 (leaving  $n=77$ ). In the aforementioned 22 runners that raced in both years, only data recorded in 2006 were included in paper 3. Thus, the final dataset comprised 185 subjects out of whom 53 were female (29%). The mean age was 61 years, but females were younger ( $59$  vs.  $62$ ,  $p<0.001$ ), lighter (BMI 23 vs.  $25 \text{ kg/m}^2$ ;  $p<0.001$ ) and slower (race duration 222 vs. 206 min;  $p<0.01$ ).

In studies IV and V, male age-matched subjects were included, stratified primarily based on previous exposure to long-distance running. Sixty-six subjects were studied (31 experienced runners: 11 (9 – 16) previous participations) and 35 beginners: 0 participations) in paper V and a subgroup comprising  $n=43$  subjects in study IV (23 experienced runners (11 (9 – 18) previous participations) and 20 beginners (0 participations)).

## Conventional blood tests and body weight

Baseline levels of all conventional blood tests were within the normal range in all participants. In paper I, the 22 participants who ran in both 2003 and 2006 were found to have higher haemoglobin, haematocrit and creatinine levels at the start of the race in 2006 than in 2003. Results of serial blood tests in paper II are shown in Table 1. Paper IV does not describe results of conventional blood testing in depth as data from the sample studied in 2008 are included in paper V. In paper V, cystatin C and creatinine increased and eGFR decreased in the overall sample ( $p<0.001$ ), especially in beginners. Potassium did not change in the overall sample ( $p=0.17$ ) but tended to be lower post-race in experienced runners (-4% vs. 0% in beginners;  $p=0.07$ ). Body weight ranged from an average of 73 kg in study III to 87 kg in the *beginner* group of study IV.

**Table 1.** Serial biochemical analyses in senior long-distance runners

	Pre-race	Post-race	Day 1	Day 6
Hemoglobin (g/L)	147 ± 9	147 ± 10	138 ± 9**	139 ± 7**
Hematocrit (%)	43 ± 2	43 ± 3	40 ± 3**	44 ± 2
CRP (mg/L)	0.32 (0.20–0.69)	0.37 (0.20–0.74)**	8.70 (5.60–10.40)**	1.30 (0.52–2.20)**
Creatinine (µM)	88 ± 9	142 ± 18**	97 ± 12**	95 ± 13
NT-proBNP (ng/L)	42 (25–117)	187 (113–464) **	165 (78–293) **	78 (37–150)
Troponin T (µg/L)	< 0.01	0.03 (0.015–0.05) **	< 0.01	< 0.01

CRP = C-reactive protein.

Overall analysis of variance  $p < 0.001$  for all. \* $p < 0.05$  and \*\* $p < 0.01$ ; both from baseline.

## Biomarkers

Baseline NT-proBNP was abnormal in a minority of runners (Paper I: 5/43 subjects, 12%; Paper II: 3/15, 20%; Paper III: 15/185, 8.1%; Paper V: 3/66, 5%). Post-race, close to one-half of subjects had NT-proBNP levels above the pre-defined upper limit of the normal range (Paper I: 21/43 subjects, 49%; Paper II: 7/15, 47%; Paper III: 79/185, 43%; Paper V: 17/66, 26%). Post-race increases in NT-proBNP are shown in Table 1. Paper I showed that larger increases in cardiac biomarkers occurred in 2006 than 2003 as shown in Table 2.

TnT was undetectable in all runners before the race in all studies. Post-race, the proportion of runners with elevation above the pre-defined normal range ( $< 0.03$  µg/L) was similar to NT-proBNP (Paper I: 19/43, 44%; Paper II: 9/15, 60%; Paper III: 75/185, 41%; Paper IV: 14/43 (33%; 10/20 (50%) beginners and 4/23 (17%) experienced runners); Paper V: 22/66 (33%; 19/35 (54%) beginners and 3/31 (10%) experienced runners). In paper V, among subjects with TnT elevation, the majority were beginners (19/22 (86%) vs. 3/22 (14%);  $\chi^2 = 14.7$ ,  $p < 0.001$ ). The kinetics of the TnT release are shown in Table 1 as studied over the first 6 days post-race in paper II.

Baseline levels of CRP were similar between papers (Paper I: 0.30 (0.21 – 5.2) mg/L; Paper II: 0.32 (0.20 – 0.69) mg/L; Paper III: 0.41 (0.20 – 0.98) mg/L; Paper IV: 0.43 (0.23 – 1.40) mg/L; Paper V: 0.42 (0.21 – 1.30) mg/L). In contrast with the small differences from baseline to immediately post-race, large increases were seen from baseline to the next day as described in papers II and V (Paper II: please refer to Table 1; Paper V: Pre-race 0.35 (0.22 – 1.30) mg/L, post-race 9.10 (5.60 – 13.60) mg/L,  $p < 0.001$ ).

Reproducibility of biomarker release was shown in paper I. In the case of NT-proBNP, pre-race, delta values and post-race levels of NT-proBNP had a low between-race coefficient of variation (6.9% - 10.9%). As for TnT, there was a strong and highly significant correlation between races ( $\rho = 0.84$ ,  $p < 0.001$ ) but due to the limit of detection of  $> 0.01$  µg/L for troponin T the between-race CV was 34.5% (as 10 runners had to be excluded from analysis due to undetectable levels).

## Echocardiography

All subjects in studies I, II and IV had normal systolic and diastolic function at rest. Conventional echocardiography showed that LV wall thickness was greater in subjects in

**Table 2.** Summary of subjects characteristics and biochemistry at the races in 2003 and 2006.

	2003	2006	<i>p</i>
Race time, min, median (IQR)	194 (177–210)	203.5 (193–225)	<0.01
Previous race participations	14.8 ± 9.7	16.8 ± 9.8	<0.01
Body Weight			
At baseline, kg	77.5 ± 12.7	75.7 ± 13.1	<0.01
Change, kg	-1.4 ± 0.9	-1.4 ± 1.1	0.93
Hematocrit			
At baseline, %	40.5 ± 2.2	43.0 ± 2.7	<0.01
Change, %	-0.6 ± 1.3	-0.2 ± 1.5	0.37
C-reactive protein			
At baseline, mg/L, median (IQR)	0.54 (0.2–1.3)	0.49 (0.2–1.3)	0.28
Change, mg/L, median (IQR)	0.02 (0–0.16)	0.05 (0–0.20)	0.68
NT-proBNP			
At baseline, ng/L, median (IQR)	62 (48–101)	41 (33–95)	0.35
After race, ng/L, median (IQR)	193 (156–305)	230 (136–308)	0.19
Change, ng/L, median (IQR)	134 (102–174)	174 (107–234)	0.05
Troponin T			
At baseline,* µg/L	<0.01	<0.01	n/a
After race, µg/L, median (IQR)	0.01 (0.01–0.04)	0.02 (0.01–0.05)	<0.01

Results are presented as mean ± SD unless otherwise specified. N=22.

\*All runners had undetectable baseline levels of troponin T on both occasions.

papers I – II (Paper I: IVSd  $10 \pm 1$ ; PWDd  $10 \pm 1$ ) than in Paper IV (IVSd  $10 \pm 2$  mm; PWDd  $8 \pm 2$  mm). It should be noted that comparisons are complicated as different methods were used to calculate LVM (Paper I and II: LVMi  $105 \pm 23$  and  $104 \pm 19$  g/m<sup>2</sup>, respectively (ASE convention); Paper IV:  $92 \pm 21$  g/m<sup>2</sup> (Penn convention)). Transmittal LV filling recorded by pulsed-wave Doppler was similar between studies (Paper I: E:  $60 \pm 14$  cm, A:  $58 \pm 15$  cm, E/A:  $1.1 \pm 0.3$ ; Paper II: E:  $64 \pm 17$  cm, A:  $52 \pm 15$  cm, E/A:  $1.3 \pm 0.2$ ; Paper IV: E:  $69$  ( $56 - 77$ ) cm, A:  $42$  ( $34 - 51$ ) cm, E/A:  $1.6$  ( $1.3 - 1.8$ )).

Cardiac functional changes induced by the race were studied in paper II by TDI as well as STI. This was done in a population of n=15 males aged  $62 \pm 5$  years. Sustained reductions in longitudinal function were seen using both techniques, both during systole and diastole, as shown in Table 3. There was also a decrease in LV end-diastolic volume which was sustained during the whole time course of the study. In contrast, echocardiography performed at baseline and on the next day post-race in 43 runners aged  $46 \pm 6.8$  years years in paper 4 showed only a non-significant tendency to reduced  $E_m$  at the lateral wall in beginners ( $p=0.06$ ).

For clinically important findings of abnormal cardiac function on echocardiography please refer till *Clinically important observations* below.

## Analyses of mechanical synchrony

Synchrony analyses were performed in n=43 runners (paper IV). In this group, a standard 12-lead ECG was obtained in 24 subjects (56%) which showed the QRS width to be normal in all subjects and similar between beginners ( $98 \pm 7$  ms) and experienced runners ( $99 \pm 10$  ms;  $p=0.82$ ).

**Table 3.** Serial echocardiographic data in senior long-distance runners.

	Pre-race	Post-race	Day 1	Day 6	ANOVA p
<b>2D</b>					
LVEDV† (mL/m <sup>2</sup> )	63 ± 9	46 ± 10**	58 ± 11	57 ± 10*	< 0.001
SV (mL)	81 ± 16	58 ± 12**	66 ± 16*	68 ± 15**	< 0.001
CO (L/min)	4.7 ± 0.9	4.2 ± 0.9	3.6 ± 1**	3.7 ± 0.8**	0.008
LVEF (%)	66 ± 7	66 ± 10.4	59 ± 7*	62 ± 9	0.02
LA† (cm <sup>2</sup> /m <sup>2</sup> )	10 ± 1.8	8.5 ± 2.4	9.7 ± 2.2	9.3 ± 1.5	Ns
<b>Pulsed-wave Doppler</b>					
E (cm/s)	64 ± 17	54 ± 17**	68 ± 18	64 ± 19	< 0.001
A (cm/s)	52 ± 15	66 ± 13**	50 ± 15	52 ± 18	< 0.001
E / A	1.3 ± 0.2	0.8 ± 0.2**	1.4 ± 0.3*	1.3 ± 0.3	< 0.001
<b>TVI Lateral</b>					
S <sub>m</sub> (cm/s)	6.7 ± 1.7	8.1 ± 1.6*	5.6 ± 1.4	6.4 ± 1.3	< 0.001
E <sub>m</sub> (cm/s)	10 ± 2.4	7.3 ± 2.5**	8.4 ± 1.8**	9.2 ± 2	0.003
A <sub>m</sub> (cm/s)	7 ± 1.7	7.3 ± 2.5	5.1 ± 1.3**	5.5 ± 1.9**	< 0.001
<b>TVI Septum</b>					
S <sub>m</sub> (cm/s)	7 ± 1	7.4 ± 1.2*	6.5 ± 0.7	6.3 ± 0.9**	0.002
E <sub>m</sub> (cm/s)	7.4 ± 1.2	6.2 ± 1.4**	7.3 ± 1.7	7 ± 1.6	0.001
A <sub>m</sub> (cm/s)	8.8 ± 1.5	9.5 ± 1.8*	7.7 ± 1*	7.5 ± 1.5**	< 0.001
<b>TVI RV</b>					
S <sub>m</sub> (cm/s)	12.5 ± 2.5	11.5 ± 2.5	11.5 ± 1.7*	11.4 ± 2.1**	0.021
E <sub>m</sub> (cm/s)	11.9 ± 2.9	8.6 ± 3*	11.6 ± 2.5	10.8 ± 2.5*	0.005
A <sub>m</sub> (cm/s)	15.2 ± 3	13.9 ± 2.4	14.4 ± 2.7	13.1 ± 2.4**	0.009
<b>Speckle Tracking</b>					
Strain (%)	19.1 ± 2.2	19.3 ± 3.9	17.2 ± 1.8*	17.9 ± 1.5**	0.013

ANOVA = Analysis of variance; LVEDV = left-ventricular end-diastolic volume; SV = stroke volume; CO = cardiac output; LVEF = left ventricular ejection fraction; LA = left atrium, end-systolic area; E = early diastolic trans-mitral filling; A = late diastolic trans-mitral filling; TVI = tissue velocity imaging; S<sub>m</sub> = peak systolic myocardial velocity; E<sub>m</sub> = early diastolic lengthening; A<sub>m</sub> = late diastolic lengthening; RV = right ventricular.

\*p < 0.05 and \*\*p < 0.01; both from baseline. †Indexed to body surface area.

The group overall did not exhibit any alterations of S-L delay from baseline to post-race (-9 (-57 – 3) vs. -15 (-68 – 11) ms; p=0.40). Baseline S-L delay differed between beginners and experienced runners in that 40% of beginners (8/20) had a positive S-L delay (peak velocity reached first in septum, followed by lateral wall) in contrast with experienced runners among whom this was seen in only 22% (5/23; p=0.01). The race tended to affect the S-L delay of the two subgroups differently, as beginners showed an increase in S-L delay (128.9 (-50 – 345.5) %) but experienced runners showed a reduction in S-L delay (-31.9 (-126.3 – 5.9) %; p=0.06).

There was no change detected in the group overall  $T_s$ -SD ( $p=0.17$ ) from baseline to post-race. At baseline, beginners and experienced runners had similar  $T_s$ -SD (31.7 (21.6 – 42.5) ms vs. 33.9 (28.9 – 45.2) ms;  $p=0.13$ ). However, post-race, a larger increase in  $T_s$ -SD occurred in beginners (13.0 (2.3 – 55.0) %) than in experienced runners (4.9 (-26.7 – 15.0) %;  $p=0.015$ ). Similar to S-L delay and  $T_s$ -SD, there was no difference in the group as a whole from baseline to post-race in terms of  $T_s$ -Diff ( $p=0.91$ ). Despite similar  $T_s$ -Diff at baseline between subgroups ( $p=0.11$ ), there was a larger increase detected in beginners (18.0 (-2.1 – 44.1) %) than in experienced runners (-1.8 (-28.4 – 13.8) %;  $p=0.014$ ).

## Vectorcardiography

Vectorcardiography was used to analyse ventricular repolarisation in paper II. Baseline VCG data recalculated into ECGs showed ECG features typical of physical training in 4 subjects (27%; left ventricular hypertrophy in 3, wandering atrial pacemaker in 1) but all ECGs were otherwise normal.

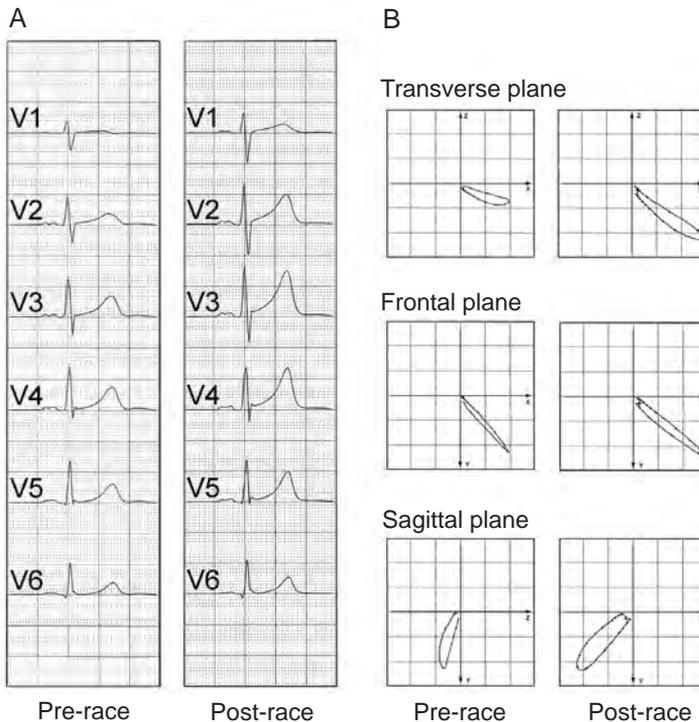
Changes in cardiac repolarisation are shown in Table 4. QTc was  $431 \pm 15$  ms at baseline and 4 runners (27%) had a QTc > 440 ms (maximum 463 ms). Post-race and on Day 1, QTc increased by an average of 4% (range -4% to 12%) and prolongation of QTc to > 440 ms occurred in 10 and 7 runners, respectively.  $T_{\text{peak-end}}$  increased by an average of 22% ( $p < 0.001$ ). Figure 11 illustrates the detected changes in ventricular repolarisation: the T vector shifted anteriorly (transverse plane; increased  $T_{\text{azimuth}}$ ) and cranially (frontal plane; increased  $T_{\text{elevation}}$ ). There was a sustained increase in  $T_{\text{area}}$  which was most evident in the sagittal plane and an increase in  $T_{\text{amplitude}}$  seen in the transverse plane. Other changes in VCG parameters include significant increases in QRS-T angle and ST-VM.  $T_{\text{avplane}}$  remained unchanged and the T loop became more elliptical on Day 1. The race did not lead to an increased QRS interval.

**Table 4.** Serial vectorcardiographic data in senior long-distance runners

	Baseline	Post-race	Day 1	Day 6	ANOVA P
HR ( $\text{min}^{-1}$ )	$59 \pm 6$	$75^{**} \pm 10$	$56^{**} \pm 5$	$55^{**} \pm 8$	< 0.001
QRS (ms)	$98 \pm 10$	$99 \pm 9,7$	$99 \pm 7,9$	$98 \pm 10,6$	Ns
QTc (ms)	$431 \pm 15$	$445^* \pm 22$	$445^{**} \pm 15$	$422 \pm 17$	< 0.001
$T_{\text{peak-to-end}}$ (ms)	$108 \pm 13$	$127^* \pm 43$	$118^* \pm 9$	$105 \pm 8$	< 0.001
ST-VM ( $\mu\text{V}$ )	$101 \pm 39$	$162^{**} \pm 51$	$115^{**} \pm 44$	$112 \pm 44$	< 0.001
QRS-T angle (deg)	$38 \pm 15$	$49^{**} \pm 17$	$35 \pm 13$	$37 \pm 14$	0.001
T azimuth (deg)	$30 \pm 167$	$41^{**} \pm 11$	$26 \pm 15$	$29 \pm 10$	0.001
T elevation (deg)	$60 \pm 15$	$72^{**} \pm 9$	$66 \pm 7$	$64 \pm 8$	< 0.001
$T_{\text{area}}$ ( $\mu\text{Vs}$ )	$75 \pm 26$	$92^* \pm 36$	$105^{**} \pm 42$	$89^* \pm 37$	< 0.001
$T_{\text{avplane}}$ ( $\mu\text{V}$ )	$0.6 \pm 0,3$	$0.7 \pm 0,3$	$0.6 \pm 0,3$	$0.6 \pm 0,3$	Ns
$T_{\text{eigenvalue}}$	$28 \pm 22$	$22 \pm 29$	$53^{**} \pm 45$	$31 \pm 25$	0.003

ANOVA = Analysis of variance; QTc = heart rate-corrected QT interval; ST-VM = ST vector magnitude.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; both from baseline.



**Figure 11.** Cardiac electrical activity before and immediately after a 30-km race. A: Precordial activity based on vectorcardiographic data. B: T loops projected onto the three orthogonal planes. Reprinted with permission from paper II of this thesis.

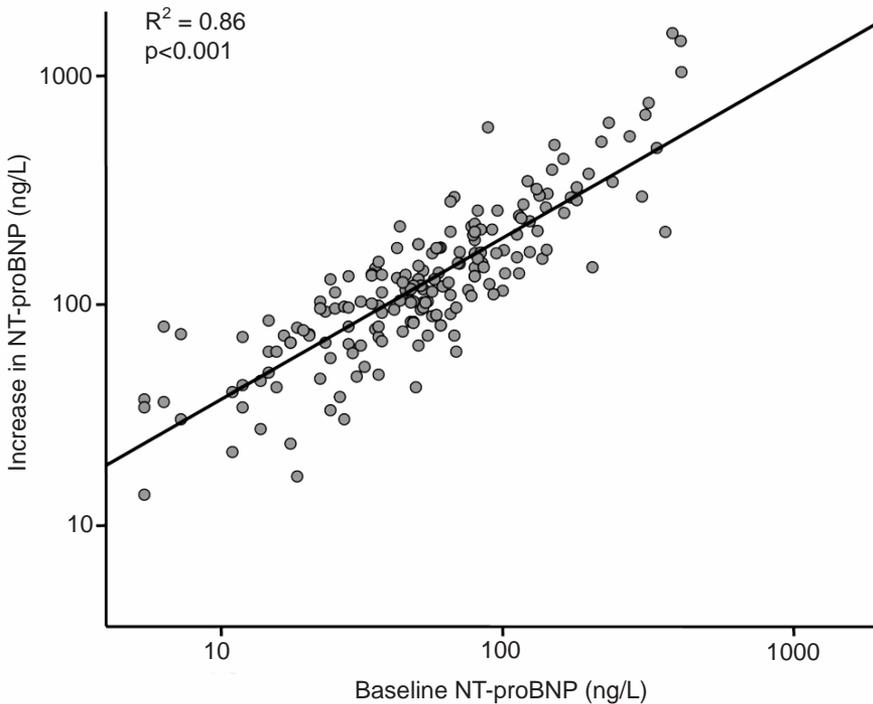
## Angiotensin-Converting Enzyme I/D gene analyses

Genetic analysis of the ACE I/D polymorphism was performed in study IV and V. The genotype frequencies adhered to Hardy-Weinberg equilibrium ( $\chi^2=2.35$ ;  $p=0.13$ ). Study V showed that there were 21 homozygotes for the I allele (II; 32%), 28 homozygotes for the D allele (DD; 43%) and 16 heterozygotes (ID; 25%). There were no differences in allele or genotype frequencies between beginners and experienced runners.

## Correlations

### *NT-proBNP*

Greater NT-proBNP release (delta) and higher post-race levels were seen in subjects with higher baseline NT-proBNP (paper I: baseline vs. delta:  $r=0.87$  and post-race:  $r=0.93$ ; paper II:  $\rho=0.91$ ; paper III: baseline vs. delta:  $R^2 = 0.86$ ;  $p<0.001$  for all) (Figure 12) and paper V ( $r=0.93$ ;  $p<0.001$ ). In paper I, NT-proBNP levels at baseline and post-race were associated with an increased left ventricular mass index ( $r=0.30$ ,  $p=0.050$  and  $r=0.32$ ,  $p=0.034$  respectively). Subjects with abnormal post-race NT-proBNP were also found to be older (62.0 vs. 59.8 years;  $p=0.041$ ), and had larger left atria (10.9 vs. 9.6  $\text{cm}^2/\text{m}^2$ ;  $p=0.024$ ). A more complete analysis of the predictors of a large NT-proBNP release was performed in the pooled dataset



**Figure 12.** Association between baseline levels of NT-proBNP and the increase detected after a 30-km cross-country race in 185 senior, healthy participants. Reprinted with permission from paper III of this thesis.

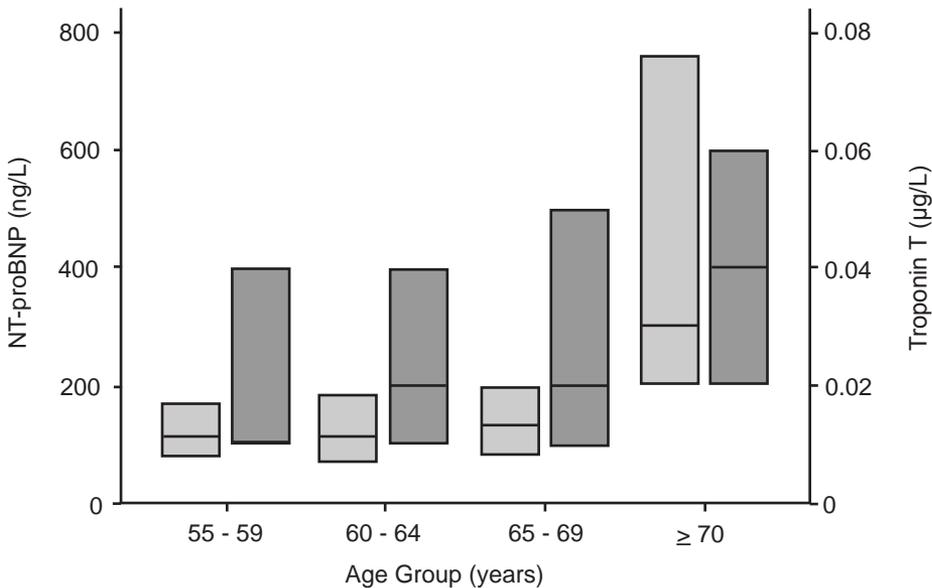
in paper III ( $n=185$ ) as shown in Table 5. The first predictor of a large NT-proBNP was: (1) a high baseline NT-proBNP ( $\beta$  0.81,  $p<0.001$ ), followed by (2) a large increase in creatinine ( $\beta$  0.19,  $p<0.001$ ). (3) Race duration ( $\beta$  0.08,  $p<0.05$ ) and (4) age ( $\beta$  0.11,  $p<0.01$ ; Figure 13) were also independent predictors for a large NT-proBNP release. Study V, which was conducted in younger participants, confirmed the association between NT-proBNP and age ( $\rho$  0.25,  $p=0.046$ ).

Runners with higher baseline NT-proBNP showed a larger increase in  $T_{\text{area}}$  after the race in paper II ( $\rho$  0.73,  $p=0.003$ ) as well as a greater reduction in tissue velocities ( $S_{\text{m}}$  lateral:  $\rho$  -0.56,  $p=0.03$ ;  $S_{\text{m}}$  septum:  $\rho$  -0.65,  $p<0.01$ ;  $E_{\text{m}}$  lateral:  $\rho$  -0.54,  $p=0.04$ ;  $E_{\text{m}}$  septum:  $\rho$  -0.68,  $p<0.01$ ).

While levels of NT-proBNP did not differ between ACE genotype groups at baseline ( $p=0.29$ ) we found that the release of NT-proBNP in the DD group (73.5 (47 – 147.5) ng/L) was larger than both the II group (46 (23 – 81) ng/L;  $p=0.02$ ) and the ID group (44 (35.5 – 80.5) ng/L;  $p=0.02$ ; overall analysis of variance  $p=0.038$ ).

### *TnT*

In paper I, TnT release was found to correlate to left atrial size, similar to NT-proBNP ( $\rho$  0.42,  $p=0.005$ ). Study III explored the independent predictors of its release as shown in Table 6: (1) the most important predictor was higher age, (Figure 13) followed by (2) large increase



**Figure 13.** Increase in biomarkers in different age groups. NT-proBNP increase is shown in light gray and post-race troponin T in dark gray. Thick horizontal bars represent median values and boxes interquartile range. Reprinted with permission from paper III of this thesis.

in creatinine. (3) Further, the number of previous participations in *Lidingöloppet* correlated inversely ( $p=0.007$ ) such that the risk of TnT elevation decreased for each quartile (OR 0.70).

In paper IV, we found that runners with  $TnT \geq 0.03 \mu\text{g/L}$  were predominantly beginners (10/14, 71% vs. 10/29, 35% among subjects with  $TnT < 0.03 \mu\text{g/L}$ ;  $p=0.02$ ), had higher BMI ( $26.3 \pm 3.1$  vs.  $24.0 \pm 2.1 \text{ kg/m}^2$ ;  $p=0.02$ ), lower LVMi ( $81 \pm 19$  vs.  $97 \pm 19 \text{ g/m}^2$ ;  $p=0.03$ ), smaller LVEDVi ( $61 \pm 12$  vs.  $68 \pm 9 \text{ mL/m}^2$ ;  $p=0.06$ ), higher LVEF ( $61 \pm 5$  vs.  $57 \pm 5\%$ ;  $p=0.04$ ) and a less negative S-L delay ( $-2$  ( $-9$  to  $34$ ) vs.  $-34$  ( $-71$  to  $-2$ ) mg;  $p=0.003$ ). In the group as a whole, subjects with elevated TnT tended to have higher diastolic blood pressure ( $p=0.08$ ), a correlation that was significant in beginners ( $\rho=0.473$ ;  $p=0.04$ ).

All three parameters used to describe LV synchrony correlated to higher TnT levels post-race: (1) more positive S-L delay at baseline ( $\rho=0.40$ ;  $p<0.01$ ) and greater augmentation of S-L delay post-race ( $\rho=0.33$ ;  $p=0.04$ ); (2) a larger increase in  $T_s\text{-SD}$  ( $\rho=0.40$ ;  $p<0.01$ ); (3) a larger increase in the maximum differences between any 2 segments ( $T_s\text{-Diff}$ ;  $\rho=0.29$ ;  $p=0.06$ ).

#### *Experience and race duration*

In paper III, troponin T release was larger in less experienced runners as demonstrated in Table 6. In paper V, *beginners* were slower ( $192 \pm 42$  vs.  $167 \pm 25 \text{ min}$ ;  $p=0.01$ ) and less lean ( $25 \pm 3$  vs.  $24 \pm 2 \text{ kg}$ ;  $p=0.01$ ) than *experienced* runners, their NT-proBNP levels increased more (55 (40 – 99) vs. 40 (29 – 81) ng/L;  $p=0.042$ ) as did their TnT (0.03 (0.01 – 0.05) vs. 0.01 (0.01 – 0.02)  $\mu\text{g/L}$ ;  $p=0.002$ ) and CRP (11 (8 – 19) vs. 7 (4 – 13) mg/L;  $p=0.027$ ).

**Table 6.** Predictors of elevated post-race troponin T levels

Variable	Univariable analysis		Multivariable analysis		
	B	OR (CI <sub>95</sub> )	B	Wald	OR (CI <sub>95</sub> )
Constant			-1.23	3.62	0.29
Age group	0.32	1.37 (1.00 – 1.89) *	0.45	6.57	1.57 (1.11 – 2.21) **
Creatinine increase	0.17	1.19 (1.03 – 1.36) *	0.18	6.05	1.20 (1.04 – 1.38) *
Previous participations	-0.35	0.71 (0.53 – 0.93) *	-0.35	5.65	0.70 (0.53 – 0.94) *

OR, odds ratio; CI<sub>95</sub>, 95% confidence interval.

Intervals for independent variables: Creatinine: increments of 10%; Age group: increments of 5 years; Previous participations: category increments based on quartiles (0 – 2; 3 – 7; 8 – 13; >13 races).

\*p<0.05; \*\*p=0.01.

### *Echocardiographic variables*

Paper II describes that a larger increase in  $T_{\text{area}}$  correlated with a greater decrease in tissue velocities ( $S_{\text{m}}$  lateral: rho -0.68, p=0.01;  $S_{\text{m}}$  septum: rho -0.57, p=0.04;  $E_{\text{m}}$  lateral: rho -0.46, p=0.10;  $E_{\text{m}}$  septum: rho -0.75, p<0.01) and in global strain (rho -0.66, p=0.01). In paper IV, conventional echocardiography showed that experienced compared with beginners had a larger LVEDVi at baseline while LVMi and LVEF were similar. Tissue velocities were similar between groups with the exception of  $E_{\text{m}}$  at the lateral wall which tended to be reduced post-race in beginners (p=0.06).

As regards TDI-based analysis of synchrony, a multivariable model in paper IV showed that the independent predictors of a higher post-race  $T_{\text{s}}\text{-SD}$  were: (1) lack of experience (p=0.005; being experienced was associated with a 12-ms lower  $T_{\text{s}}\text{-SD}$  than being a beginner); (2) lower  $S_{\text{m}}$  in the basal septum (p=0.011; each decrement of 1 cm/s was associated with an increment in  $T_{\text{s}}\text{-SD}$  of 5 ms); (3) more copies of the D-allele (p=0.048, each additional copy was associated with a 5.5 ms increase in  $T_{\text{s}}\text{-SD}$  (Figure 14)).

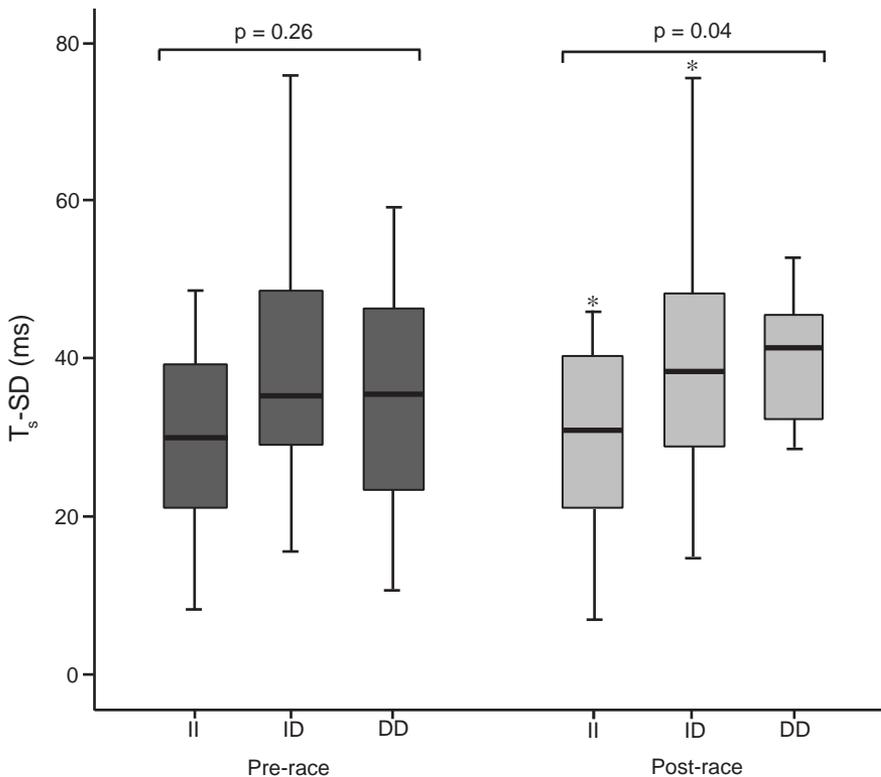
Higher  $S_{\text{m}}$  both at the septum and lateral wall correlated to a shorter  $T_{\text{s}}$  at the corresponding segment (septal  $S_{\text{m}}$  vs.  $T_{\text{s}}$ : rho=-0.63; p<0.001; lateral  $S_{\text{m}}$  vs.  $T_{\text{s}}$ : rho=-0.37; p=0.016). The change detected in  $S_{\text{m}}$  (post-race – pre-race value) correlated to the change in  $T_{\text{s}}$  (septal  $S_{\text{m}}$  vs.  $T_{\text{s}}$ : rho=-0.55; p<0.001; lateral  $S_{\text{m}}$  vs.  $T_{\text{s}}$ : rho=-.26; p=0.09).

### *Clinically important observations*

Although runners with a known history of cardiovascular disease and treatment for medical disorders were excluded using a screening questionnaire, a minority of participants did harbour important cardiovascular disease.

Paper III describes findings made in 15 runners (13 male, 2 female; aged  $65 \pm 6$  years) with abnormal baseline NT-proBNP (8.1% of whole study population) which led us to refer these subjects for routine cardiac work-up. There was evidence of a cardiac abnormality in all but 4 subjects, ranging from moderate tricuspid insufficiency to severe heart disease. Investigations showed especially severe cardiac disease in the following subjects: (1) one male subject (NT-proBNP 2250 ng/L, CRP 2.6 mg/L) had severe biventricular cardiac impairment and asymptomatic atrial fibrillation with left bundle branch block. (2) A second male runner

(NT-proBNP 339 ng/L, CRP 2.4 mg/L) gave a history suggesting accelerating angina pectoris and had severe coronary artery disease on cardiac workup, requiring coronary artery bypass surgery. (3) One male athlete (NT-proBNP 219 ng/L, CRP 0.2 mg/L) who underwent non-invasive workup was found to have an ascending aortic aneurysm (47 mm). This was likely to be secondary to severe hypertensive cardiovascular disease as evidenced by severe hypertension (175/110 mm Hg) and left ventricular hypertrophy associated with abnormal relaxation. These three runners were advised to stop running until their cardiac disease had been brought under control. (4) One male subject (NT-proBNP 363 ng/L, CRP 2.0) died suddenly whilst training, a few months after the race. The post-mortem examination showed severe left ventricular hypertrophy and myocardial fibrosis. There was widespread evidence of severe coronary artery disease and an old myocardial scar (6 x 4 cm) suggesting a past history of asymptomatic myocardial infarction.



**Figure 14.** Greater intra-left ventricular dyssynchrony post-race in long-distance runners carrying the angiotensin-converting enzyme D allele.

\*p<0.05 vs. DD.

# GENERAL DISCUSSION

This thesis examined the impact of prolonged exercise on different aspects of cardiac function. Serum levels of cardiac biomarkers were found to increase significantly and reproducibly. Higher concentrations of NT-proBNP and TnT were seen especially in subjects of higher age. Exertional increase in NT-proBNP correlated strongly with levels at baseline and higher TnT was seen in those unaccustomed to endurance sports. Attenuation of systolic and diastolic function was evident on post-exercise echocardiography, and occurred in parallel with altered ventricular repolarisation especially in runners with high baseline levels of NT-proBNP. Analysis of the ACE I/D polymorphism showed that carriers of the D allele developed higher levels of NT-proBNP and had greater intra-left ventricular mechanical dyssynchrony post-exercise. Finally, in a minority of senior long-distance runners with elevated NT-proBNP at rest, serious cardiac dysfunction was found on clinical work-up.

## Biomarkers

### *Troponin T*

In this thesis, all five papers describe that endurance exercise leads to a significant elevation of TnT immediately post-race. In senior runners studied in paper III, post-race levels exceeded the pre-determined normal range in 41%, while 33% of younger runners studied in paper V had elevated levels. A meta-analysis of 26 studies (n=1120 cases) published by Shave and co-workers showed that 47% (confidence interval 39 – 56) had increased levels of troponin T after endurance exercise with considerable heterogeneity between studies.<sup>107</sup>

In clinical practice, exercise-induced increases in cardiac troponin may become clinically important in the setting of e.g. a runner who seeks medical attention after an endurance event for conditions unrelated to ischaemic heart disease and is, incidentally, found to have raised troponin levels. Such troponin elevation may complicate management and can lead to unnecessary hospital admission and inappropriate invasive diagnostic procedures. Given the increasing popularity of long-distance races, it is desirable that members of the medical profession are aware of these phenomena.<sup>108</sup>

In a multivariable model, we found that TnT release was independently predicted by both higher age and lack of previous endurance experience. The paper that showed the largest release was number II in which the study cohort had undetectable pre-race levels but post-race TnT of 0.03 (0.015 – 0.05) µg/L. It is noteworthy that the participants in study II were aged an average of 62 years and almost one-half of the study population had never participated in the *Lidingöloppet* previously. These observations led us to design study V in which we compared novices to experienced runners and controlled for age in a matched design. The impact of inexperience was confirmed as the subgroup of beginners at *Lidingöloppet* released TnT to significantly higher levels than did age-matched experienced runners.

The underlying cause for the release of troponin has been subject to considerable debate. While the first troponin assays to become available were hampered by cross-reactivity with skeletal muscle troponin, the Roche Diagnostics 3<sup>rd</sup>-generation assay uses antibodies

directed to an epitope present only on the cardiac TnT isoform.<sup>67</sup> In clinical practice, cross-reactivity does therefore not occur. In endurance running, however, massive skeletal muscle breakdown occurs and serum levels of many muscle proteins are often dramatically elevated. It is conceivable that a very low degree of cross-reactivity, which may be unimportant in clinical cardiology, may become important in this setting. This question was specifically addressed by Roth and co-workers who studied participants in one of the toughest endurance races in the world: the Death Valley race in California, U.S.A. Despite large post-race elevations of muscle cell proteins there were no increases in cardiac troponin T.<sup>109</sup> The key point made in their article is that cross-reactivity is unlikely to occur with the 3<sup>rd</sup> generation troponin assay as this would have led to false-positives which they did not see. Data from an animal model also support the notion that the troponin detected after prolonged exertion is indeed of cardiac origin as seen following stressful exercise in rats after 5 hours<sup>110</sup> but not after less prolonged exercise (1 – 3 hours).<sup>111</sup>

The troponin release kinetics were studied in paper II of this thesis by performing serial analyses for TnT during the days after an endurance event. An early peak in levels immediately post-race was followed by a similarly rapid drop into the undetectable range within 24 hours of finishing the race which is similar to the kinetics described by other authors performing serial troponin testing.<sup>112</sup> While destruction of contractile elements typically leads to a sustained release of cardiac troponin, the findings in paper II suggest that TnT is released from the cytosolic pool of non-complex bound troponin, perhaps due to transient impairment of cardiomyocyte cell membrane integrity.<sup>113,114</sup> Although it has previously been speculated that this may be a consequence of exercise-induced generation of reactive oxygen species, there is no convincing evidence that this mechanism plays a role in humans.<sup>115</sup>

### *NT-proBNP*

Levels of NT-proBNP were examined before and after prolonged exercise in papers I – III and V and were found to increase significantly and reproducibly. As shown in the multivariable analysis in paper III, the most important predictor of a large NT-proBNP release was the level of NT-proBNP present at baseline. Although this observation had allegedly been made by other researchers<sup>116</sup> it had never previously been reported in athletes. In studies of heart failure patients, a brief bout of exercise has previously been shown to induce release of natriuretics peptides which is both larger than in healthy controls,<sup>117</sup> and also associated with baseline levels.<sup>118</sup> In long-distance runners, it would therefore appear that the factors that determine resting NT-proBNP also determine the occurrence and magnitude of NT-proBNP release after prolonged exercise.

NT-proBNP is released by the cardiac wall in response to stretching of cardiomyocytes as shown in cardiac muscle preparations *in vitro*.<sup>119</sup> Elevation of filling pressures leads to natriuretic peptide release *in vivo* which corresponds to augmented LV wall stress<sup>120,121</sup> and LV stiffness.<sup>122</sup> In the populations studied in this thesis, it is conceivable that minor cardiac structural alterations were present at baseline which predisposed to small variations in resting diastolic function and cardiac filling. In the setting of prolonged exercise such subtle differences could conceivably cause large alterations of cardiac function. This notion is partly supported by the findings presented in paper II: higher baseline levels of NT-proBNP predisposed participants to greater attenuation of longitudinal velocities ( $S_m$  as well as  $E_m$ ). We found higher levels of baseline NT-proBNP in runners recruited in 2003 and 2006 as compared with 2008, which is likely to reflect the higher age of those subjects.<sup>123</sup> Indeed,

a multivariable model in athletes aged an average of 61 years, as presented in paper III, confirms that higher age is an independent predictor for NT-proBNP in this setting. The association between NT-proBNP and age was re-confirmed in paper V despite the lower mean age of 43 years in this paper. Levels seen in our senior subjects were similar to a group of marathon runners aged an average of 48 years whose NT-proBNP increased from 48 to 183 ng/L<sup>124</sup> and higher than in runners aged an average of 40 years whose NT-proBNP increased from 44 to 137 ng/L.<sup>125</sup> NT-proBNP levels have previously been shown to be positively correlated to age both in healthy volunteers<sup>126</sup> and in endurance athletes.<sup>127</sup> It has been described that NT-proBNP rises in parallel with ‘cardiac ageing’ as manifested by attenuated longitudinal function in higher age and an associated increased tendency to diastolic impairment.<sup>51,128</sup>

In the senior athletes studied in paper I, we found associations between NT-proBNP and both LVMi as well as LA size. This is in keeping with an association between NT-proBNP and filling pressures.<sup>129,130</sup> Whether the high mean age in study I may have played a role is uncertain but it is conceivable that age acted as a confounder in this setting (a larger LA and LVMi being present in senior runners whose NT-proBNP was also higher). If so, it should be noted that high NT-proBNP levels in an aged person are no less important than in a young subject. In fact, age-related NT-proBNP increase should probably best be viewed as a reflection of augmentation of cardiac chamber stiffness leading to clinically important elevation of filling pressures.<sup>128,131</sup>

There is one line of evidence which argues that filling pressures, and not age itself, may explain the link between LA size and NT-proBNP in these subjects. In an unpublished study from our echocardiographic laboratory we examined 20 senior runners with high NT-proBNP levels in a matched design against 20 with low NT-proBNP (aged 65 vs. 65 years; NT-proBNP 209 (147 – 329) ng/L vs. 46 (23 – 80) ng/L). We found that runners with high NT-proBNP had larger left atria ( $13 \pm 2$  vs.  $11 \pm 2$  cm<sup>2</sup>/m<sup>2</sup>,  $p < 0.05$ ) as well as higher diastolic ( $79 \pm 11$  vs.  $72 \pm 9$  mm Hg,  $p < 0.05$ ) but not systolic blood pressure ( $p = ns$ ). This firstly shows that, after correcting for age, an association remains between NT-proBNP and left atrial size. Furthermore, the differences in blood pressure between the two groups suggest that minor functional and/or structural differences in LV wall composition may predispose some athletes to a slightly stiffer and less compliant LV. This was specifically investigated using a non-invasive method to interrogate the LV wall for presence of interstitial matrix: integrated backscatter analysis. Higher backscatter (i.e. more interstitial matrix) tended to be present in basal segments of the high NT-proBNP group, reaching statistical significance at the lateral wall ( $100 \pm 26$  vs.  $83 \pm 20$  dB,  $p < 0.05$ ) but not the septum ( $127 \pm 29$  vs.  $114 \pm 27$  dB,  $p = ns$ ). (Data on file)

## Cardiac fatigue

In this thesis, we examined the impact of endurance running on cardiac mechanical function using echocardiography before and after prolonged exercise in papers II and IV. These two studies differed considerably both in study design and with regards to outcome. In paper II, the protocol included echocardiograms immediately post-race as well as on the next day and on day 6. There was evidence of attenuated cardiac longitudinal velocities both during systole ( $S_m$ ) and in early ( $E_m$ ) and late ( $A_m$ ) diastole. In contrast, paper IV describes only a non-significant tendency to decreased tissue velocities in early diastole at the lateral wall of inexperienced runners, one day post-race. The key difference between these two studies is likely to be the 16-year difference in the mean age of participants (study II: 62 years; study IV: 46 years). The published literature in this field has not previously examined the

impact of age on exercise-induced cardiac fatigue. Some of the key mechanisms thought to be important for this phenomenon (as reviewed below) are likely to be influenced by age.

#### *Importance of cardiac loading*

Several important perturbations occur in the cardiovascular system after prolonged exercise, including changes in heart rate (typically elevated immediately post-race), cardiac pre-load (commonly reduced post-race due to depletion of intra-vascular volume leading to reduced cardiac venous return), after-load (reduced due to a fall in systolic blood pressure and total peripheral vascular resistance, as blood redistributes into skin and exercising muscle).<sup>132</sup> One commonly used proxy for cardiac pre-load is LVEDV which in study II was reduced on day 6 post-race. This suggests that a sustained reduction in cardiac pre-load may have occurred which needs to be borne in mind when interpreting these data. In fact, there are surprisingly few published experimental reports that have specifically examined the importance of pre-load for cardiac fatigue in the setting of prolonged exercise. Hassan and co-workers studied subjects post-endurance racing and compared conventional echocardiograms performed supine with the Trendelburg position (legs raised). Based on conventional echocardiographic measurements, the authors found attenuation of diastolic, but not systolic, function.<sup>133</sup> These findings were confirmed in a recent study by Hart and co-workers who repeated the same study design but used more sensitive tissue Doppler imaging.<sup>134</sup> Two other studies showing that systolic dysfunction did not arise when cardiac pre-load was maintained by oral and intravenous fluid replacement<sup>135,136</sup> have been criticised for having an insufficiently strenuous study protocol.<sup>137</sup>

In this thesis, blood pressures were taken at baseline but we did not have the benefit of serial recordings in either study II or IV. Blood pressure can otherwise be used in two important ways: firstly, systolic blood pressure may act as a proxy for cardiac after-load. Secondly, blood pressure can be used to calculate cardiac wall stress.<sup>138</sup> It is likely that future advances in cardiac imaging will enable non-invasive assessment of cardiac function which is less dependent, or ideally completely independent, of cardiac loading. This is a crucial requirement which must be met in order to accurately quantify changes in contractility and diastology.<sup>14</sup>

#### *Importance of cardiac autonomic tone*

In paper II, there was a sustained depression of cardiac systolic function which was not accompanied by compensatory augmentation of heart rate. This is interesting as it somewhat contradicts the view advanced by some researchers that cardiovascular drift occurs as a direct consequence of a fatiguing process of the heart, and not solely due to reduction or redistribution of circulating volume.<sup>139</sup> It is conceivable that heightened vagal tone was present post-race which may explain not only the subtly depressed heart rates present post-race, but also the altered cardiac repolarisation seen. Indeed, the augmented sympathetic tone present during physical exercise is withdrawn within minutes of cessation and is accompanied by vagal activation.<sup>140</sup> Prolonged exercise has been suggested to lead to more sustained alterations of cardiovascular autonomic tone including vagal excess for at least 24 hours.<sup>141</sup> Altered sympatho-vagal balance may therefore play a role at least for the interpretation of ventricular repolarisation changes in paper II. Interestingly, higher NT-proBNP was associated with greater alterations of ventricular repolarisation. It may be speculated that direct sympatho-inhibitory effects by natriuretic peptides may contribute to greater vagal predominance post-race in runners with higher NT-proBNP.<sup>142</sup> On the contrary, the greater attenuation of cardiac longitudinal velocities seen in runners with higher NT-proBNP is difficult to explain, unless heightened vagal tone had negative effects on cardiac inotropy and contractility too.

This question was addressed by Hart and co-workers who gave runners the vagolytic drug glycopyrrolate post-exercise. The intervention had no impact on post-exertional cardiac fatigue which suggests that this phenomenon is not caused by vagal excess post-race.<sup>143</sup>

#### *Other mechanisms*

In paper IV, we found increased intra-left ventricular mechanical dyssynchrony in race beginners, reflecting heterogeneous prolongation of times-to-peak across the segments of the left ventricle. A higher peak longitudinal velocity of a segment was associated with a shorter time-to-peak, and accordingly a larger reduction of velocity was associated with greater prolongation of time-to-peak. It may therefore be speculated that the 12-segment standard deviation method for measuring ventricular synchrony ( $T_s$ -SD) acted as an index of excitation-contraction coupling in our study population of healthy male long-distance runners: its impairment or at least its inter-segmental heterogeneity. It is noteworthy that calcium handling, which is a key regulator of excitation-contraction coupling, has been demonstrated to be involved in cardiac fatigue in several articles in human and animal models.<sup>144,145</sup>

Interestingly, in paper IV we were also able to describe that an independent predictor of higher post-race  $T_s$ -SD was having more copies of the ACE D allele. The ACE D allele is known to predispose carriers to unfavourable remodelling in circumstances where activation of the renin-angiotensin system occurs, leading to muscle cell hypertrophy and fibrosis of bodily tissues in general and the heart in particular. Myocardial fibrosis contributes to greater heterogeneity of cardiac function and may therefore augment dyssynchrony. Alternatively, it is known that angiotensin II (which is produced in larger amounts by carriers of the D allele) acts to increase sympathetic transmission at the neuromuscular junction. This has been shown to lead to impaired diastolic calcium clearance, an important feature in clinical heart failure.<sup>146</sup> If this is indeed something that occurs in athlete's cardiac fatigue, it would indicate that this condition has phenomenological similarities to clinical heart failure, albeit on a very mild scale.

The greater functional impairment seen in senior runners with higher NT-proBNP in paper II is interesting as this suggests fatigue develops especially in those with elevated cardiac wall stress. It is conceivable that subtle changes related to age or sub-clinical hypertension may contribute to intermediately elevated NT-proBNP and wall stress in this sample population. As wall stress is an important determinant of myocardial oxygen requirements, it is conceivable that a degree of LV hypertrophy may predispose runners to mismatch between myocardial oxygen supply and demand. Interestingly, this notion is supported by some data from a canine model where LV hypertrophy has been associated with myocardial stunning post-exercise.<sup>147</sup> Other mechanisms that have received attention include desensitisation of cardiac  $\beta_1$ -adrenoceptors which occurs in clinical heart failure<sup>148</sup> and appears to also play a role for athletes' cardiac fatigue in a human model.<sup>143</sup> Prolonged fatty acid metabolism has been implicated,<sup>149</sup> as has generation of reactive oxygen species. However, as mentioned above there is little evidence that such mechanisms actually play a role in humans.<sup>115</sup>

It is apparent from papers III – V in this thesis that the impact of prolonged exercise is greatest in relatively less experienced runners. Physical training has multiple effects on muscle structure and function, including increased muscle capillarisation as well as enhanced function of the sarcoplasmic reticulum and improved calcium handling.<sup>150,151</sup> It is also conceivable that inexperienced participants are more liable to develop an intracellular 'energy crisis' after prolonged exertion. In skeletal muscle, energy shortage may reflect either an insufficient ability of the cell to manufacture the energy required to run the race.

Alternatively, the available energy stores may be inadequate.<sup>152</sup> As regards fatigue of cardiac muscle, the mitochondria of the heart are chronically maintained in a maximally upregulated state. Therefore, cardiomyocyte oxidative capacity (which is three-fold higher than in skeletal muscle slow-twitch fibres under untrained conditions) and mitochondrial volume, density or function, are therefore not changed by physical training.<sup>153</sup> There are some experimental data that shows that glycogen availability influences skeletal muscle fatigue. Future research will need to address the possibility that an 'energy crisis' occurs in endurance runners which predisposes inexperienced participants in particular to greater alterations of cardiac function.<sup>154</sup>

## **Impact of Angiotensin-Converting Enzyme I/D genotype on cardiac biomarkers**

Strenuous physical exercise leads to marked activation of the renin-angiotensin-aldosterone system. As the magnitude of activation is dependent on ACE I/D genotype, sports physiologists have paid considerable attention to this polymorphism. Montgomery and co-workers used echocardiography to examine 140 military recruits who underwent a physically demanding training programme during 10 weeks. All subjects had genetic testing for ACE I/D genotype and a subgroup were tested for natriuretic peptide levels (n=49). It was found that the training programme induced cardiac hypertrophy which was more pronounced in carriers of the D allele, especially homozygotes. In the subgroup whose BNP was analysed, higher baseline levels were seen post-training in carriers of the D allele. Importantly, all subjects underwent the same training programme and D allele carriers were therefore no more likely than others to have recently engaged in a bout of training prior to testing. This key study showed for the first time that physical exercise leads to myocardial hypertrophy associated with elevation of resting BNP in certain individuals but not in everybody.

It has previously been suggested that athletes' levels of NT-proBNP and cardiac troponin have some degree of overlap. This is likely to partially reflect the fact that certain underlying predictors of release are shared between the two biomarkers, such as inexperience (shown in paper V), higher age and larger left atrium (shown in papers I and III). Based on the aforementioned reports that inexperienced runners may predominantly be carriers of the D allele,<sup>77</sup> we investigated whether the ACE I/D genotype may also constitute such a shared predictor: conceivably, the greater release of troponin in the inexperienced runners may reflect an over-representation of D allele-carriers in this group. Study V examined this question, but while experienced runners and beginners differed markedly in terms of post-race biomarkers, there was no evidence of a skewed allele distribution in the two subgroups. However, study V had limited power to address this question in view of its small sample size.

Study V did not show an association between post-race troponin and ACE allele distribution. However, we did find that post-race levels of NT-proBNP were highest in homozygote carriers of the D allele. This extends the findings of Montgomery and co-workers who measured NT-proBNP at rest and found higher levels in D allele carriers.<sup>155</sup> It is likely that this partly reflects an underlying association between pre-race and post-race levels of NT-proBNP as described in paper I. The fact that there was a difference post-race but not at baseline argues that groups may have had a differential response to exercise depending on I/D allele status. Several studies have assessed the importance of ACE I/D status in the setting of a brief bout of exercise. Hagberg and co-workers found that postmenopausal women with the D allele have a lower peak  $\text{Vo}_2$  than I carriers, secondary to either differences in maximal

arteriovenous O<sub>2</sub> difference<sup>156</sup> or in peak heart rate and cardiac output.<sup>157</sup> Another study by the same group failed to replicate these findings in young women.<sup>158</sup> The importance of ACE I/D status for the cardiovascular response to prolonged exercise was studied by Ashley and co-workers. Carriers of the I allele exhibited greater depression in fractional shortening after a 90 – 120 hour ultra-endurance race. The authors speculate this may relate to increased sympathetic activity (assessed using blood pressure and heart rate variability) in D allele carriers, which may conceivably have acted to limit post-race decline in cardiac function in these runners.<sup>159</sup>

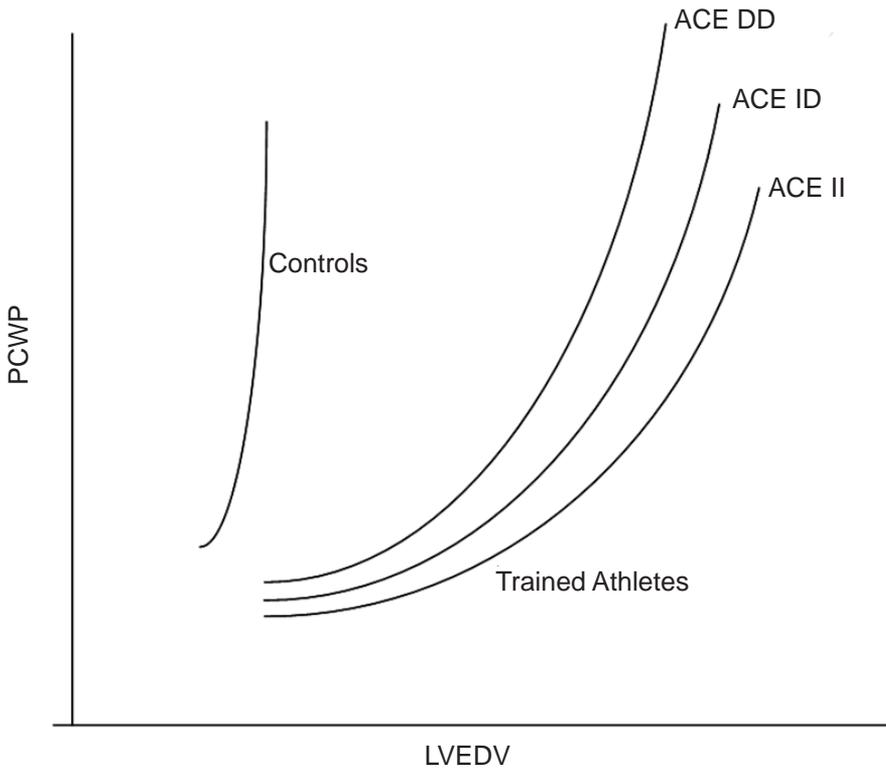
Collins and co-workers found an over-representation of I allele carriers among the fastest finishers of an Ironman race.<sup>160</sup> The possibility that the I allele predisposes carriers to superior endurance performance has been discussed extensively in the scientific community. It has been suggested that cardiac work is performed less energy-efficiently by carriers of the D allele which may be a consequence of either elevated Ang II levels or depressed bradykinin levels.<sup>161,162</sup> Another possibility is that higher levels of Ang II and associated deposition of extra-cellular matrix, as discussed above, may conceivably render the cardiac wall of certain athletes slightly less compliant. Previous studies using serum markers for cardiac fibrosis as well as post-mortem findings in long-distance runners have shown that myocardial fibrosis appears to occur in certain senior athletes.<sup>163,164</sup> Whether this has any clinical relevance has not been established.<sup>165</sup> Based on the aforementioned findings from our laboratory that higher NT-proBNP is associated with more backscatter in senior athletes, it may be hypothesised that the ACE D allele predisposes its carriers to such altered wall composition, leading to slight changes in LV stiffness and cardiac chamber compliance which may serve to explain the association with NT-proBNP. A theoretical model for the association between ACE I/D status and NT-proBNP is presented in Figure 15, based partly on experimental data by Levine et al.<sup>166</sup> Of course, this hypothetical explanation requires experimental validation in an invasive study design.

## Clinical implications

There is overwhelming evidence that moderate, regular exercise has beneficial effects for many different aspects of human physiology.<sup>167</sup> However, the effects of exercise on health are more complex and contradictory than they might seem at first: while exercise causes an overall reduction in an individual's cardiovascular risk, there is a short-term increase in risk during and immediately after exercise which is more pronounced in untrained individuals.<sup>168-170</sup> This phenomenon, commonly referred to as the *exercise paradox*, has received considerable attention and has been compared to a two-edged sword.<sup>171</sup>

Endurance exercise, as studied in this thesis, constitutes an extreme form of exertion characterised by prolonged cardiovascular stress. It is likely that the vast majority of participants have a benign cardiovascular risk profile overall, as demonstrated by epidemiological data showing that ex-athletes have a lower incidence of many cardiovascular and other disorders in later life.<sup>8</sup> Nevertheless, some authors have suggested a non-linear dose-response association between amount of exercise and cardiovascular events.<sup>165</sup> Some limited data suggest an L-shaped association (suggesting a threshold effect),<sup>172</sup> or even a U-shaped association<sup>173-175</sup> between levels of physical activity and adverse clinical outcomes.

An L-shaped association between exercise volume and outcomes would suggest that a change from sedentary lifestyle to mild exercise, or from mild to moderate exercise, may yield considerably greater health benefits than a shift from moderate to hard or extreme



**Figure 15.** Putative association between ACE I/D polymorphism and LV compliance of trained athletes which may explain differences in NT-proBNP between allele groups. Adapted from reference 166.

exercise as in many of the subjects studied in this thesis. However, if the association is in fact U-shaped, one would need to consider the possibility that endurance exercise has the potential to inflict damage upon the coronary arteries or myocardium. This has been proposed based on findings made in the on-going “Marathon Study” in Essen, Germany<sup>176,177</sup> but remains, as yet, largely conjectural. In response to these reports, some authors have proposed that long-distance running may uncover or accelerate incipient coronary artery disease. If this should be true, it would lead to a fundamental change in our view of exercise and its cardiovascular effects. Future research in this field will need to approach and explore these complex issues very carefully.<sup>178</sup>

Irrespective of whether repeated bouts of long-distance running has ‘pathogenic’ potential over time, published data indicate that approximately 1 in 50,000 marathon runners develop an acute life-threatening arrhythmia in the setting of a marathon race.<sup>179,180</sup> The leading cause is underlying coronary artery disease which is present in the majority of deceased athletes.<sup>181,182</sup> There may be several explanations why physical exercise acutely increases cardiovascular risk: pre-existing<sup>183</sup> or *de novo* arising erosions or ulcerations of atherosclerotic plaques may predispose to thrombotic coronary occlusion owing in part to platelet activation.<sup>184</sup>

Senior athletes are known to have limited augmentation of LVEF during exercise and therefore rely predominantly on LV enlargement to increase cardiac output.<sup>185</sup> It has been suggested that this plays a role as underlying coronary atherosclerotic changes will be subjected to considerable mechanical stretch when the exercising heart dilates. Plaque rupture is more likely to occur in this setting, a sequence of events sometimes referred to as *Black's crack in the plaque* after an early report by Black and co-workers.<sup>186</sup> It is also conceivable that pain thresholds may be altered due to increased endorphin levels, leading to ischaemic symptoms to remain unnoticed by the runner.<sup>187</sup> Elevated catecholamine levels may increase myocardial excitability and associated electrolyte disturbances may further facilitate arrhythmogenicity.<sup>188</sup>

In this thesis, the repolarisation changes seen in paper II are especially interesting as it shows for the first time that endurance exercise leads to electrophysiological alterations that last for several days post-race. Previous studies in this field have attributed cardiac events to exercise if occurring during or within 30 – 60 minutes after exercise cessation.<sup>168,170</sup> Based on this paper, it is possible that an arrhythmogenic tendency is present beyond the first hours post-race. Importantly, there was a positive correlation seen between baseline levels of NT-proBNP and the degree of repolarisation change. This should be interpreted in the light of the very powerful ability of NT-proBNP to prognosticate which has been shown in several large epidemiological studies. In fact, NT-proBNP predicts cardiovascular events in the general population at levels that are well below commonly used diagnostic cut-offs.<sup>62</sup>

In paper III we found that 8% of senior *Lidingöloppet* participants had elevated levels of NT-proBNP above the upper limit of normal (194 ng/L). Among these 15 runners, 4 had severe cardiovascular disorders including 1 male runner who died of sudden heart death within months after the race. Based on this relatively small observational study, it would appear that self-reportedly healthy, senior athletes with elevated NT-proBNP have a 'risk' of harbouring a severe cardiovascular disorder (i.e. a proportion of disorders) of 4/15 (27%).

An important question is whether blood tests for baseline NT-proBNP would allow those senior athletes to be identified in whom exercise causes the greatest perturbations to cardiac function. This may be attractive especially given the current interest in screening athletes to avoid exercise-induced cardiac events.<sup>189-192</sup> The idea of using NT-proBNP to identify athletes at elevated risk is further supported by the findings made in the aforementioned, unpublished study of 20 senior runners with high NT-proBNP who were compared to 20 with low levels. Those with high NT-proBNP were characterised by a significantly longer QTc interval ( $445 \pm 36$  vs.  $425 \pm 22$  ms,  $p < 0.05$ ) which suggests these individuals had a higher risk of ventricular arrhythmias.<sup>89</sup>

There is one published study where NT-proBNP was tested for the purpose of pre-participation screening. Daniels and co-workers examined 457 college athletes in the U.S.A. with a median age of 19 and a median NT-proBNP of 21 ng/L. Although no cases had major structural abnormalities in their study population<sup>193</sup> it is possible that a similar study design in senior participants would identify a minority of runners with asymptomatic left ventricular dysfunction. The positive predictive value of a raised NT-proBNP will need to be examined in a carefully designed prospective screening approach and compared with existing pre-participation screening protocols.<sup>189</sup>

# CONCLUSIONS

The following conclusions are based on examinations performed in participants exposed to prolonged exercise at the 30-km cross-country race *Lidingöloppet*.

- Endurance exercise leads to increased levels of cardiac biomarkers NT-proBNP and TnT.
- The magnitude of biomarker increase is reproducible in the individual athlete and larger in inexperienced runners.
- As for NT-proBNP, the magnitude of the increase is mainly determined by the pre-race levels.
- Release of TnT and NT-proBNP is independently predicted by a large increase in creatinine, suggesting an association with greater exertion.
- A small proportion of senior long-distance runners have serious, undiagnosed cardiovascular disease. Senior runners whose NT-proBNP is elevated may be at higher risk of harbouring such conditions.
- Prolonged exercise in senior athletes induces alterations in ventricular repolarisation which are sustained up to 6 days. These changes occur in parallel with attenuation of ventricular systolic and diastolic longitudinal function.
- Altered repolarisation and attenuation of longitudinal function are both greater in runners with higher levels of NT-proBNP at baseline.
- Inexperienced subjects whose troponin T is elevated after prolonged exercise exhibit greater intra-left ventricular mechanical dyssynchrony after prolonged exercise.
- Greater intra-left ventricular mechanical dyssynchrony is seen after prolonged exercise in carriers of the ACE D allele.
- Runners carrying the ACE D allele exhibit significantly larger increases in NT-proBNP.

# ACKNOWLEDGEMENTS

Associate Professor Frieder Braunschweig. For all the support and encouragement he has given me during the last few years. Thanks to his readiness and availability whatever the time of day, this project was able to progress smoothly. His dedication, hard work and patience will always be admired.

Associate Professor Reidar Winter. His knowledge of echocardiography and cardiac physiology is enormous and his enthusiasm is inspiring.

Professor Cecilia Linde. For providing valuable input during the course of this thesis. As Head of the Cardiology Department she has always ensured excellent working conditions and research facilities.

Professor Lennart Bergfeldt whose knowledge of cardiac electrophysiology is profound, and whose hard work with my vectorcardiography manuscript paid off in the end.

Aigars Rubulis, for performing the original MIDA 1000 recordings and also for helping me retrieve data later from the not-so-user-friendly computer and its Dos-based user interface.

Kambiz Shahgaldi, for performing high-quality echos at high speed.

Per Jacobsen, for taking the trouble to read what seemed like a thousand echos with me.

Jan E Svensson and Thomas P Gustafsson, for taking part in the research network around the Lidingöloppet project before my time.

Eva Wallgren and her crew in Gröna Barracken. Helena Karlsson, David Ersgård and Magnus Mossfeldt all played an important role in this project, especially David for doing the layout of the final thesis.

Marcus Ståhlberg and Philip Aagaard whose help during this project enabled me to gather important data while drinking Singapore Slings at the Raffles Long Bar.

Britta Lind and Aristomenis Manouras, for helping to do echocardiograms.

Anna Aminoff, Ewa Ehrenborg and Per Eriksson, for performing genetic analyses.

Thomas Fux, for helping out in the changing room-turned-echo laboratory at Lidingöwallen.

Per Tornvall, for important input and advice.

Kickan Österblom and the others at the Lidingöloppet race secretariat, for having a positive and helpful attitude and sending me innumerable lists of Lidingöloppet race entrants.

John Öhrvik and Elisabeth Berg, for giving valuable statistical advice.

Per Hellström, for providing guidance and constructive advice.

Tony Marklund and the others at Roche Diagnostics in Bromma, for generously donating the assays used to measure biomarkers in these papers.

John Pernow, for always maintaining an academic focus in the department.

Karin Schenck-Gustafsson, for her positive and enthusiastic attitude in general, and in particular for taking time to sit down with me on her first day back from annual leave to sign my application for defending the thesis.

Peter Matha, Lena Sandlund and the many other members of staff involved with handling our specimens in the Department of Clinical Chemistry.

My mother Kerstin, for baby-sitting on an almost daily basis while I was busy writing. My father Hans, for his support and encouragement.

Vinu, who has put up with my nonsense for many years now... Filip, who is new to it.

And last but not least, to all the participants at the Lidingöloppet who willingly took part in these studies and thereby made this thesis possible in the first place.

This work has been supported by grants from the Swedish Heart-Lung Foundation, the Swedish Centre for Research in Sport and the Folksam Foundation.

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