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Doctoral Thesis

Aspects of Regular Long-Term Endurance Exercise in Adolescents, with Focus on Cardiac Size and Function, Hormones, and the Immune System

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“May the space between where I am and
where I want to be inspire me”

Tracee Ellis Ross

Abstract

The long-term effects of starting high-intensity training at younger ages are largely unknown. The present studies focused on adolescents who had performed regular endurance exercise for several years at an elite level, and compared those subjects with a control group of adolescents of similar age and sex who had not engaged in regular exercise. The knowledges generated by this research will contribute to further understanding of some of the physiological effects of strenuous regular exercise during adolescence.

Aim: The overall aim of this research was to investigate endurance-trained adolescents, focusing on cardiac size and function, hormones associated with growth and metabolism, and impact on the immune system.

Methods: All participants underwent echocardiography at rest as well as immediately and 15 minutes after a maximal cardiopulmonary exercise test. Blood samples were taken at rest and analyzed for biomarkers such as hormones, immune cell surface markers, and secreted cytokines and chemokines. The study design was cross-sectional (Papers I, III, and IV) and comparative, with a quantitative approach in all four studies. The evaluation in Paper II used a pre-posttest design with measurements of cardiac parameters before and after a maximal treadmill test. The studies in Papers I–III compared endurance-trained (active group) and untrained (controls) adolescents matched by age and sex, whereas the analysis in Paper IV considered differences between the sexes in the endurance-trained adolescents.

Results: Compared with controls, the endurance-trained adolescents showed increased size of all four heart chambers, as well as improved cardiac systolic function at rest. Considering cardiac changes from baseline to immediately after exercise, the systolic and diastolic patterns were similar in both groups, although the diastolic function was more enhanced in the active group. Strong associations between peak oxygen uptake and cardiac size and function could be seen both

at rest and after exercise. Circulating hormones at rest did not differ between the two groups. No correlation between insulin-like growth factor 1 and cardiac size was found among the endurance-trained adolescents. Compared with endurance-trained girls, endurance-trained boys exhibited an elevated immune response to a potent type 1 diabetes-related autoantigen. Conversely, compared with the trained boys, the trained girls showed an increased number of circulating immune cells and increased secretion of C-peptide and proinsulin.

Conclusions: There are many benefits associated with regular exercise, and the present research did not provide any data to oppose that statement. Factors such as increased cardiac size at rest and exercise-related effects on cardiac functions do occur and therefore should be expected in endurance-trained adolescents with high peak oxygen uptake. Indeed, this should be interpreted as a sign of physiological adaptation and not as pathophysiology. The greater cardiac dimensions observed in these subjects could not be related to increased resting levels of hormones associated with growth and metabolism. The endurance-trained adolescents did show some sex-related differences with regard to their immune response at rest.

Keywords: adolescents, endurance exercise, cardiac size, cardiac systolic function, cardiac diastolic function, growth hormone, immune response, echocardiography, biomarkers, cytokines, chemokines, insulin-like growth factor 1, type 1 diabetes-related autoantigen, proinsulin

Contents

List of Papers

Paper I

L. Rundqvist, J. Engvall, M. Faresjö, E. Carlsson, P Blomstrand. (2017).

Regular endurance training in adolescents impacts atrial and ventricular size and function. *European Heart Journal – Cardiovascular Imaging:* 18(6):681–687. doi:10.1093/ehjci/jew150.

Paper II

L. Rundqvist, J. Engvall, M. Faresjö, P. Blomstrand. (2018).

Left ventricular diastolic function is enhanced after peak exercise in endurance-trained adolescents as well as in their non-trained controls. *Clinical Physiology and Functional Imaging.* doi: 10.1111/cpf.12534

Paper III

L. Rundqvist, J. Engvall, P. Blomstrand, E. Carlsson, M. Faresjö.

Resting level of insulin-like growth factor 1 is not at play in cardiac enlargement in endurance-trained adolescents. *Submitted.*

Paper IV

E. Carlsson, L. Rundqvist, P. Blomstrand, M. Faresjö.

Enhanced immune response to a potent type 1 diabetes-related autoantigen is observed in endurance-trained boys. *Submitted.*

Abbreviations

2D	two-dimensional
A	late mitral or tricuspid inflow filling velocity
a'	late diastolic peak myocardial velocity
BMI	body mass index
BMR	basal metabolic rate
BSA	body surface area
CCL2	chemokine (C-C Motif) ligand 2
CD	cluster of differentiation
CPET	cardiopulmonary exercise test
E	early mitral or tricuspid inflow filling velocity
e'	early diastolic peak myocardial velocity
E/A	ratio of early to late diastolic mitral flow velocity
E/e'	ratio of early diastolic transmitral inflow filling velocity to peak myocardial velocity
ECG	electrocardiogram
FAC	fractional area change
FACS	fluorescence-activated cell sorters
FSH	follicle-stimulating hormone
GABA	gamma amino butyric acid
GAD	glutamic acid decarboxylase
GH	growth hormone
HR	heart rate
IA-2	tyrosine phosphatase
IFN-ɣ	interferon gamma
IGF	insulin-like growth factor
IL	interleukin
IVS	interventricular septum
LA	left atrium
LH	luteinizing hormone
LV	left ventricle
LVEDV	left ventricular end-diastolic volume
LVEF	left ventricular ejection fraction

LVGLS	left ventricular global longitudinal strain
LVID	left ventricular internal diameter
LVM	left ventricular mass
LVPWT	left ventricular posterior wall thickness
MAPSE	mitral annular plane systolic excursion
MET	metabolic equivalent of task
M-mode	motion mode echocardiography
NK	natural killer
PBMC	peripheral blood mononuclear cell
RA	right atrium
RER	respiratory exchange ratio
RLU	relative light unit
ROI	region of interest
RV	right ventricle
RVD1	right ventricular basal diameter
RVFAC	right ventricular fractional area change
RVOTprox	proximal right ventricular outflow tract
s'	systolic peak myocardial velocity
SBP	systolic blood pressure
SSC	side-scatter channel
T1D	type 1 diabetes
T2D	type 2 diabetes
TAPSE	tricuspid annular plane systolic excursion
TNF-α	tumor necrosis factor alpha
TSH	thyroid-stimulating hormone
TT	tissue tracking
VCO_2	carbon dioxide elimination
VO_2	oxygen uptake
VO_{2max}	maximal oxygen uptake

Introduction

Regular exercise is necessary for good health and physical fitness. In general, a physically active lifestyle has a positive impact on the cardiovascular system, body composition, and bone health, and also leads to reduced stress and improved psychological well-being, along with many other valuable effects. To achieve these essential health benefits in children and adolescents, the physical activity should be at least 60 minutes per day and of moderate to high intensity (Poitras et al., 2016). In a global perspective, 80% of adolescents do not comply with the public health guidelines recommended for physical activity, but, on the other hand, the long-term effects of being highly physically active during adolescence have not been studied to the same extent. A dose-response relationship has been demonstrated in school-aged children and adolescents, indicating that the more intensive the physical activity, the greater the physical, social, and mental health benefits (Janssen & Leblanc, 2010). Nonetheless, the existence of a dose-response benefit of regular strenuous endurance exercise is more uncertain (Sanchis-Gomar, Perez, Joyner, Lollgen, & Lucia, 2016).

During exercise, the body undergoes multiple physiological adjustments, including constantly regulation of cardiovascular and respiratory functions to meet the demands. In adult elite athletes, we know that adaptations of the cardiovascular system to regular endurance exercise include increased cardiac volume and wall thickness, as well as impacts on heart functions. A comprehensive approach for imaging the athlete's heart aims to differentiate the physiological changes induced by intensive training from the similar morphological features caused by serious cardiac diseases (Pelliccia, Maron, & Maron, 2012). In addition, as the body transitions from a resting to an active state, the rate of metabolism increases to provide necessary energy. The physiological response to increased metabolism is primarily the responsibility of the endocrine system, which is constantly monitoring the body's internal environment. This system recognizes all changes and can rapidly release hormones to ensure

homeostasis and aid internal processes that support physical activity (Kenney, Wilmore, & Costill, 2015).

Due to the increased popularity of youth sports and emphasis on the benefits of physical fitness in children and adolescents, it is essential to understand the impact of exercise on physiological aspects of growth and development. Youngsters must not be regarded as mere miniature versions of adults, as was assumed in the past. Indeed, the effect of regular exercise on the heart, hormones, and inflammatory mediators is particularly important during childhood and adolescence, considering the puberty-related growth spurt that occurs during this period in life (Kenney et al., 2015; Riddell, 2008). Any exercise-associated cardiac load, as well as hormonal and/or inflammatory effects, may have profound consequences for growth and development, especially if maintained for long periods (Eliakim & Nemet, 2010). Yet few studies have explored the impact of long-term endurance exercise on adolescents, and most investigations in this area have been performed on adults. Therefore, the studies underlying the present thesis focused on adolescents aged 13–19 years with a history of several years of intense endurance exercise, in particular considering the heart, the hormones, and the immune system.

Background

Exercise and health in young people

According to the World Health Organization (WHO, 1995), "*health* is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". It is well-known and widely accepted that exercise is associated with improved physical and mental well-being among children and adolescents. In addition to an average of 60 minutes of moderate to vigorous physical activity per day, the majority of the physical exercise in this age group should consist primarily of aerobic activities, because activities that stress the cardiovascular and respiratory systems have the greatest health benefits (Janssen & Leblanc, 2010). Healthcare professionals and rehabilitation professionals should support these exercise recommendations for children and adolescents as a consistent part of their message to young patients and their families, care providers, and school personnel. Furthermore, in addition to improving physical health and quality of life, it is plausible that physical activity can lead to better grades in school and that it possesses real merit for raising academic prowess and enhancing well-being in terms of happiness and the ability to keep moving towards a goal (Archer & Garcia, 2014).

Approaches to prevent obesity and disorders such as cardiovascular diseases and type 2 diabetes (T2D) are often too narrow in scope and are initiated too late. However, a majority of adolescents are however free of both cardiovascular disorders and T2D, whereas far fewer are free of the risk factors for these conditions, especially lifestyle factors like poor exercise and dietary habits (Chung, Touloumtzis, & Gooding, 2015). Throughout the world, public health guidelines for physical activity address the exercise needs of children and adolescents. The necessity of physical activity at home and in the community is abundantly evident. Still, with increased pressure on school systems to decrease their costs and increase their academic

performance, physical education – and thus physical activity – has been deemed expendable. Fortunately, it appears that organized sports contribute to the proportion of children and adolescents who meet recommendations for physical activity and increase the time that individuals in this age group spend on moderate and vigorous exercise. Notwithstanding, as a barrier to exercise, it has been pointed out that the high levels of competitiveness and intensity of organized sports may deter youngsters from participating. Furthermore, being involved in organized sports is not a guarantee for reducing the odds of being classified as overweight or obese, or for lowering the time spent sedentary (Landry & Driscoll, 2012; Marques, Ekelund, & Sardinha, 2016). Understanding the causes of physical activity behavior is essential for development and improvement of public health interventions, because an effective program in this context will target factors known to cause inactivity. Research on both children and adults has shown that physical activity is associated with factors such as age (inversely), male sex, education level, overweight (inversely), motivation, stress (inversely), and social support. Obviously, the greatest challenge in this field will be to translate research results into public health actions (Bauman et al., 2012).

Definitions of exercise and peak VO_2

The terms *exercise* and *training* are traditionally defined as physical activity that is planned, structured, and repetitive in a manner that leads to improvement or maintenance of fitness (E. T. Howley, 2001). Thus p*hysical activity* is not synonymous with exercise but is instead a global term that is defined as bodily movement produced by skeletal muscle contractions that results in increased energy consumption (Caspersen, Powell, & Christenson, 1985).

Maximal oxygen uptake (VO_{2max}) stands for the highest achievable oxygen consumption rate during exercise and is considered to be the best single measure of aerobic fitness in youngsters. In children and adolescents of both sexes, VO_{2max} is higher in those who practice endurance exercise compared with untrained peers, although $VO_{2max.}$

is also affected by age and sex. VO_{2max} is approximately 10% higher in boys than in girls during childhood, and it is about 35% greater in males at the age of 16 years (Armstrong, Tomkinson, & Ekelund, 2011; Rowell, 1974). It has long been know that VO_{2max} is strongly correlated with body mass, and hence it is usually controlled for with respect to whole body mass, to mass$^{0.67}$ (as the mass specific VO_{2max} is higher in small than in large subjects), or in some cases to the subject's height (Sjodin & Svedenhag, 1992; Whipp, 2010). In healthy subjects, it is assumed that VO_{2max} is reflected by a limit being reached in the cardiac output. A combination of ventricular volume, ventricular mass, and heart rate reserve (calculated as maximal heart rate – resting heart rate) explains much of the variance in VO_{2max} (La Gerche, Burns, Taylor, et al., 2012). VO_{2max} occurs when the uptake of oxygen does not continue to rise despite increases in work rate noted as a plateau in a plot during an exercise test. If there is no demonstrable evidence that the plateau criterion has been met, the maximum value attained represents the subject's *peak* VO_2 (Edward T Howley, Bassett, & Welch, 1995; Whipp, 2010).

Moderate exercise intensity can be defined as 50% of a given subject's VO_{2max}, which means 50% of the maximum ability to take in, transport, and use oxygen (Albouaini, Egred, Alahmar, & Wright, 2007; Yanagisawa et al., 2010). However, the intensity of exercise is rarely defined in studies of training and physical activity in either adults or adolescents. Metabolic equivalent of task (MET) can also be used to describe the differences between various intensities. MET expresses energy expenditure in multiples of resting energy cost: 1 MET is defined as the basal (or resting-) metabolic rate (BMR) during 1 minute of quiet seated rest, which is equivalent to an oxygen uptake of 3.5 mL per kg of body weight, and a value of $\geq$ 12 METs indicates heavy vigorous exercise (Henry, 2005; Jette, Sidney, & Blumchen, 1990; Schnohr, O'Keefe, Marott, Lange, & Jensen, 2015; Vanhees et al., 2012). An important limitation of MET is that it does not apply well to all individuals or to all population subgroups, because it varies due to differences in aspects such as body mass, adiposity, age, and

sex. Indiscriminate use of conventional MET values is likely to bias the true relative energy cost of exercise (Ainsworth et al., 2000; Melzer et al., 2016).

The heart and its response to exercise

Since the late 19th century, it has been known that highly trained athletes have enlarged hearts and a lower resting heart rate (HR) compared with non-athletes. The understanding of this syndrome has gradually expanded due to the introduction of echocardiography for more than 40 years ago, and subsequently also electrocardiography (ECG) and cardiac magnetic resonance, techniques that together have enabled quantitative assessment of cardiac remodeling associated with regular exercise (B. J. Maron & Pelliccia, 2006). The term *athletic heart* is used to describe the complex development of morphological, functional, and electrical remodeling of the heart that is associated with regular athletic training. These cardiac alterations are all of significance, because they represent physiological adaptations that will substantially help athletes perform better in physically demanding situations (Barry J Maron, 1986; Prior & La Gerche, 2012). Furthermore, during an intense exercise session, several interrelated cardiovascular changes occur that are intended to enhance delivery of oxygen to meet the requirements of the exercising muscles. These alterations include higher systemic blood pressure (SBP) and increased cardiac output in response to higher HR and stroke volume, which have been widely studied in both young and adult athletes, as well as sedentary equals (Henriksen, Sundstedt, & Hedberg, 2008; La Gerche, Burns, Mooney, et al., 2012; Liang et al., 2017; Neilan et al., 2006; Rodeheffer et al., 1984; Santoro et al., 2015; Sanz-de la Garza et al., 2017). As emphasized above, children and adolescents are not small adults, and thus exercise-trained young people may be physiologically distinct from athletic adults and must therefore be considered in a different manner (Kenney et al., 2015). However, there is insufficient knowledge about how the growing heart in athletic adolescents normally responds to regular intense exercise.

Cardiac structure

The heart is a muscular organ that is divided by a septum into a right and a left half, each of which consists of an atrium and a ventricle (Figure 1). The atrioventricular plane lies between the atria and ventricles and is attached to a fibrous skeleton called the anulus fibrosus cordis. The walls of the heart are composed of the myocardium, which is substantially thicker in the left ventricle (LV) than in the right ventricle (RV). The inner surface area of the cardiac chambers is lined with endothelial cells. The heart contains four valves, which are called the mitral, tricuspid, pulmonary, and aortic valves (Widmaier, Raff, & Strang, 2014). The chordae tendineae connect the mitral and tricuspid valves to the papillary muscles, which project from the walls of the RV and LV into those cavities. This connection prevents movement of the valve leaflets into the atria during ventricular contraction. The variability in number and arrangement of papillary muscles of the right side of the heart distinguish the tricuspid valve from the mitral valve, which is supported by a more regular arrangement of two groups of papillary muscles (Ho & Nihoyannopoulos, 2006; Madu & D'Cruz, 1997).

Contraction of the myocardium is induced by an electrical impulse that is generated by the sinus node, which consists of special pacemaker cells located in the myocardium of the right atrium (RA). This impulse spreads throughout the atria and to the atrioventricular node, which in turn conducts the impulse from the atria to the ventricles. This causes a heartbeat that initially comprises an atrial contraction and thereafter a ventricular contraction (Widmaier et al., 2014). Maximal HR is higher in children than in adults but decreases linearly with age: it is approximately 210 beats/minute at the age of around 10 years but about 195 beats/minute at age 20 years. It has been suggested that the decrease in maximal HR throughout life is due to a decrease in sensitivity of the cardiac β-adrenergic receptors (Christou & Seals, 2008; Kenney et al., 2015; Rodeheffer et al., 1984).

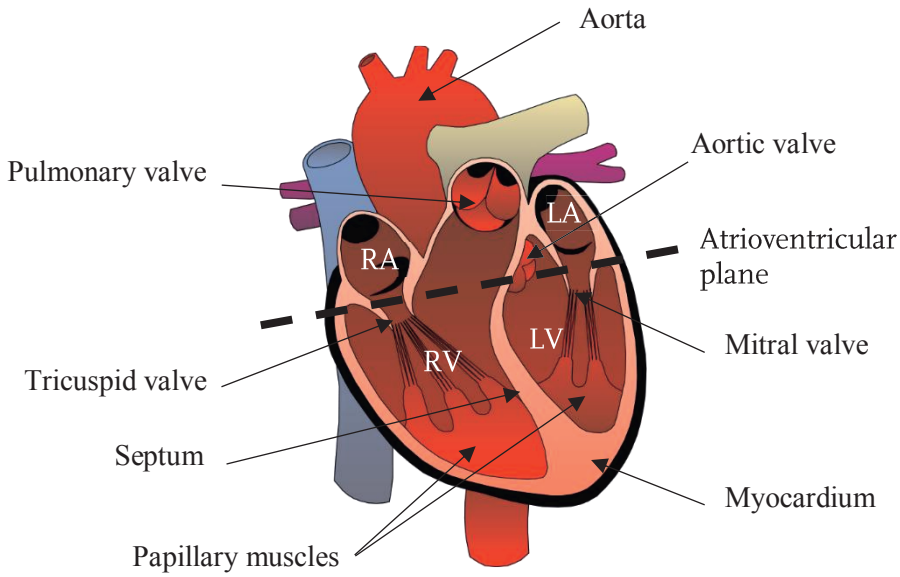

Figure 1. Anatomy of the heart. RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle (part of the illustration is from http://www.freestockphotos.biz/stockphoto/14157).

It is well-known that long-term hemodynamic changes caused by regular exercise lead to an increase in both left ventricular internal diameter (LVID) and LV wall thickness to normalize LV wall stress (Galderisi et al., 2015; Barry J Maron, 1986). This results in an increase in calculated left ventricular mass (LVM), which allows a greater force of contraction, as has been shown in both adolescent and adult athletes compared with non-athletes (Hedman et al., 2015; Obert, Stecken, Courteix, Lecoq, & Guenon, 1998; Sharma et al., 2002; Utomi et al., 2013). The upper limit of normal LVM indexed by body surface area (BSA) in adults is 95 g/m^2 in women and 115 g/m^2 in men, whereas the pediatric limit measured by magnetic resonance is slightly lower than the adult limit (Lang et al., 2015; Lorenz, 2000). Accordingly, the increased left ventricular posterior wall thickness (LVPWT) seen in athletes rarely exceeds the upper normal limit of 13 mm in adults (Barry J Maron, 1986; Pelliccia et al., 2012) or 12 mm in

youths (Sharma et al., 2002). Besides sporting discipline and exercise habits, the remodeling of heart size is affected by several additional factors, such as age and sex, and even more so by BSA, which has been reported to be the strongest determinant (Pelliccia et al., 2012). The different cardiac dimensions are illustrated in Figure 5 (in section headed *Echocardiographic analysis*).

Volume and wall thickness in the RV are affected by endurance exercise in a similar way as in the LV. Greater right ventricular end-diastolic basal diameter (RVD1) (Figure 6, in section headed *Echocardiographic analysis*) and RV area, as well as increased diameter of the proximal right ventricular outflow tract (RVOTprox) and inflow tract are observed in highly trained adult athletes compared with untrained controls (D'Andrea, La Gerche, Golia, Teske, et al., 2015; D'Andrea et al., 2013). The enlargement of the RV is associated with an enhancement of early diastolic ventricular function and with the presence of normal systolic parameters. In addition, the LV stroke volume and the pulmonary artery systolic pressure have been shown to be strong predictors of the dimensions of both the RV and the RA (D'Andrea et al., 2003; D'Andrea, La Gerche, Golia, Teske, et al., 2015).

Considering the atria, biatrial enlargement is observed in highly trained adult athletes. (D'Andrea et al., 2010; D'Andrea et al., 2013; D'Ascenzi et al., 2014; Hedman et al., 2015). Increased dimensions and volumes are in general proportional to the enlargement of the ventricles and are also affected by the type of training that is undertaken (Pelliccia et al., 2012; Prior & La Gerche, 2012). It has been suggested that the dynamic component of training is the primary driver of both LA and RA adaptation in adult athletes (McClean et al., 2015). A recent study has also shown biatrial enlargement in preadolescent athletes compared to sedentary controls (D'Ascenzi et al., 2016).

Increased cardiac dimensions in both adults and youngsters are positively related to VO_{2max} in athletes as well as in individuals who

do not exercise regularly. It has been suggested that left ventricular end-diastolic volume (LVEDV) can explain 50% of the variability in VO_{2max} in adult athletes (La Gerche, Burns, Taylor, et al., 2012). However, it has also been noted that LVM, RV dimensions, and atrial size can predict VO_{2max}, which establishes that it is cardiac structural remodeling rather than functional remodeling that enables greater oxygen consumption during exercise (Hedman et al., 2015).

Cardiac function

Systolic function

Systole is the period of ventricular contraction during witch blood is ejected into the aorta and pulmonary trunk. A ventricular contraction has three components that are defined as longitudinal, radial, and circumferential based on the composite myocardial fiber orientation (Figure 2). The longitudinal contraction represents motion from base to apex via atrioventricular plane displacement; the radial contraction entails a radial shortening from outer to inner position; and the circumferential contraction consists of a rotational movement. Together, these segmental movements result in a complex pattern of ventricular twisting motion, which leads to decreased longitudinal and radial length. Longitudinal atrioventricular plane displacement is the primary contributor to LV pumping and accounts for approximately 60% of the stroke volume (Blessberger & Binder, 2010; M. Carlsson, Ugander, Mosen, Buhre, & Arheden, 2007; Ingels Jr, 1997).

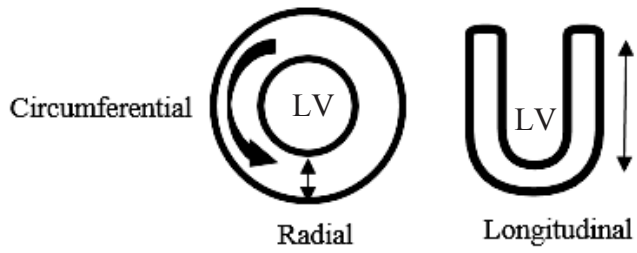

Figure 2. Components of the ventricular contraction in three vectors. LV, left ventricle.

Left ventricular ejection fraction (LVEF) has become one of the parameters most commonly used to assess LV systolic function. LVEF reflects the percentage of blood that is pumped out of a filled ventricle during systole, and an LVEF of 53–73% is classified as normal. LVEF is not significantly related to sex, age, or body size, and it is calculated using LVEDV and left ventricular end systolic volume (LVESV) as follows (Lang et al., 2005):

LVEF = (LVEDV–LVESV)/LVEDV

Athletes generally have LVEF values close to those noted in the general populations (Prior & La Gerche, 2012). In addition, systematic reviews have reported that trained and untrained subjects show similar systolic responses to exercise, including rises in LVEF (Armstrong et al., 2011; Rowland, 2009).

Direct measurement of myocardial systolic function allows quantification of strain and strain rate. Strain is defined as the relative change in length of a material related to its original length, given in percent. Strain rate describes the rate of shortening or lengthening of the temporal change in strain (Leitman et al., 2004; Prior & La Gerche, 2012). Strain and strain rate deformation parameters are not only a measure of intrinsic myocardial contractility but are also influenced by changes in cardiac load and structure (Ferferieva et al., 2012). For the LV, global longitudinal strain (GLS) is commonly used, which describes the relative length deformation of the LV myocardium between end diastole and end systole. There are no recommended universal normal values of GLS, since differences between vendors and software packages are too large. However, to provide some guidance, a peak GLS in the range of 20% can be expected in a healthy person. For the RV, total longitudinal strain represents the percentage of systolic shortening of the lateral wall of the chamber (Lang et al., 2015). It is assumed that the peak systolic strain rate is more relevant than strain for noninvasive assessment of myocardial contractile function (Blessberger & Binder, 2010; Ferferieva et al., 2012).

To gain further insight into LV and RV systolic function, it is of interest to study the displacement of the atrioventricular planes by investigating mitral and tricuspid annular plane systolic excursion (MAPSE and TAPSE, respectively). Also, the velocity of longitudinal shortening of the myocardium in systole can be measured as annular systolic peak myocardial velocity (s') (Lang et al., 2005; Otto, Schwaegler, & Freeman, 2016). Right ventricular fractional area change (RVFAC) expresses the percentage change in RV area between end diastole and end systole, which provides a further estimate of the global RV systolic function (Lang et al., 2015). No differences in the systolic function of the LV or RV at rest have been documented between athletes and untrained controls (D'Andrea, La Gerche, Golia, Teske, et al., 2015; Galderisi et al., 2015). However, RV systolic dysfunction can develop immediately after a strenuous exercise session, although in a review published by La Gerche et. al (La Gerche & Claessen, 2015), it was concluded that this impairment of RV function is transient and is normalized within days.

Circulatory responses related to exercise are also influenced by body posture. Exercise in an upright body position requires different circulatory adjustments to acquire an optimal cardiac output and blood supply for the body, as compared with a supine position in which the effects of gravity are removed. Rowland et al. (Rowland et al., 2009), demonstrated that stroke volume in both young swimmers and untrained control subjects did not increase during progressive exercise on a swim bench, an observation that may be important to consider when studying heart volumes and function in athletes.

Diastolic function

Diastole is the period of the cardiac cycle when the ventricles relax and fill with blood. There are several parameters that can be used to describe different aspects of diastolic function, but there is no single measure that can be applied to describe the overall function. Diastole can be divided into four phases: isovolumic relaxation, an early rapid diastolic filling phase, diastasis, and late diastolic filling caused by

atrial contraction. During isovolumic relaxation, the ventricular pressure falls below atrial pressure, and atrioventricular valves open. The blood then flows from the atrium to the ventricle during the early rapid diastolic filling phase, which is influenced by atrial pressure, ventricular relaxation, and compliance of the chambers. In diastasis, ventricular and atrial pressure are equalized, there is little movement of blood, and the atrioventricular valves remain in a semi-open position. The duration of diastasis depends on the HR, being longer at a slow HR and entirely absent at a higher HR. Finally, the atrial contraction causes higher atrial pressure, resulting in a second pulse of ventricular filling that comprises about 20% of the total ventricular filling. The early inflow filling velocity (E) and the late inflow filling velocity (A) through the mitral and tricuspid valves reflect the pattern of diastolic filling of the LV and the RV, respectively, in other words, E and A provide information about the early and late filling phases (Appleton, Hatle, & Popp, 1988; Arques, Roux, & Luccioni, 2007; Otto et al., 2016).

The ratio of E to A also reflects the diastolic function. The contribution of atrial contraction under resting conditions is lower in adult athletes than in untrained controls. Therefore, the A wave is decreased in athletes because the ventricular filling occurs mainly during early diastole, which leads to a higher E/A ratio compared with controls. In general, the E wave velocity does not differ between athletes and controls, most likely because the heart of an athlete has increased chamber size and a prolonged diastolic phase (Caselli, Di Paolo, Pisicchio, Pandian, & Pelliccia, 2015).

The velocity of the myocardial longitudinal lengthening during diastole can be recorded near the atrioventricular plane and is given as the mitral and tricuspid early (e') and late (a') diastolic peak myocardial velocities. These parameters are less dependent on preload than transvalvular flow velocities and thus are useful measures for evaluation of diastolic function. The velocity of e' corresponds to the early diastolic relaxation of the myocardium, and a' corresponds to the

second myocardial velocity following atrial contraction (Elliott et al., 2014). The ratio of transvalvular early peak velocity to e' (E/e') is assumed to overcome the influence of ventricular relaxation on peak E velocity. The E/e' ratio for the LV is used as a non-invasive method to reflect the LV filling pressure of this chamber, which is equivalent to the preload of the heart (Burgess, Jenkins, Sharman, & Marwick, 2006; Ommen et al., 2000; Otto et al., 2016). The greater the preload, the greater volume of blood in the heart, which provides a greater stroke volume due to Starling's law. An E/e' of < 8 usually indicates normal LV filling pressure, whereas an elevated E/e' value (lateral wall E/e' > 13 and/or septal wall E/e' > 15) is considered abnormal, reflecting a diastolic dysfunction (Nagueh et al., 2016). Values between 8–15 (grey zone) are regarded as indeterminate. Most studies of diastolic function have confirmed that structural remodeling, seen as part of an athlete's heart, is not associated with an impairment of diastolic filling (Caselli, Montesanti, et al., 2015; Prior & La Gerche, 2012).

During high-intensity exercise, increased cardiac output in untrained subjects depends largely on a higher HR caused by a plateau in stroke volume. By comparison, in athletes, the possibility of further increase in stroke volume continues to contribute to the rise in cardiac output during exercise, which suggests that augmented diastolic filling is the underlying mechanism (Rowland, 2009). A rapid filling rate is necessary during exercise, because the ventricular filling time decreases due to the higher HR, and hence an improved myocardial relaxation may be important in endurance-trained subjects. The ability to increase LV inflow during diastole enables larger stroke volume without further increase in filling pressure (Nagueh et al., 2016; Sundstedt, Hedberg, Jonason, Ringqvist, & Henriksen, 2007). Therefore, larger heart chambers are essential for adequate filling of the athlete's ventricles (Prior & La Gerche, 2012). In healthy children and adults (both athletes and non-athletes), increased mitral E and mitral e' have been demonstrated at peak exercise. However, data on E/e' are somewhat conflicting in that some studies have reported that

this ratio is not affected during and after exercise compared with the resting state (Neilan et al., 2006; Punn et al., 2012; Rowland, Heffernan, Jae, Echols, & Fernhall, 2006; Santoro et al., 2015), whereas other investigations have demonstrated that E/e' is elevated but still within normal limits (Cifra et al., 2016; Studer Bruengger et al., 2014). However, thus far no studies have compared the E/e' response exhibited by endurance-trained and untrained adolescents.

Atrial function

LA function is conventionally divided into three integrated phases designated reservoir, conduit, and contractile. The reservoir phase entails expansion occurring during LV systole and storage of pulmonary venous return in the LA; the conduit phase involves the passive transfer of blood into the LV during diastole; the contractile phase comprises contraction of the LA during the end of diastole (Mehrzad, Rajab, & Spodick, 2014; Pagel et al., 2003). Total LA strain can be measured to evaluate these three components of LA function (Saraiva et al., 2010). Very few studies have considered atrial function in adolescents engaged in regular endurance exercise, although one investigation did find preserved biatrial function in pre-adolescent athletes despite cavity enlargement (D'Ascenzi et al., 2016). In adult athletes, research results concerning atrial function are conflicting, because atrial strain has been reported both with and without discrepancies between athletes and controls (D'Ascenzi et al., 2013; D'Ascenzi et al., 2014; McClean et al., 2015).

Hormones related to growth and metabolism, and their response to exercise

Hormones are involved in most physiological processes and influence the regeneration phase after exercise. They are secreted in an intermittent manner from endocrine glands into the blood stream, where they act as chemical signals throughout the body. The anterior pituitary gland is one of the major endocrine glands, and it responds strongly to exercise by increasing the release of growth hormone (GH), prolactin, and thyroid-stimulating hormone (TSH). The anterior

pituitary gland also secretes follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in response to exercise, although to a lesser extent. Cortisol, which is secreted by the adrenal cortex, is a steroid hormone that, among many other effects, is essential for the ability to adapt to exercise. However, the plasma concentration of cortisol increases only in relation to exercise duration of more than 1 hour (Kenney et al., 2015; Pedersen & Hoffman-Goetz, 2000).

In normal puberty, release of hormones changes dramatically, and this includes enhanced secretion of gonadotropin-releasing hormone. The primary function of this hormone is to regulate the growth, development, and function of the testes in boys and the ovaries in girls by sending signals to the anterior pituitary gland to secrete LH and FSH. In boys, LH stimulates testosterone production, while FSH promotes sperm production. In girls, both LH and FSH stimulate the ovaries to produce estrogen and progesterone, which are necessary for normal menstruation (Pinyerd & Zipf, 2005). The Tanner scale (I-V) is a five-stage clinical scoring system that is often used to describe pubertal development, and in which each stage represents the extent of pubic hair growth and breast or genitalia development, in girls and boys, respectively (Pinyerd & Zipf, 2005; Tanner & Whitehouse, 1976).

Insulin-like growth factor 1 (IGF-1) is a hormone secreted mainly by the liver in response to GH, in what is known as the GH–IGF-1 axis. IGF-1 is also secreted by multiple tissues for autocrine and paracrine functions. Along with thyroid hormones, cortisol, and gonadal steroid hormones (e.g., estradiol and testosterone), IGF-1 is crucial for the hormonal control of growth during childhood and adolescence (Rogol, Roemmich, & Clark, 2002). Particularly in the heart, IGF-1 has been most extensively characterized as the cellular signaling pathway responsible for inducing physiological cardiac hypertrophy, but also for promoting formation of cardiomyocytes, and protecting against cell death (Neri Serneri et al., 2001; Troncoso, Ibarra, Vicencio, Jaimovich, & Lavandero, 2014). Mechanical stretch of the cardiac

myocytes can stimulate local synthesis of IGF-1 within the myocardium, which in turn has an autocrine effect (Godfrey, Madgwick, & Whyte, 2003). High-intensity exercise has long been known to be a potent stimulus of the GH–IGF-1 axis in both prepubertal and adolescent subjects (Eliakim & Nemet, 2010). Reduced levels of IGF-1 are independently associated with T2D and abdominal obesity (Puche & Castilla-Cortazar, 2012).

Insulin-like growth factor 2 (IGF-2) is another hormone secreted mainly by the liver, but even by the utero in pregnant women. This hormone is a large contributor to intrauterine growth and it is suggested to be a marker for childhood obesity, however, current data is both limited and contradictory (Kadakia & Josefson, 2016).

Hormones associated with pubertal development and energy metabolism during exercise also affect glucose homeostasis in muscle cells and triglyceride balance in adipose tissue. Insulin and glucagon are the main glucoregulatory hormones during prolonged moderate-intensity exercise (Riddell, 2008). Insulin is a peptide hormone synthesized by β-cells in the islets of Langerhans in the pancreas. The role of this hormone is to increase glucose uptake by muscle cells and the liver, where the glucose can be stored as glycogen. In type 1 diabetes (T1D), insulin is completely or almost completely absent in the plasma due to the total or near-total destruction of the pancreatic β-cells by the body's own leukocytes (i.e., an autoimmune disease). In contrast to insulin, during stress or high-intensity exertion, the peptide hormone glucagon sends signals to the liver and skeletal muscles to induce glycogenolysis, which is the breakdown of glycogen stores into glucose to maintain blood glucose homeostasis and provide energy to the muscles. Hepatic glycogenolysis also increases the circulatory levels of cortisol and GH (Kanungo, Wells, Tribett, & El-Gharbawy, 2018; Steinacker, Lormes, Reissnecker, & Liu, 2004; Widmaier et al., 2014).

It is known that some degree of insulin resistance occurs during puberty, and that pubertal adolescents also show insulin resistance during exercise. The cause of lower insulin sensitivity is not entirely clear, although it might be the result of increased levels of circulating GH and IGF-1 (Amiel, Sherwin, Simonson, Lauritano, & Tamborlane, 1986; Riddell, 2008). Nonetheless, this transient insulin resistance during exercise is of important, because maintenance of blood glucose levels during exercise is critical for the glucose-dependent brain, considering that without such balance exercising muscles might consume all available glucose with lethal consequences (Steinacker et al., 2004). Moreover, long-term endurance exercise is effective in maintaining normal insulin sensitivity and β-cell function with aging, despite a reduced training volume in the later stages of life (Kusy, Zielinski, & Pilaczynska-Szczesniak, 2013). It has been argued that β-cell stress (i.e., increased stimulation of the β-cells) may arise during periods of rapid growth, when the demand for insulin is great. Also, the β-cells may be stimulated by regular overeating, any infections, and psychological stress (Ludvigsson, 2006). Still, there have been no reports indicating that regular excessive exercise during adolescence affects the function of the β-cells.

The immune system and its response to exercise

The immune system consists of cells, mostly leukocytes, that are involved in defending the body against infections caused by viruses, bacteria, parasites, and other pathogens. A large number of cells and a far larger number of chemical messengers participate in the immune defenses. The immune system can be classified into two categories called the innate and the adaptive immune system, which interact with each other. The innate immune system consists of immune cells including granulocytes, macrophages, and natural killer (NK) cells, which protect us against foreign substances without specifically recognizing them. In contrast, the adaptive immune system depends on specific recognition of the cells or substances to be attacked. Any foreign protein molecule or polysaccharide that triggers the immune

system is termed an *antigen*. Occasionally, a normal tissue component can be the target of an immune response, as seen in autoimmune diseases, and in such cases the antigen is called an *autoantigen* (Coico & Sunshine, 2015).

Lymphocytes such as B- and T-cells play key roles in the adaptive immune system, and they have antigen-specific receptors on the surface of their cell membranes. Essentially, B-cells produce antibodies, and T-cells produce cytokines. The immune response involving secretion of antibodies from B-cells is denoted humoral immunity, whereas the immune response initiated by substances such as cytokines and chemokines is called cell-mediated immunity. There is constant interplay between humoral and cell-mediated immunity (Coico & Sunshine, 2015). The immune system matures gradually from childhood to adulthood. Children acquire infections that must be fought off and controlled by immune responses, which over time results in an immunological memory in which features of the adaptive immune system evolve (Simon, Hollander, & McMichael, 2015).

Cluster of differentiation (CD) molecules are markers expressed on the cell surface that are commonly used to identify and characterize leukocytes and other cells relevant for the immune system. This approach has led to characterization and formal designation of more than 400 different molecules. For example, T cells are identified as $CD3^+$, and the two major subgroups of T cells are designated $CD4^+$ and $CD8^+$. $CD4^+$ cells can be further divided into, for example, T helper (Th) 1 and 2 cells. $CD8^+$ cells are known as T cytotoxic cells and can also be further subdivided. B cells are identified as $CD19^+$, and NK cells as $CD16^+$ and $CD56^+$. The symbol "+" with a CD number indicates the presence of a surface molecule on a cell, and a "–" indicates the absence of such molecule (Engel et al., 2015; Gianchecchi, Delfino, & Fierabracci, 2018; IUIS-WHO Nomenclature Subcommittee IUIS-WHO, 1984; Sharif et al., 2018). An intensive

bout of exercise induces mobilization of all lymphocyte subpopulations into the blood. After prolonged intense exercise, with long duration, the size of the lymphocyte subpopulation declines, and that effect lasts for at least 1 hour (Pedersen & Toft, 2000).

Cytokines and chemokines

Immune cells release large quantities of *cytokines,* which are protein messengers that link the components of the immune system together in a way that enables communication between the immune cells. Most cytokines are secreted by more than one type of immune cells, and there is often a cascade of secretion. There are several subgroups of cytokines: interleukins (IL), tumor necrosis factor (TNF), interferon (IFN), and *chemokines* (Coico & Sunshine, 2015). Chemokines are the largest subgroup, and they are classified into four major subfamilies, including those designated CXC and CC. Chemokines play a crucial role in coordinating adaptive immune responses as key activators of adhesion molecules and in driving leukocyte migration to inflammatory sites, and therefore they are primarily considered to be pro-inflammatory mediators (Karin & Wildbaum, 2015; Robertson, 2002). CXC ligand (CXCL) 10 is secreted by $CD4^+$, $CD8^+$, and NK cells and appears to contribute to the pathogenesis of many autoimmune diseases, such as T1D and autoimmune thyroiditis (Antonelli et al., 2014).

During exercise, production and release of pro-inflammatory cytokines increase as a local inflammatory response to tissue injury. These cytokines include IL-1β, -6, -8, -17, TNF-α, IFN-ɣ, and chemokine CC ligand (CCL) 2, which facilitate an influx of leukocytes that participate in healing of the tissue (Pedersen & Hoffman-Goetz, 2000; Sugama, Suzuki, Yoshitani, Shiraishi, & Kometani, 2012; Suzuki et al., 2002). However, this increased release of pro-inflammatory cytokines is rapidly counteracted by anti-inflammatory IL-10, a cytokine produced by the immunosuppressive type 1 regulatory cells. IL-10 has been classified as an interleukin that is closely involved in regulation of the immune system (Handzlik,

Shaw, Dungey, Bishop, & Gleeson, 2013). Moreover, the release of IL-10 is increased during and after exercise, and, in addition to inhibiting the production of several pro-inflammatory cytokines, IL-10 protects against diseases associated with long-term and low-grade systemic inflammation (de Waal Malefyt, Abrams, Bennett, Figdor, & de Vries, 1991; Petersen & Pedersen, 2005; Schild et al., 2016).

Type 1 diabetes biomarkers and related autoantigens

Within the β-cells, the precursor molecule proinsulin is cleaved into insulin and C-peptide. The insulin and C-peptide produced are released from the β-cells in equal amounts, whereas only a minor amount of uncleaved intact proinsulin is released to the circulation in healthy individuals. Consequently, it has been suggested that elevated level of circulating proinsulin constitute a biomarker for secretory β-cell dysfunction. However, neither β-cell dysfunction nor proinsulin secretion is correlated with development of diabetes (Pfutzner & Forst, 2011). It has been shown that detecting the humoral response (i.e., secretion of autoantibodies in reaction) to the autoantigens insulin, tyrosine phosphatase (IA-2), and glutamic acid decarboxylase (GAD_{65}) can be a useful tool to predict T1D. The physiological function of IA-2 is incompletely defined, but it may be involved in the fine regulation of β-cell function in the pancreas and contribute to the regulation of insulin granule content (Seissler, Nguyen, Aust, Steinbrenner, & Scherbaum, 2000; Torii, 2009). GAD_{65} is involved in the synthesis of gamma amino butyric acid (GABA), which is a potent inhibitory neurotransmitter in the central nervous system. GAD_{65} is also detected in certain non-neural cells (e.g., in the pancreas, where its functional relevance is not yet known), although it may be related to paracrine effects in the modulation of glucagon. However, there are still areas that remain to be elucidated regarding the physiological effects of GAD_{65} (Towns & Pietropaolo, 2011). In most cases, a positive observation with a single autoantibody specificity represents harmless non-progressive β-cell autoimmunity, whereas positivity for multiple (≥ 2) autoantibodies usually reflects progression to disease within a year of the initial appearance of the autoantibody reactivity

(Knip & Siljander, 2008). A previous study analyzed 5-year-old children regarding cell-mediated response after *in vitro* stimulation with type 1 diabetes-related autoantigens (E. Carlsson, Ludvigsson, Huus, & Faresjo, 2015), and the results showed decreased spontaneous immune activity for several different cytokines and also a low immune response to diabetes-related autoantigens in the children with high physical activity compared to those with low and/or average physical activity. However, such investigations of older children and adolescents are scarce.

Methodological background

Echocardiography

Echocardiography is the most frequently used noninvasive examination to obtain detailed anatomical and physiological information about the heart (Lang et al., 2015). Echocardiographic imaging is based on ultrasound waves that are produced by a transducer, typically with frequencies of 1–20 megahertz. In short, the ultrasound waves are sent to a chosen part of the body and are reflected at tissue interfaces back to the transducer. The signal then undergoes complex processing, including conversion into electrical impulses that can be assessed by the ultrasound scanner, which finally generates an echocardiographic image on a screen. Cardiac measurements can be done in one-, two-, and three- dimensional views. In M-mode, also called one-dimensional echocardiography, the reflections from the ultrasound beam are along a time axis, which generates a gray-scale level (Otto et al., 2016). An M-mode image is presented in Figure 3.

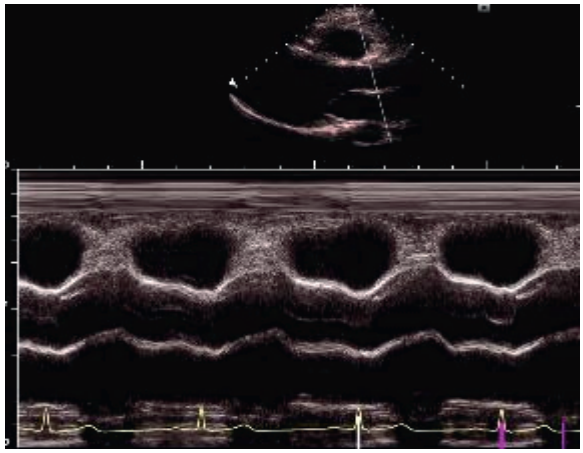

Figure 3. Echocardiographic M-mode image.

In two-dimensional (2D) echocardiography, the ultrasound sweeps across a tomographic plane (a sector) to produce 2D images (Figure 4). The frame rate, which is the number of ultrasound images displayed per second, varies with the depth of interest. For cardiac applications, a rate of ≥ 30 frames per second is desirable (Lang et al., 2015; Otto et al., 2016).

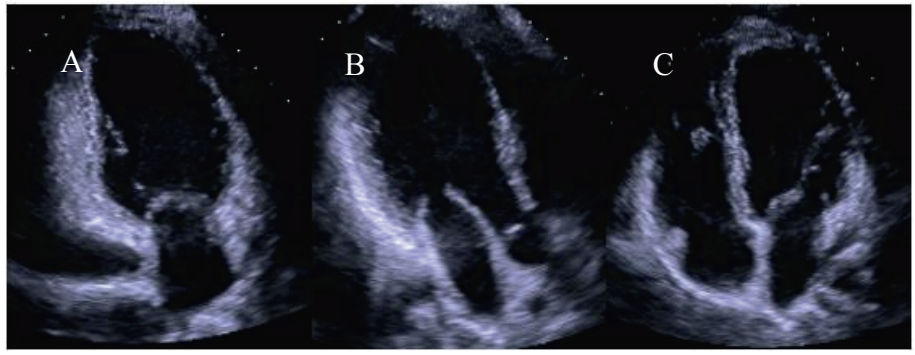

Figure 4. Two-dimensional echocardiographic images showing two-, three-, and four-chamber views (A–C, respectively).

Use of speckle tracking echocardiography enables offline analyses and quantification of wall motion from digitally recorded cine loops with ECG gating. A region of interest (ROI) is defined between the endocardial and epicardial borders. The myocardium is usually divided into apical, mid, and basal segments, where unique fingerprints, or "speckles", are tracked. Detection of the spatial movement of these speckles during the heart cycle allows direct calculation of Lagrangian strain. The tissue velocity also generates the strain rate (Blessberger & Binder, 2010).

The principle of Doppler analysis is the change in frequency of the ultrasound signal reflected from a moving target, such as the blood cells for assessment of blood flow and the myocardium for evaluation of wall motion. Pulsed, continuous, and color tissue Doppler can be used. Pulsed Doppler allows sampling of blood flow velocities from a specific intracardiac region, and the typical sample volume length is 5 mm. Continuous Doppler reflects the transmitted ultrasound without interruption, which offers the major advantage of enabling measurement of very high velocities (Otto et al., 2016). Color tissue Doppler is calculated based on tissue velocities and directions. Compared with tissue Doppler, the speckle tracking method is angle independent and does not require such a high frame rate (Blessberger & Binder, 2010).

Cardiopulmonary exercise test and peak VO_2

The cardiopulmonary exercise test (CPET) performed on a treadmill or a bicycle ergometer allows analysis of gas exchange during exercise by breath-by-breath measurements of VO_2 and carbon dioxide output (VCO_2). CPET can also be used to measure ventilatory flow. Peak or maximal VO_2 is important in CPET, because this variable defines the limit of the cardiopulmonary system and reflects

the maximum ability of a person to take in, transport, and use oxygen (Albouaini et al., 2007). VO_{2max} is calculated by the Fick equation as the product of cardiac output (HR and stroke volume) and arteriovenous oxygen difference (C[a-v] O_2) at peak exercise as follows (Balady et al., 2010):

VO_{2max} = (HR × stroke volume) × (C[a-v] O_2)

It is evident that with the Fick's principle, values can be determined by using VCO_2 and VO_2 when applying the CPET technique (Sun et al., 2000).

The respiratory exchange ratio (RER) is the ratio between VCO_2 and VO_2 during exercise, and it is obtained exclusively by ventilatory expired gas analysis. Most subjects attain a ventilatory threshold when the RER is ≥ 1.0. Thus, higher exercise intensities result in lactic acid buffering, which in turn increases the VCO_2 output at a faster rate than VO_2, leading to increased RER during the progress. A peak RER of ≥ 1.1 is generally considered to be an indication of excellent effort during CPET (Balady et al., 2010).

Hormonal and immunological analysis

Flow cytometry

Flow cytometry can be used to measure and analyze multiple physical characteristics of isolated single cells, such as T and B lymphocytes and NK cells. Both the number of intrinsically fluorescent compounds within each cell and the information they provide are limited. Therefore, the cells to be assessed are stained with fluorescent dyes called fluorochromes that can reveal the presence of components that otherwise would not be visible. To label proteins covalently, cells are subjected to fluorescence staining with conjugated monoclonal antibodies and then passed in a fluid through a laser beam in which they scatter the laser light, and this light scattering is directly related to structural and morphological properties of the cell. The scattered and emitted fluorescent light is collected by optical detectors, which

convert the registered optical signals into electronic signals. The fluorescence emission derived from the fluorochromes is proportional to the amount of fluorochromes bound to the cell or cellular component of interest. A fluorescent-activated cell sorter (FACS) is a flow cytometer that has the capacity to separate fluorescent-labeled cells from a mixed cell population (Adan, Alizada, Kiraz, Baran, & Nalbant, 2017; Gross et al., 2015).

Multiplex fluorochrome technique (Luminex)

The fluorochrome multiplexing technique (Luminex) is a preferred testing method that enables simultaneous detection of multiple immune markers such as cytokines and chemokines within a single sample. Key components of the Luminex technology are the microspheres that are individually dyed with two or three spectrally distinct fluorochromes. The surface of microspheres is coated with monoclonal antibodies directed against the analyte of interest, and serum or cell supernatants from a source such as *in vitro* stimulated peripheral blood mononuclear cells (PBMCs) can be added to the antibody coated microspheres. The analyte of interest is detected by a secondary antibody labeled preferably with streptavidin-phycoerythrin. Finally, assessment of the microsphere-complex to detect the cytokines is done in a dual-laser flow analyzer, where precision fluidics align the beads in a single stream through a flow cell in which the lasers excite the fluorochromes individually. First there is a red classification laser that scans the dyes in each bead and identifies the microspheres specific for the analyte that is being detected. Then a green reporter laser excites the reporter fluorochrome molecule. The intensity of the signal emitted from the reporter fluorochrome (streptavidin-phycoerythrin) is in direct proportion to the amount of bound analyte (Wild & John, 2013).

Chemiluminescence Microparticle Immunoassay (CMIA)

CMIA is a technique used for quantitative determination of biomarkers in serum, such as cortisol. This method is based on a microparticle sandwich immunoassay that measures chemiluminescence as relative light units (RLUs). To detect cortisol, the magnetic microparticles coated with anti-cortisol are used to bind cortisol in the sample and cortisol acridinium-labeled conjugate. The amount of unlabeled analyte is determined by detection of the RLU signal from the acridinium-labeled conjugate in the immunoreactions. The detected RLU signal is inversely related to the analyte concentration (Iranifam, 2013).

Peripheral blood mononuclear cells (PBMCs)

PBMCs represent a diverse population of cells that participate in the body's immune defense, such as lymphocytes, monocytes, and dendritic cells. These cells can be stimulated *in vitro*, and, when activated, they secrete substances like cytokines and chemokines, which can be detected by various methods (e.g., the multiplex fluorochrome technique [Luminex]) (Hamot, Ammerlaan, Mathay, Kofanova, & Betsou, 2015).

Rationale

For many years, it was assumed that children and adolescents respond and adapt to exercise in the same way as adults, and thus few studies focused on the younger age groups. Today, understanding of both the similarities and the differences between athletic youngsters and adults has increased in many fields, because exercise research has also been focused on young people. In addition, knowledge regarding the differences in response to exercise between athletic and non-athletic adolescents has increased. Notwithstanding, the long-term effects of starting exercise at the elite level and with high intensity at a young age are still largely unknown.

In general, it is important to understand how children and adolescents respond and adapt to exercise, because physical activity is vital to battle childhood obesity and to teach children to develop lifelong healthy habits (Kenney et al., 2015). Specifically, further research is needed to determine whether regular intense physical training at a young age is associated only with benefits. Studies of the athlete's heart have been undertaken, especially in adults. This is beneficial for a number of reasons, including to explain how cardiac adaptation contributes to improved athletic performance, and to make it possible to differentiate the normal condition of the athlete's heart from important cardiac diseases. This thesis includes cross-sectional studies of cardiac dimensions and function, and also assessments of part of the body's hormones and immune system in adolescents who participated in regular endurance exercise for at least 2 years. These endurance-trained adolescents are compared with a control group consisting of boys and girls in a similar age span who did not take part in regular physical training. Adolescence is an important period in life during which good health habits should be established and maintained. The data generated by the present research will contribute to further understanding of some of the physiological effects of intense regular exercise performed at an elite level at a young age.

Overall and specific aims

The overall aim of the research presented in this thesis was to investigate adolescents practicing regular and long-term endurance exercise, with a focus on cardiac size and function, hormones, and the immune system.

The specific aims were as follows:

- To compare atrial and ventricular size and function at rest in endurance-trained adolescents and a non-trained control group of similar age and sex.

- To compare the extent and the temporal development of systolic and diastolic functional changes associated with a maximal exercise test in the trained and untrained subjects.

- To explore how cardiac dimensions and function in adolescents are related to peak VO_2 at rest and after peak exercise.

- To compare the subjects who participated in regular active training and those who did not (controls) with regard to resting levels of several circulating hormones associated with growth and metabolism.

- To determine whether cardiac dimensions are positively related to resting levels of growth and metabolic hormones, with emphasis on the growth factor IGF-1.

- To evaluate the impact of sex on the immune system in endurance-trained adolescents, with emphasis on type 1 diabetes-related autoimmunity.

Materials and methods

Design

All four of the studies included in this thesis used a quantitative approach. Three of the studies had a cross-sectional design (Papers I, III, and IV) and one had a pre-post-test design (Paper II). Furthermore, the studies reported in Papers I-III compared endurance-trained subjects with non-trained controls, whereas the last study (Paper IV) involved comparison between the sexes only in endurance-trained subjects (Table 1).

Table 1. Overview of the studies

Paper	I	II	III	IV
Design	Cross-sectional Comparative Quantitative Age and sex matched	Pre-post test Comparative Quantitative Age and sex matched	Cross-sectional Comparative Quantitative Age and sex matched	Cross-sectional Comparative Quantitative
Participants	27 athletes and 27 controls, 32 boys + 22 girls	27 athletes and 27 controls, 32 boys + 22 girls	24 athletes and 24 controls, 28 boys + 20 girls	44 athletes, 24 boys + 20 girls
Data collection	Echocardiographic examination at rest; CPET; offline analysis of data by EchoPAC	Echocardiographic examination at rest, immediately, and 15 min after CPET; offline analysis of data by EchoPAC	Blood samples taken at rest analyzed by Luminex and CMIA; echocardiographic examination at rest; CPET; offline analysis of data by EchoPAC	Blood samples taken at rest, analyzed by flow cytometry, Luminex, and CMIA; CPET
Data analysis	Non-parametric Wilcoxon matched-pair signed-rank test; linear regression	Non-parametric Wilcoxon matched-pair signed-rank test; multiple linear regression with multiple imputation	Non-parametric Wilcoxon matched-paired signed-rank test; bivariate correlation and linear regression	Non-parametric Mann-Whitney U test; chi-square test

CPET, cardiopulmonary exercise test; CMIA, chemiluminescent microparticle immunoassay

Participants

The Exercise Project at Jönköping University, Sweden, includes a total of 72 adolescents aged 13–19 years divided into one active group (n = 45) and one control group (n = 27). The exercise-practicing participants were recruited from orienteering and cross-country ski clubs located in southern Sweden, and the controls were selected at public schools in the same area. All of the active subjects had exercised and competed at an elite level in their sports for at least two years prior to study enrollment; on average, they exercised 5 days a week for at least 30 minutes on each occasion, and this was in addition to compulsory physical education in school. The controls included healthy adolescents who were not engaged in regular exercise during leisure time.

Each of the studies reported in Papers I and II included 54 adolescents; all 27 subjects in the project control group, and 27 athletes who were selected from the project active group and were completely matched by age and sex with the controls. In Paper III, all 24 of the project controls with available blood samples were included and compared with 24 subjects from the active group who were completely matched by age and sex. The investigation described in Paper IV was based on all endurance-trained participants with available blood samples (n = 44).

Data collection procedure

Collection of data was performed from November 2013 to May 2015 at the Department of Clinical Physiology and the Department of Laboratory Medicine, Region Jönköping County, Jönköping, Sweden. The study subjects were instructed to refrain from exercise on the day testing was performed. All participants completed a questionnaire that covered exercise habits, medical conditions, and smoking habits. Weight and height were recorded, and a resting 12-lead ECG was performed with MAC 5500HD version 10 (GE Healthcare, Milwaukee, WI, USA). Systolic and diastolic blood pressure was

measured in supine position after at least 5 minutes of rest. Blood samples were drawn by an experienced biomedical scientist.

Cardiopulmonary exercise test (CPET)

Peak VO_2 was assessed by a maximal CPET. Prior to each test, a calibration of ambient conditions was performed, and the automatic volume and gas analyzer was also calibrated. The CPET was performed on a treadmill (RL2500E; Rodby, Vänge, Sweden) according to the modified Bruce protocol (McInnis & Balady, 1994) using a Jaeger Oxycon Pro device (Viasys Healthcare, Hoechberg, Germany). Exhaled air was analyzed on a breath-by-breath basis for O_2 and CO_2 content, and ventilatory data were presented as 30-second averages. To ensure maximal exertion for all participants, the criteria for termination of exercise were exhaustion and/or RER > 1.1. Peak VO_2 was related to kg^{-1}.

Echocardiography

Echocardiographic examinations were performed according to clinical routine by two experienced investigators, with the subjects lying in left lateral position (see Echocardiographic Study Protocol, Appendix I). All echocardiographic investigations were conducted in a transthoracic manner. The procedure was performed using an ultrasound scanner (Vivid E9, GE Healthcare, Horten, Norway) equipped with an M5S probe and ECG gating using 3-lead ECG, and this was done three times: at rest before the CPET (baseline), immediately (within 1-2 minutes) after CPET, and 15 minutes after CPET. Baseline examination proceeded for approximately 15 minutes whereas the second and third examinations were performed for 8–10 minutes each. Two-dimensional echocardiographic images were acquired from the parasternal long- and short-axis views, and from apical two-, three-, and four-chamber views at a rate of > 40 frames/second. In addition, a modified four-chamber view focused on the RV and RA was obtained. Pulsed-wave Doppler recordings of subvalvular aortic and mitral flow were also made. Color tissue Doppler imaging loops were obtained in the apical two- and four-

chamber view at a rate of > 100 frames/second. M-mode tracing was obtained. All images were recorded to DVD in a raw Digital Imaging and Communications in Medicine (DICOM) format.

Echocardiographic analysis

Echocardiographic data were analyzed offline by a single operator using EchoPAC PC version 110.0 (GE Healthcare, Horten, Norway).

Cardiac dimensions and volumes

Interventricular septum thickness (IVS), LVPWT, and LVID at end diastole were measured by 2D echocardiography (Figure 5). LVM was calculated by the linear method at the parasternal long axis approach (Lang et al., 2015) as follows:

$$LVM = 0.8 \times 1.04 \times [(LVID + LVPWT + IVS)^3 - LVID^3] + 0.6$$

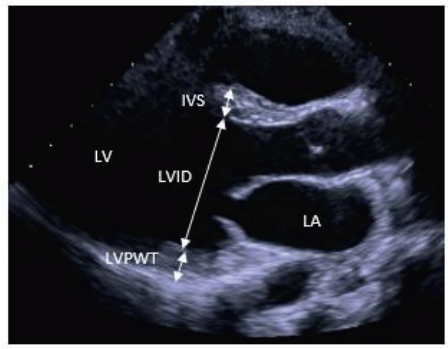

Figure 5. Echocardiographic parasternal long-axis view of the left ventricle and atrium in diastole. LV, left ventricle; LA, left atrium; IVS, interventricular septum thickness; LVID, left ventricular internal diameter; LVPWT, left ventricular posterior wall thickness.

LVEDV and LVESV were calculated by the biplane disk summation technique. Briefly, in this method, the LV volume is based on tracings of the blood–tissue interface of the endocardial border within the LV cavity. At the mitral valve level, the tracing is connected manually to a

straight line from the lateral to the septal annulus in both four- and two-chamber views. The length of the ventricle is measured from the middle point of the line at the annulus to the most distant point of the line in the ventricle (i.e., the apex) (Lang et al., 2015).

RVD1 (Figure 6) and RV area were obtained at end diastole in the RV-focused 2D view. RVOTprox was measured from the anterior wall of the RV to the aortic valve in the parasternal short-axis view at end diastole. Assessment of the internal diameter of the LA was achieved by M-mode tracing using leading edge to leading edge convention. The disk summation technique was used to calculate the LA volume (Lang et al., 2015). The RA diameter was estimated by measuring the distance between the lateral RA wall and septum at the midatrial level. RA area was assessed at end systole.

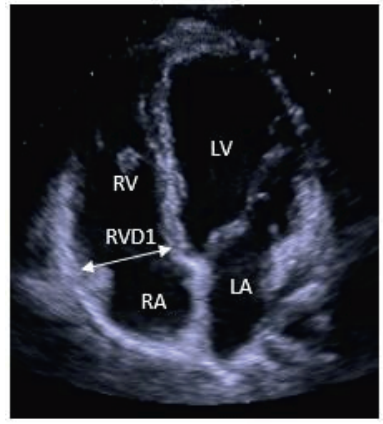

Figure 6. Echocardiographic four-chamber apical view in diastole. RV, right ventricle; RA, right atrium; RVD1, right ventricular basal diameter; LV, left ventricle; LA, left atrium.

Cardiac systolic function

LVEF was calculated by the biplane disk summation technique (modified Simpson's rule) (Lang et al., 2015). Peak left ventricular global longitudinal strain (LVGLS) was measured from the three

standard apical views and averaged from 18 segments (six in each level: base, mid, apex) using speckle tracking echocardiography. The speckle-tracking ROI was manually adjusted along the endocardial border to exclude the pericardium. Measurements from segments of good tracking quality were automatically accepted by the software, whereas segments with poor tracking were excluded. From the modified 2D four-chamber view, longitudinal strain of the lateral free wall of the RV was determined by averaging the three segments (base, mid, apex), and fractional area change (FAC) of the RV was calculated using measurements of end diastole and end systole.

Color tissue Doppler was used to acquire the LV s' and RV s' from the base of the ventricle (at the septal, lateral, anterior, and posterior walls in the LV, and at the lateral free wall in the RV). Measurements were averaged over three cardiac cycles. Figure 7 illustrates the measurements of s' in systole and e' and a' in diastole. MAPSE and TAPSE were measured with the tissue-tracking (TT) algorithm, that is s' of the LV and the RV, respectively, measured by color tissue Doppler integrated over time (Brodin, van der Linden, & Olstad, 1998; Van Orman, Connelly, Albinmousa, & Tousignant, 2016). MAPSE was averaged from three consecutive heartbeats at the septal, lateral, inferior, and anterior aspects of the mitral annulus in the apical two- and four-chamber views. TAPSE, in turn, was averaged from three consecutive heartbeats at the lateral aspect of the tricuspid annulus, in the apical four-chamber view focused on the RV.

Cardiac diastolic function

Values of e' and a' (Figure 7) were obtained from the base of the ventricle (septum, lateral, anterior, and posterior walls in the LV, and the free lateral wall in the RV) using color tissue Doppler. In the apical view, at the level of the tip of the mitral leaflets, pulsed Doppler was utilized to measure early and late diastolic flow velocity across the mitral valve specified as E and A wave, respectively (Figure 8). The ratios of E/A and E/e' were also calculated. LA total strain was determined by speckle tracking echocardiography in four-chamber

view with the beginning of the P wave as the reference point, which made it possible to obtain the sum of peak negative and peak positive values (Lang et al., 2015). LA total strain corresponds to contractile, conduit, and reservoir function combined (Saraiva et al., 2010).

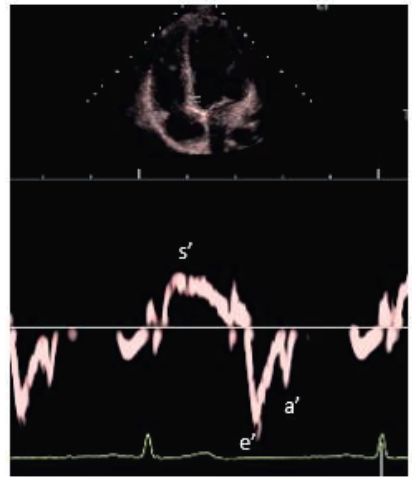

Figure 7. Echocardiographic apical view with tissue Doppler recorded from the basal septum. s', peak myocardial systolic velocity; e', early diastolic peak myocardial velocity; a', late diastolic peak myocardial velocity.

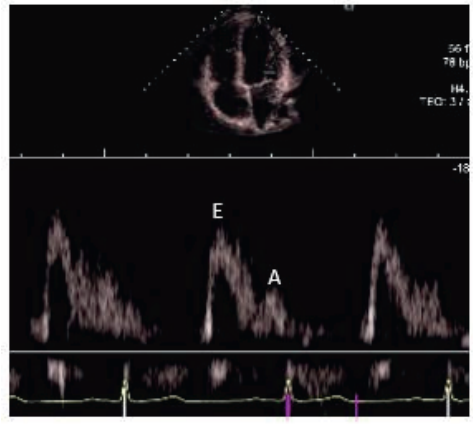

Figure 8. Echocardiographic apical view with pulsed Doppler across the mitral valve. E, early mitral inflow filling velocity; A, late mitral inflow filling velocity.

Analysis of biomarkers

From 44 individuals in the active group and 24 controls, non-fasting venous blood samples were collected in tubes supplemented with EDTA or sodium-heparin, or without anticoagulants. Whole blood supplemented with EDTA was prepared for flow cytometry, and sodium-heparinized blood was used for preparation for PBMC. Serum (without anticoagulant) was stored at –80 °C until analyzed for cytokines and hormones.

Immune cells

In EDTA-supplemented whole blood, lymphocytes were stained with fluorochrome-conjugated monoclonal antibodies for $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+/CD16^+$ before analysis on a FACS Canto flow cytometer (both antibodies and the cytometer from BD Biosciences, San Jose, CA, USA). After sample preparation, data were collected using a side-scatter channel (SSC) gate on lymphocyte region and SSC/CD45 dot plots where the count stopped when it reached 50,000 lymphocytes. T cells were gated from lymphocytes based on SSC/CD3 dot plots and on CD4/CD8, and B and NK cells were gated from dot plots based on CD19 and on CD56/CD16 (Figure 9).

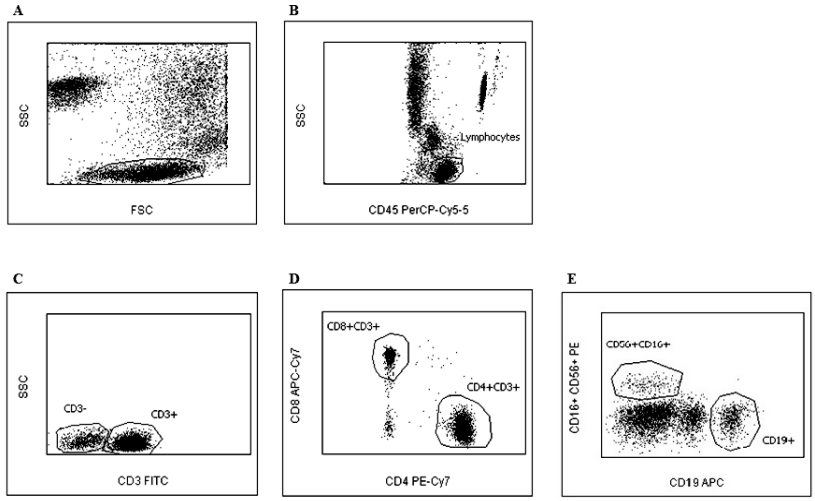

Figure 9. Gating strategies for lymphocyte subsets.

Cytokines and chemokines

From sodium-heparinized venous blood, PBMCs were separated by Ficoll Paque density centrifugation (Pharmacia Biotech, Sollentuna, Sweden). Thereafter, the PBMCs were incubated at 37 °C in a humified atmosphere with 5% CO_2 in serum-free medium only or in such medium with either of the diabetes-related autoantigens IA-2 (a.a. 853–872, 10 ug/mL; New England Peptide, Gardner, MA) or GAD_{65} (5 µL/mL of whole protein, Diamyd, Medical AB, Stockholm Sweden). Cell supernatant and PBMCs were harvested after 72 hours of *in vitro* stimulation. To measure the secretion of cytokines and chemokines that depended solely on antigen stimulation, the spontaneous secretion of cytokines was measured and subtracted from *in vitro* stimulated PBMC secretion.

The Luminex technique (Bio-Rad Laboratories, USA) was used to detect and quantify the secreted cytokines (IL-1β, -5, -6, -8, -10, -17, IFN-γ, and TNF-α) and chemokines (CCL2 and CXCL10) in cell supernatants from the *in vitro* cultured PBMCs. Cytokines and chemokines were analyzed with the Human Cytokine 10-Plex Group I Assay (Bio-Rad Laboratories, USA). A Bio-Plex 200™ system (Luminex xMAP™ Technology, USA) was used to identify and quantify each parameter.

Hormones

The Luminex technique was used to detect and quantify the hormones IGF-1, IGF-2, proinsulin, FSH, GH, LH, prolactin, and TSH in serum. The hormones were assessed with the Human Metabolic and Hormone Assays (Bio-Rad Laboratories, USA) and the Bio-Plex 200™ system (Luminex xMAP™ Technology, USA).

Cortisol was measured in non-fasting serum by CMIA using Architect i2000 (Abbott, Chicago, Illinois, USA). After incubation, cortisol acridinium-labeled conjugate was added to the reaction mixture. After a second incubation, the microparticles were washed, and then pre-trigger and trigger solutions were added to the reaction mixture. The chemiluminescent emitted was measured as RLUs.

Data analysis

All statistical methods were performed with Statistical Packages for the Social Sciences (SPSS) versions 19 and 21. Non-parametric tests were used, with data presented as median values in all four studies.

The differences in echocardiographic, hormonal, and immunological parameters between the groups were examined by the Wilcoxon matched-pairs signed-rank test (Papers I–III) or the Mann-Whitney U-test (Paper IV). Also, in the analysis reported in Paper IV, the chi-square test was used for categorical variables. In the four studies, statistical significance was set at $P < 0.05$. Bivariate correlations and linear regression analysis were performed to determine the correlation between peak VO_2 and cardiac dimensions and functions, and between hormones and cardiac dimensions (Papers I–III). In the analyses described in Paper IV, correlations were determined using Spearman's rank correlation coefficient. For additional analysis presented in Table 7, the cytokines/chemokines were ln-transformed with zero skewness log transformation. The resulting variables were analyzed by linear regressions with the BSA, sex, active group/controls, and the interaction between the sexes and active/controls as independent variables.

Due to the difficulty in performing optimal echocardiographic examination immediately after peak exercise, image quality was not the best in all views at that time point. Therefore, multiple imputation of missing data was used in correlation and regression analyses in Paper II. If that had not been done, inadequate handling of the missing data might have led to biased and/or inefficient estimates of parameters such as regression coefficients. Multiple imputation is a statistical technique that uses the distribution of the observed data to generate a set of estimated plausible values for the missing data (White, Royston, & Wood, 2011).

The values of the cardiac dimensions given in Papers I and II were indexed with BSA, which was calculated by the DuBois formula, where W = weight and H = height:

$$BSA = 0.007184 \times W^{0.425} \times H^{0.725}$$

The reproducibility of the offline echocardiographic analysis with EchoPAC was tested in randomly chosen subjects, ten from the active group and ten from the control group. Inter- and intraobserver variability were determined from selected echocardiographic measurements with Pearson's bivariate two-tailed correlation and with the interclass correlation coefficient using a two-way mixed, absolute agreement model. Interobserver variability was tested against a second experienced investigator.

Ethical considerations

All four of the studies included in this thesis adhered to the ethical conduct for human research described in the Helsinki Declaration ("World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects," 2013) and by the Swedish Research Council (Hermerén, 2011). The studies also took into account the ethical guidelines outlined by the Council for International Organizations of Medical Science (CIOMS) ("International ethical guidelines for biomedical research involving human subjects," 2002) as well as principles of biomedical ethics (Beauchamp, 2003). All of these proclamations refer to the following fundamental ethical principles: the principle of autonomy, which includes respect for person and protects a person's right to self-determination; the principle of beneficence and non-maleficence, which involves the desire to do good and minimize harm; and the principle of justice, which concerns the selection of participants and asserts that all groups of people should be included in research. The Exercise Project at Jönköping University, Sweden, was approved by the Central Ethical Review Board in Linköping, Sweden (Dnr 2013/89-31).

Written informed consent was obtained from all of the adolescents themselves and also from their parents of those who were under the age of 18 years. A concise, easy-to-read letter about the project was compiled to ensure that both the adolescents and their parents would receive the same information. Before the actual start of data collection, the design of the letter and the questionnaire had been discussed and approved by 14 adolescents who later did or did not participate in the project. The letter described the purpose of the research and explained that the physical examination process would include painless investigations of the heart, but would also comprise venipuncture to collect blood samples, which might cause some discomfort and hence could be excluded if preferred. The letter also stated that participants could withdraw from the project without any

negative consequences. In addition, information was given regarding contact persons for any questions that might arise and explaining that examinations would be performed in a way that would guarantee confidentiality.

All investigations were performed at the Department of Clinical Physiology and the Department of Laboratory Medicine, Region Jönköping County, Jönköping, Sweden. To ensure safety, all blood sampling, echocardiographic examinations, and CPETs were carried out by experienced investigators using proven equipment. The participants' questionnaires, blood samples, and test results were all assigned code numbers to protect the confidentiality, and all identifiable information was removed from reports and scientific publications. The completed questionnaires were stored in locked cabinets at Jönköping University; the echocardiographic images were kept at Department of Clinical Physiology, Region Jönköping County; and the biological samples were stored in a registered biobank at Department of Laboratory Medicine, Region Jönköping County.

Obviously, it was possible that previously unknown heart defects would be detected during the examinations. After discussion with a pediatric cardiologist, we decided that even if any abnormality was revealed by the echocardiographic examination and/or the ECG, the participant could still carry out the maximal running test. The pediatric cardiologist considered it unlikely that any extreme pathology would be identified that would prohibit performance of the treadmill test, because such an abnormality would certainly have been detected earlier during the adolescent's life. Moreover, if any abnormality had been found, we would have contacted the pediatric cardiologist, and the adolescent would have been invited to visit the pediatric clinic for further examinations and follow-ups within a week after the detection.

There is some risk of injury during the maximal treadmill test (i.e., the CPET). To reduce that risk, a thick padded mattress was hung on the wall behind the treadmill in case the participants lost their balance and fell backwards during the test, and the treadmill was also equipped with an emergency stop. Furthermore, we emphasized that it is important to stop running on the treadmill before becoming completely exhausted due to the increased risk of falling at the end of the test.

After conclusion of the investigations, all participants were given oral information about the results of their echocardiographic and ECG examinations and their peak oxygen uptake. However, the participants were not informed of the results of their blood tests, because they probably did not have the knowledge necessary to understand the outcome of such analyses.

Results

Characteristics of the subjects

A total of 72 adolescents participated in this project: 41 boys (25 in the active group) and 31 girls (20 in the active group). Demographic characteristics of the participants according to study group and sex are presented in Table 2. Compared with the controls, the participants in the active group showed significantly lower resting HR and higher peak VO_2 ($P = 0.003$ and $P < 0.001$, respectively).

Cardiac dimensions and volumes

At baseline, cardiac dimensions and volumes indexed by BSA were larger in all four chambers in the active group compared with age- and sex-matched controls (Table 3). The values for all the participants were within recommended ranges. The statistical significances between the groups remained even after controlling for sex and age.

Immediately after exercise (i.e., within 1–2 minutes of concluding the treadmill test), structural values for the LV and LA were obtained, including LVEDV, LVESV, and LA volume (all indexed by BSA), and LV length (Table 3). LVEDV did not change from rest to immediately after exercise in either the active or the control group, thus the difference between the two groups persisted even after exercise. LVESV and LA volume decreased immediately after exercise compared to baseline in both groups ($P < 0.001$ for both). For LA volume, the difference between the groups remained immediately after exercise. After 15 minutes of rest, LVEDV and LVESV were on the same level as baseline, while LA volume was still was decreased in both groups. LV length at rest differed between the groups, with higher values in the active subjects. LV length decreased significantly compared to baseline in the active group both immediately and 15 minutes after peak exercise ($P = 0.009$ and $P = 0.017$, respectively), whereas the controls showed no changes in LV length between baseline and after exercise.

Cardiac functions

Systolic function

At rest, LV stroke volume, LVEF, RV s', and TAPSE indexed by BSA were higher in the active group compared with age- and sex-matched controls (Table 4). Also, differences between the two groups were found immediately after exercise with respect to increased LV stroke volume and LVEF in the active group.

In comparisons between rest and immediately after exercise, both groups showed similar changes observed as higher values for LV stroke volume, LVEF, LVGLS, LV strain rate, LV s', RV strain rate, and RVFAC (Table 4). At 15 minutes after exercise, most systolic parameters had returned to baseline, except for LVGLS and MAPSE, which at that time point were even lower than in the resting state, and these findings apply to both the active group and the controls (LVGLS, 20% in both groups; MAPSE, 7.7mm/m^2 in the active group and 7.4mm/m^2 in the controls).

Diastolic function

Considering the diastolic parameters, none of the results at rest differed between the age- and sex-matched groups, except for the mitral E/A ratio, which was higher in the active group (2.5 compared to 2.1 in controls, $P < 0.046$). The A wave could only be measured at rest, that is, it could not be discerned after CPET due to fusion with the E wave.

Within their respective groups, the active and control subjects exhibited similar changes after exercise including higher LA total strain, LV a', mitral E, E/e' ratio, and RV a' compared to the resting state (Table 5). The E/e' ratio was significantly higher in the active group than in the control group both immediately and 15 minutes after the treadmill test ($P = 0.012$ and $P = 0.008$, respectively). After 15 minutes of rest, E/e' was still enhanced in the active group compared to baseline ($P = 0.001$), whereas the controls had returned to the resting value.

Table 2. Demographics of the participants in total and in subgroups according to sex

	Active group	Controls
Number (n)	45	27
Boys	25	16
Girls	20	11
Age (years)	15.7 ± 1.8	15.6 ± 1.8
Boys	15.7 ± 1.8	15.8 ± 1.9
Girls	15.7 ± 1.9	15.4 ± 1.6
Height (m)	1.71 ± 0.09	1.73 ± 0.12
Boys	1.74 ± 0.10	1.76 ± 0.14
Girls	1.67 ± 0.04	1.67 ± 0.06
Weight (kg)	57.5 ± 8.4	61.0 ± 14.8
Boys	59 ± 10	63 ± 18
Girls	55 ± 6	58 ± 8
BMI (kg/m^2)	19.6 ± 1.7	20.2 ± 3.3
Boys	19.3 ± 1.4	20.0 ± 3.7
Girls	19.9 ± 2.0	20.6 ± 2.8
BSA (m^2)	1.67 ± 0.16	1.72± 0.25
Boys	1.71 ± 0.19	1.77 ± 0.31
Girls	1.62 ± 0.09	1.65 ± 0.11
Duration CPET (min)	15.4 ± 1.6	11.6 ± 1.8
Boys	16.4 ± 1.2	12.3 ± 1.9
Girls	14.1 ± 0.9	10.5 ± 0.8
HR at rest (beats/min)	60 ± 10	69 ± 12
Boys	60 ± 11	68 ± 12
Girls	61 ± 10	70 ± 11
HR max (beats/min)	196 ± 8	195 ± 9
Boys	197 ± 9	197 ± 10
Girls	195 ± 7	192 ± 5
Peak VO_2 (L/min)	3.5 ± 0.7	2.7 ± 0.9
Boys	3.9 ± 0.7	3.1 ± 1.1
Girls	3.0 ± 0.3	2.2 ± 0.3
Peak VO_2 (mL/kg/min)	61 ± 7	45 ± 9
Boys	66 ± 5	49 ± 8
Girls	55 ± 4	39 ± 5

Data are presented as mean ± standard deviation. BMI, body mass index; BSA, body surface area; CPET, cardiopulmonary exercise test; HR, heart rate; VO_2, oxygen uptake.

Table 3. Cardiac dimensions and volumes in age- and gender-matched groups (Papers I and II)

	Active group n = 27	Controls n = 27	*P*-value
LVEDV (mL/m²)			
At rest	60 (50–80)	50 (38–72)	**<0.001**
1–2min post exercise	60 (51–85)	52 (40–69)	**<0.001**
LVESV (mL/m²)			
At rest	24 (18–31)	20 (15–29)	**0.002**
1–2min post exercise	16 (11–29)	14 (11–19)	NS
LV length (cm)			
At rest	8.4 (6.9–10)	7.8 (6.0–9.5)	**0.036**
1–2min post exercise	8.1 (7.1–9.4)	7.7 (6.4–9.4)	NS
IVS (mm/m²)			
At rest	3.7 (2.9–4.8)	3.1 (2.5–4.9)	**<0.001**
LVPWT (mm/m²)			
At rest	4.5 (3.8–6.7)	4.0 (3.2–4.9)	**<0.001**
LVID (mm/m²)			
At rest	29 (26–37)	26 (22–33)	**<0.001**
LVM (g/m²)			
At rest	67 (48–100)	45 (37–73)	**<0.001**
RVD1 (mm/m²)			
At rest	23 (19–28)	20 (17–28)	**<0.001**
RVOT prox (mm/m²)			
At rest	16 (14–23)	15 (11–19)	**0.006**
RV area (cm²/m²)			
At rest	15 (11–21)	13 (9–17)	**<0.001**
LA volume (mL/m²)			
At rest	27 (21–36)	19 (14–31)	**<0.001**
1–2min post exercise	17 (10–23)	12 (9–21)	**0.032**
LA diameter (mm/m²)			
At rest	21 (17–27)	19 (12–25)	**0.001**
RA diameter (mm/m²)			
At rest	23 (17–28)	20 (15–27)	**0.008**
RA area (cm²/m²)			
At rest	9.1 (6.6–11.4)	7.2 (5.1–8.7)	**<0.001**

Data are presented as median with the range in parentheses. *P*-values are between active group and controls. All variables except LV length are indexed by body surface area. Boldface denotes statistical significance. NS, not significant: for other abbreviations, see section headed *Abbreviations*.

Table 4. Cardiac systolic function in age- and gender-matched groups (Papers I and II)

	Active group n = 27	Controls n = 27	*P*-value between the groups
LV stroke volume (mL)			
At rest	58 (50–94)	48 (33–87)	**0.001**
1–2min post exercise	69 (58–117)	61 (40–101)	**0.005**
P-value within each group	**< 0.001**	**< 0.001**	
LVEF (%)			
At rest	61 (57–67)	59 (54–67)	**0.036**
1–2min post exercise	73 (66–79)	72 (69–77)	**0.001**
P-value within each group	**< 0.001**	**< 0.001**	
LVGLS (%)			
At rest	22 (19–25)	21 (19–27)	NS
1–2min post exercise	24 (20–27)	24 (18–29)	NS
P-value within each group	**0.001**	**0.015**	
LV strain rate (s^{-1})			
At rest	1.2 (1.0–1.6)	1.3 (1.0–1.6)	NS
1–2min post exercise	2.0 (1.5–2.8)	2.2 (1.5–2.7)	NS
P-value within each group	**< 0.001**	**< 0.001**	
LV s' (cm/s)			
At rest	7.5 (5.3–9.3)	7.8 (6.4–11.3)	NS
1–2min post exercise	10.3 (6.4–14.4)	10.1 (8.1–14.9)	NS
P-value within each group	**< 0.001**	**< 0.001**	
MAPSE index (mm/m^2)			
At rest	8 (6–11)	8 (6–11)	NS
1–2min post exercise	9 (7–11)	9 (7–12)	NS
P-value within each group	NS	**0.002**	
RV strain (%)			
At rest	27 (19–34)	28 (19–33)	NS
1–2min post exercise	23 (16–35)	28 (16–41)	NS
P-value within each group	NS	NS	
RV strain rate (s^{-1})			
At rest	1.4 (1.0–2.2)	1.5 (1.1–2.4)	NS
1–2min post exercise	2.2 (1.5–3.3)	2.4 (1.4–3.4)	NS
P-value within each group	**< 0.001**	**< 0.001**	
RV s' (cm/s)			
At rest	11 (7–15)	10 (8–14)	**0.031**
1–2min post exercise	12 (7–18)	13 (7–17)	NS
P-value within each group	NS	**< 0.001**	
TAPSE index (mm/m^2)			
At rest	12 (7–16)	10 (8–16)	**0.008**
1–2min post exercise	12 (7–15)	11 (7–18)	NS
P-value within each group	NS	**0.002**	
RVFAC (%)			
At rest	42 (36–49)	41 (35–48)	NS
1–2min post exercise	58 (41–69)	56 (43–66)	NS
P-value within each group	**< 0.001**	**< 0.001**	

Data are presented as median with range in parentheses. MAPSE and TAPSE are indexed by body surface area. Boldface denotes statistical significance. NS, not significant: for other abbreviations, see section headed *Abbreviations*.

Table 5. Cardiac diastolic function in age- and gender-matched groups (Papers I and II)

	Active group n = 27	Controls n = 27	*P*-value between the groups
LA total strain (%)			
At rest	39 (31–53)	38 (28–52)	NS
1-2min post exercise	45 (37–61)	43 (27–96)	NS
P-value within each group	**0.001**	**0.034**	
LV e' (cm/s)			
At rest	14 (12–18)	13 (10–15)	NS
1-2min post exercise	13 (9–18)	14 (9–17)	NS
P-value within each group	NS	NS	
LV a' (cm/s)			
At rest	4.7 (2.5–7.2)	4.9 (3.2–8.2)	NS
1-2min post exercise	6.8 (3.6–9.3)	6.5 (4.0–10.6)	NS
P-value within each group	**< 0.001**	**< 0.001**	
Transmitral E-wave (cm/s)			
At rest	102 (76–125)	96 (72–117)	NS
1-2min post exercise	122 (90–159)	115 (75–154)	NS
P-value within each group	**< 0.001**	**0.001**	
Mitral E/e'-ratio			
At rest	7.6 (5.8–9.6)	7.5 (5.7–9.5)	NS
1-2min post exercise	9.0 (7.3–18.4)	8.1 (5.4–11.7)	**0.012**
P-value within each group	**< 0.001**	**0.005**	
RV e' (cm/s)			
At rest	11 (7–16)	11 (8–15)	NS
1-2min post exercise	11 (5–14)	12 (7–20)	**0.031**
P-value within each group	NS	NS	
RV a' (cm/s)			
At rest	6.3 (3.4–10.6)	5.3 (2.3–10.2)	NS
1-2min post exercise	12.2 (5.1–25.1)	13.8 (2.6–27.1)	NS
P-value within each group	**< 0.001**	**< 0.001**	

Data are presented as median with range in parentheses. Boldface denotes statistical significance. NS, not significant: for other abbreviations, see section headed *Abbreviations*.

Associations with cardiac dimensions and/or function

Associations with peak VO_2

At rest, cardiac parameters of all four chambers, such as LVEDV, LVM, RVD1, RVOTprox, LA volume, and RA area, all indexed by BSA, were strongly correlated with peak VO_2 (r = 0.77, 0.74, 0.61, 0.58, 0.78, and 0.78, respectively, and $P < 0.001$ for all). Among the systolic parameters at rest, correlation with peak VO_2 was observed for the RV, including RVFAC (r = 0.42, $P = 0.002$) and TAPSE indexed by BSA (r = 0.26, $P = 0.035$). Among the cardiac variables measured immediately after the treadmill test, septal E/e' (r = 0.34, $P = 0.013$) and LVEDV indexed to BSA (r = 0.67, $P < 0.001$) showed the strongest correlation with peak VO_2. LVEF (r = 0.41, $P = 0.002$) and LA volume indexed by BSA (r = 0.35, $P = 0.010$) were also correlated with peak VO_2, albeit to a lesser extent at the multiple linear regression analysis performed in different steps.

Associations with growth and metabolic factors

IGF-1 was correlated with the following cardiac dimensions ($P < 0.05$ for all): LVID (r = 0.42), LVM (r = 0.34), LA diameter (r = 0.29), LA volume (r = 0.31), RA diameter (r = 0.38), and RA area (r = 0.37). However, when subjects were separated into an active group and an age- and sex-matched control group, the association with IGF-1 was found only in the control group, although the correlation disappeared even in this group after controlling for BSA. The other hormones that were analyzed (i.e., cortisol, IGF-2, FSH, GH, LH, prolactin, and TSH) were not associated with any ventricular or atrial dimension, except FSH, which was correlated with LA diameter (r = 0.29, $P = 0.043$).

Hormones

Resting levels of circulating cortisol, IGF-1, IGF-2, FSH, GH, LH, and TSH for the subjects are presented in Table 6. The levels of these hormones did not differ significantly between the active group and the control group, except for prolactin, which was found at a slightly higher level in the active group ($P = 0.049$). When the participants were separated into subgroups according to sex, the active boys showed higher LH and prolactin levels compared to the untrained boys ($P = 0.031$ and $P = 0.039$, respectively). No other differences were found between the subgroups.

The immune system

Circulating levels of cytokines and chemokines at rest are presented for all participants in Table 6. When studying the entire active group divided into subgroups according to sex, girls showed increased numbers of circulating $CD3^+$ and $CD4^+$ T cells ($P < 0.05$ for both), as well as higher level of spontaneously secreted IL-17 ($P < 0.01$). No differences between the sexes were found regarding spontaneously secreted cytokines such as IL-1β, -5, -6, -8, -10, IFN-γ, and TNF-α or the chemokines CCL2 and CXCL10. The circulating numbers of $CD56^+/CD16^+$ NK cells and $CD19^+$ B cells did not differ between the groups.

Regarding the diabetes-related immune response, compared with the girls, the boys had increased levels of IL-1β and IL-6 after *in vitro* stimulation with the IA-2 peptide ($P < 0.05$ for both). No differences could be noted between the sexes after *in vitro* stimulation with GAD_{65}. Levels of C-peptide and proinsulin were higher in girls than in boys ($P = 0.05$ and $P < 0.05$, respectively). When comparing boys and girls in the control group regarding changes in secreted IL-17 and *in vitro* IA-2-induced IL-1β and IL-6, these girls also had higher levels of IL-17 compared with boys. However, differences between the sexes in diabetes-related immune response could not be found in the controls such as in the active group (Table 7).

Intra- and interobserver variability

Intra- and interobserver variability were assessed by off-line analysis of selected echocardiographic parameters: LVEDV, LVESV, LA volume, RA area, and RV strain of the lateral free wall of the RV. Pearson's correlation values for the listed parameters were, respectively, 0.97, 0.92, 0.98, 0.91, and 0.75 for intraobserver variability, and 0.95, 0.88, 0.93, 0.96, and 0.75 for interobserver variability. The corresponding values for the intraobserver intraclass correlation coefficient were 0.83, 0.67, 0.94, 0.91, and 0.74, and for the interobserver interclass correlation coefficient 0.93, 0.79, 0.92, 0.94, and 0.72.

Table 6. Circulating levels of hormones, cytokines, and chemokines at rest, spontaneously secreted, and stimulated by IA-2

	Active group		Controls	
	Boys (n = 24)	Girls (n = 20)	Boys (n = 14)	Girls (n = 10)
Cortisol (nmol/L)	141 (40–312)	172 (48–360)	120 (33–286)	108 (37–330)
IGF-1 (ng/mL)	28 (11–47)	21 (7.6–51)	25 (14–48)	19 (4–29)
IGF-2 (ng/mL)	1.1 (0.0–25)	0.0 (0.0–5.0)	0.0 (0.0–8.0)	0.0 (0.0–3.0)
FSH (mLU/mL)	5.5 (1.8–17)	7.4 (2.1–18)	4.1 (1.4–9.1)	6.3 (3.8–12)
GH (ng/mL)	1.5 (0.1–26)	1.2 (0.5–12)	3.2 (0.2–29)	1.8 (0.5–14)
LH (mLU/mL)	1.5 (0.0–12)	2.0 (0.0–28.0)	0.7 (0.0–4.6)	2.0 (0.6–6.1)
Prolactin (ng/mL)	1.1 (0.1–4.8)	0.8 (0.3–5.5)	0.9 (0.0–4.3)	0.9 (0.0–2.8)
TSH (μLU/mL)	3.5 (0.9–11)	2.6 (0.8–28)	2.8 (1.2–12)	2.5 (1.3–9.8)
IL-1β (pg/mL)	0.0 (0.0–0.3)	0.0 (0.0–6.3)	0.0 (0.0–0.5)	0.0 (0.0–1.1)
IL-5 (pg/mL)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-6 (pg/mL)	0.0 (0.0–0.0)	0.0 (0.0–3.3)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-8 (pg/mL)	152 (20–726)	203 (0.0–3715)	47 (12–274)	124 (8–3086)
IL-10 (pg/mL)	0.0 (0.0–0.9)	0.0 (0.0–1.4)	0.0 (0.0–0.7)	0.0 (0.0–0.3)
IL-17 (pg/mL)	0.0 (0.0–3.2)	1.3 (0.0–5.1)	0.0 (0.0–3.2)	0.8 (0.0–4.2)
IFN-γ (pg/mL)	0.0 (0.0–16)	0.0 (0.0–13)	0.0 (0.0–8.2)	0.0 (0.0–3.1)
CCL2 (pg/mL)	0.0 (0.0–19)	2.6 (0.0–49)	1.57 (0.0–15)	7.8 (0.5–24)
TNF-α (pg/mL)	0.0 (0.0–8.3)	0.0 (0.0–6.4)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-1β by IA-2 (pg/mL)	1.3 (0.2–6.3)	0.7 (0.0–28)	0.9 (0.2–6.1)	0.9 (0.2–9.1)
IL-5 by IA-2 (pg/mL)	0.0 (0.0–8.7)	0.0 (0.0–2.7)	0.0 (0.0–0.0)	0.0 (0.0–2.7)
IL-6 by IA-2 (pg/mL)	3.8 (0.0–8.7)	1.7 (0.0–23)	2.8 (0.0–5.8)	2.8 (0.6–8.7)
IL-8 by IA-2 (pg/mL)	616 (199–1295)	452 (30–1943)	309 (120–854)	290 (95–1871)
IL-10 by IA-2 (pg/mL)	0.0 (-0.9–5.3)	0.0 (–1.4–1.7)	0.0 (–0.7–1.7)	0.0 (0.0–3.8)
IL-17 by IA-2 (pg/mL)	0.0 (–2.3–9.7)	–0.2 (–4.6–23)	0.0 (–3.2–22)	0.0 (–2.3–24)
IFN-γ by IA-2 (pg/mL)	0.0 (–16–0.0)	0.0 (–13–0.0)	0.0 (–8.2–0.0)	0.0 (–3.1–0.0)
CCL2 by IA-2 (pg/mL)	30.8 (0.0–101)	25.7 (–3.2–425)	32.0 (0.0–141)	46.5 (–2.6–104)
TNF-α by IA-2 (pg/mL)	0.0 (–8.3–13)	0.0 (0.0–10)	0.0 (0.0–13)	0.0 (0.0–8.4)

Data are presented as median with the ranges in parentheses. All abbreviations are explained in the section headed *Abbreviations*.

Table 7. Regressions analysis of differences between boys and girls with regard to three cytokines

	Active group	*P*-value	Controls	*P*-value	*P*-value*
IL-6 by IA-2	–0.30	**0.009**	0.008	0.582	**0.040**
IL-1β by IA-2	–0.72	**0.003**	0.19	0.538	**0.020**
IL-17	3.67	**0.002**	2.77	**0.080**	0.637

Girls compared to boys are presented as unstandardized beta values for the active group and the control group. *Last column presents the *P*-value showing the interaction between sex and each of the cytokine, i.e. if the difference between boys and girls are significantly different between active and controls. Boldface denotes statistical significance. IL, interleukin; IA-2, tyrosine phosphatase.

Discussion

This thesis presents the results of examinations of adolescents who had practiced intense regular endurance exercise for years, with focus on cardiac size and function, hormones, and the immune system at rest, and partly considered in relation to a maximal exercise test. The findings included here show considerable biatrial and biventricular anatomic remodeling and functional remodeling of the RV at rest in endurance-trained adolescents compared with untrained controls (Paper I). These observations indicate that adolescents adapt well to the same type of endurance training routine as performed by adults, because the cardiac morphological changes in all four heart chambers that have previously been demonstrated in athletic adults could also be shown in adolescents. The current results also revealed that the cardiac response to a peak exercise test was similar in endurance-trained and untrained adolescents (Paper II). Temporal cardiac changes were seen in both systolic and diastolic echocardiographic parameters, as well as cardiac volumes, which suggests that the functional response to an exercise bout is not affected by the degree of endurance exercise practice. However, the diastolic function was enhanced to a larger extent during exercise in the endurance-trained subjects than in the controls. Moreover, the results reported in Papers I and II showed positive associations between peak VO_2 and cardiac dimensions and functions both at rest and after exercise. These data suggest that much of the variation in peak VO_2 can be explained by a combination of increased cardiac size and enhanced filling pressure.

As outlined in Paper III, when not controlling for body size, an association between circulating growth factor IGF-1 at rest and cardiac dimensions could not be detected in the active group but was found in the untrained subjects. We can only speculate that the regular increase in volume load achieved by exercise and/or the autocrine impact of locally synthesized IGF-1 from the myocardial cells during exercise may have a greater effect than circulating growth factors at rest, considering the cardiac growth that occurs in athletic adolescents.

In the fourth study (Paper IV), we analyzed immune responses exclusively in the endurance-trained subjects and found that the response to a T1D-related autoantigen was increased in boys compared with girls. This implies that the immune response may differ in relation to gender in a young athletic population. The girls however had increased secretion of C-peptide and proinsulin compared with the boys which may suggests that intense regular endurance training can have an impact on the pancreatic β-cells during adolescence.

Exercise and health

Young competitive athletes are generally considered to be healthy individuals with a unique lifestyle, who seem to be invulnerable and often also capable of extraordinary physical performance. The physiological adaptation to exercise or to mechanical load occurs through neurohormonal activation and growth stimulation, and this may lead to cardiac hypertrophy, which is regarded as a benign remodeling when it arises in response to exercise (Maisch, 2015). Still, can you get too much of a good thing? Is it possible that regular intense exercise harms the heart? Clinical knowledge of the athlete's heart has expanded considerably in recent years as a result of greater accessibility to large populations of trained athletes. Consequently, there is increased recognition of the impact that prolonged endurance exercise has on cardiac remodeling, which eventually may mimic certain pathological conditions that can potentially lead to sudden cardiac death or disease progression (B. J. Maron & Pelliccia, 2006). It is also plausible that healthy individuals who are engaged in strenuous endurance exercise can exhibit transient cardiological features that are associated with cardiac diseases. Therefore, it is important to be aware that in most cases these alterations represent transient physiological responses rather than pathological status (Sanchis-Gomar et al., 2016).

No randomized controlled trials have assessed the health effects of intense prolonged exercise, neither has any definitive evidence of harm been demonstrated. Thus, studies using end points and preclinical data to evaluate cohorts of adults have suggested that prolonged intense endurance exercise (approximately 15–40 hours per week) can be associated with an excess of arrhythmias (La Gerche & Heidbuchel, 2014). However, there are no results indicating that remodeling of the athlete's heart leads to any disease progression, cardiovascular disability, or sudden cardiac death (B. J. Maron & Pelliccia, 2006).

The most frequent cause of sudden cardiac death among young athletes is hypertrophic cardiomyopathy, but this is extremely rare (Chandra, Bastiaenen, Papadakis, & Sharma, 2013). Systematic pre-participation screening in young athletes has the potential to identify those who are at risk of this condition and thus reduce mortality. However, there is significant debate among cardiologists in Europe and the United States about such screening, with regard to its efficacy, the impact of false-positive results, and the cost-effectiveness of this assessment. The evidence available to date indicates that ECG screening is an efficient health strategy to prevent sudden cardiac death in young athletes (aged ≤ 35 years). The pre-participation screening should also cover family history of cardiovascular diseases, personal history with aspects such as chest pain and/or syncope during exercise, and physical examination including detection of heart murmur. It is recommended that the screening be repeated every second year, and that it instructs the athletes to avoid exercise during infections to reduce the risk of myocarditis (Corrado et al., 2011; Fritsch et al., 2017).

In summary, compared with inactivity, exercise offers numerous benefits that indeed outweigh any potentially negative effects. Nevertheless, the possibility that intense endurance exercise offers a dose-response benefit compared with less intense exercise is more uncertain. No dose-related harm of exercise has been convincingly

demonstrated, which means that health professionals should not have the overall impression that strenuous endurance exercise is either hazardous or shortens life expectancy (Sanchis-Gomar et al., 2016).

Discussion of the results focused on the heart

It is generally assumed that the heart of trained athletes shows a benign increase in cardiac mass involving specific circulatory and cardiac morphological alterations, and that this represents a physiological adaptation to systematic training (B. J. Maron & Pelliccia, 2006). Considering cardiovascular parameters, children and adolescents differ from adults at both submaximal and maximal levels of exercise, possibly due to the smaller heart and blood volume, greater stimulation of peripheral chemoreceptors, and a lower responsiveness of β-adrenergic receptors (de Prado, Dias, & Trombetta, 2006). The research presented in this thesis aimed to elucidate the aspects of the adolescents' perspective.

Left ventricle

Larger LV dimensions and volumes in the active group compared with the controls were reported in Paper I, which agrees with previous studies of both adolescent and adult athletes (Hedman et al., 2015; Sharma et al., 2002; Utomi et al., 2013). There is a general consensus in the literature that LVM is increased in an athletic population, and that this can be attributed to increases in both wall thickness and cavity size of the LV as a consequence of prolonged repetitive volume and pressure overload (Naylor, George, O'Driscoll, & Green, 2008). It has also been established that, despite the significant changes in anthropometry that occur during maturation, young athletes have significantly larger cardiac diameters, wall thicknesses, and LVM than young non-athletes even after adjusting for age (McClean et al., 2018). None of the participants in our studies exceeded the upper limits of maximal wall thickness ≥ 12 mm or LVID ≥ 55 mm. Values above these limits in trained children and adolescents should be

viewed with suspicion, particularly in the young athletes who also are present with a family history of sudden cardiac death and/or abnormal ECG results, such as T wave inversion, tachyarrhythmias, and prolonged QT interval (McClean et al., 2018; Sharma et al., 2018).

As outlined in Papers I and II, no differences in LV systolic functions were found between the active participants compared with the controls, except for a higher stroke volume both at rest and after exercise in the active group. According to a literature review (Armstrong et al., 2011), earlier studies have consistently reported larger stroke volume in trained children and adolescents compared with less trained equals, indicating that the higher peak VO_2 in young athletes is a function of enhanced stroke volume. It seems reasonable to suggest that regular exercise can enhance stroke volume through a more effective peripheral muscle pump and/or plasma volume expansion (i.e., by achieving an increased venous return), although empirical support for this hypothesis has not yet been established.

In standing posture, blood volume in the legs increases. Upon onset of upright leg exercise, this blood is mobilized by the skeletal muscle pump, leading to increased central circulation and thereby increased stroke volume. Rowland et al. (Rowland et al., 2009) did not observe any change in stroke volume in children performing a progressive exercise test in a prone position on a specially constructed swim bench. Those investigators pointed out their failure to observe a pattern of 20–40% increased stroke volume, as is typically seen at the onset of upright leg exercise, which supports the concept that increased stroke volume during upright exercise actually represents a refilling phenomenon rather than a contribution to the circulatory demands of exercise. The participants in our project performed a maximal exercise test upright on a treadmill, whereas all echocardiographic examinations were done with the subjects in a supine position, which may have affected the stroke volume values. Nevertheless, as described in Paper II, we found that both the active and the control group showed significantly increased stroke volume

immediately after exercise compared to baseline, which indicates that the refilling phenomenon that is supposed to occur during upright exercise will continue to influence the stroke volume for a certain amount of time after completion of physical activity, irrespectively of body position.

Conflicting results have been reported concerning the pattern of stroke volume during progressive exercise in endurance-trained athletes. Some researchers have found an initial rise at the onset of exercise and thereafter a plateau persisting until exhaustion in both endurance-trained and sedentary subjects (Whipp, 2010). However, other investigators have noted a continuous rise in stroke volume until exhaustion in endurance-trained athletes, and this non-plateau pattern was suggested to be due to an augmented diastolic filling mechanism that relies on the Frank-Starling mechanism (Rowland, 2009). Nonetheless, this topic was outside the scope of our studies, because we collected echocardiographic data before and after, not during, the exercise test.

When comparing temporal changes in LV systolic function within each group before and after exercise (Paper II), we found that both the active group and the controls showed similar patterns with higher LVEF, strain, strain rate, and s' of the LV immediately after exercise compared to baseline. These findings were expected, because cardiac output is increased during exercise, an observation that has also been made in previous studies of adults and youngsters (Cifra et al., 2016; La Gerche, Burns, Taylor, et al., 2012). One of those investigations (Cifra et al., 2016) also showed that systolic velocities in the lateral wall and septum, as well as strain, increased linearly with increasing HR in the pediatric cohort under evaluation. However, available echocardiographic data obtained during exercise are limited for children and adolescents, compared with adults. Moreover, two of our studies (Papers I and II) revealed only a marginal difference in LV diastolic function (seen as an increased E/A ratio in the active group) between the active participants and the controls at baseline. Together,

these findings indicate that regular endurance exercise has less effect on the function of the LV at rest than on the dimension parameters of the LV, which concurs with a previous small study focused on the LV in prepubertal children practicing endurance exercise (Obert et al., 1998). Furthermore, a study of adults under resting conditions showed altered LV diastolic properties including decreased A wave and increased E/A ratio in highly trained athletes compared to controls (Caselli, Di Paolo, et al., 2015). Thus, it is possible that the adolescents in our studies had not been practicing endurance exercise long enough to develop detectable changes in LV diastolic function at rest.

Considering diastolic functional response to exercise (Paper II), the physically trained and untrained adolescents exhibited a similar pattern, for example, seen as higher values of mitral E wave, LV a', and E/e' ratio immediately after exercise compared to the resting levels. In other words, the exercise-induced E/e' enhancement was not unique to the athletes, although the E/e' ratio was significantly higher in the active group than in the controls. Overall, there was an enhancement of diastolic function in both groups, which appeared to be more pronounced in the endurance-trained athletes. Very few studies have evaluated diastolic function during and after exercise in young individuals, in particular regarding comparison of trained and untrained subjects. Punn et al. (Punn et al., 2012) have reported an unaltered E/e' ratio after exercise compared to baseline in healthy children and adolescents not divided into groups based on exercise history, whereas another study of subjects of similar age found a higher E/e' at peak exercise (Cifra et al., 2016).

When assessing diastolic dysfunction, determination of the presence of elevated LV filling pressure is recommended as a first step. Inasmuch as the value of E/e' reflects the LV diastolic filling pressure, it might be assumed that in our subjects a diastolic dysfunction would have occurred in response to exercise, and been more pronounced in the active group. However, although determination of the E/e' ratio is

recommended to simplify the assessment, diastolic function should always be interpreted in a wider context that includes clinical status and other 2D and Doppler parameters, such as annular e' velocity, LA volume index, and peak tricuspid regurgitation velocity (Nagueh et al., 2016). Therefore, in the evaluation of our subjects, the higher E/e' in response to exercise was described as an enhanced diastolic function and not as a diastolic dysfunction.

Right ventricle

In the study reported in Paper I, the RV dimensions including RVD1, RVOT prox, and RV area (all indexed by BSA) were larger in the active group than in the controls, results that are similar to those recorded for the LV. The structural remodeling of the RV in response to regular exercise is a physiological process that is affected by the type and volume of the exercise performed. Other investigations have found RV dimensions and volumes to be larger in endurance-trained athletes compared to less trained controls and athletes based on strength disciplines (D'Andrea et al., 2013; Utomi et al., 2013). However, those observations were made on adult populations, and the indicated aspects have rarely been studied in young athletes.

In our studies, the values for the systolic parameters RV s' and TAPSE at rest were higher for the active adolescents than for the controls, whereas RV total longitudinal strain and RVFAC did not differ between the two groups. This might simply be interpreted as the similar changes in the left side of the heart: larger dimensions require higher wall velocity but not necessarily greater strain or an increased right ventricular ejection fraction. Previous studies of adults have suggested that regular exercise has the greatest cardiac impact on the RV (La Gerche, Burns, Mooney, et al., 2012; Scharhag et al., 2002; Simsek, Tas, Gunay, & Degirmenci, 2013). In addition, La Gerche et al. (La Gerche, Burns, Mooney, et al., 2012) concluded that RV remodeling was more prevalent in athletes with a longer history of competition in endurance sports, which might explain the difference in the extent to which the RV is affected in athletic adolescents

compared to adults, because the former obviously have a shorter history of exercise. Recently, D'Ascenzi et al. (D'Ascenzi et al., 2017) studied male pre-adolescent athletes and proposed that intensive endurance training might influence the growing heart by leading to an additive increase in RV dimensions but with maintained normal systolic and diastolic function. This suggestion supports the hypothesis of a purely physiological adaptation of the heart and is consistent with the results obtained in our studies. Still, the presence of a dilated RV in children and/or adolescents raises suspicion of an underlying cardiovascular disease. Nonetheless, in asymptomatic endurance-trained athletes with a negative family history of cardiomyopathies and/or sudden cardiac death, an increase in RV dimensions associated with normal RV function probably represents a physiological expression of the athlete's heart that should not be misinterpreted or expressed as an incipient RV cardiomyopathy.

In Paper II, the systolic and diastolic response of the RV to the maximal exercise test showed that similar changes occurred in the groups with higher values for RV strain rate, RV s' (however not significant in the controls), RVFAC, and RV a' immediately after exercise. These findings are consistent with the results of a previous study of a healthy pediatric cohort in which the subjects however were not divided into well-trained and less trained (Cifra et al., 2016). Other investigations of RV response to exercise have been conducted exclusively on adults. La Gerche et al. (La Gerche et al., 2011) used a combination of magnetic resonance and echocardiography and found that adult endurance-trained athletes showed exercise-induced increases in RV end-systolic wall stress and volume and a lower RV ejection fraction compared with the same parameters for the LV. The data published by these authors also concur with the conclusion that structural and functional remodeling of the RV, such as RV enlargement and reduced RV ejection fraction, should be expected in athletes and should not necessarily be interpreted as a sign of pathology.

The pulmonary circulation and accordingly also the pulmonary artery systolic pressure increase substantially during exercise, which may create a significant burden on the contractile reserve of the RV and thereby possibly limit the output from that chamber (D'Andrea, La Gerche, Golia, Padalino, et al., 2015; La Gerche et al., 2011). However, it is suggested that the healthy RV has a contractile reserve to meet this demand, at least for a while, because it appears that the systolic function increases proportionally to the increase in pulmonary artery pressure during intense exercise of short duration. On the other hand, prolonged exercise such as a marathon race or an ultra-endurance triathlons may induce cardiac fatigue during which the RV seems to be more susceptible than the LV, indicating that the magnitude of these effect may be related to both the intensity and the duration of the exercise performed (D'Andrea, La Gerche, Golia, Padalino, et al., 2015; Oxborough et al., 2011).

Atria

We observed biatrial enlargement at rest in terms of greater diameters and volume/area indexed by BSA in our physically active participants compared with the control group, which is consistent with previous findings in both adolescent and adult athletes (D'Andrea et al., 2010; D'Ascenzi et al., 2015; D'Ascenzi et al., 2016; McClean et al., 2015; Sharma et al., 2002). This suggests that both the ventricles and the atria will become morphologically adapted during the early phases of the sports career of an athlete. Increased size of the LA could potentially be caused by either LV dysfunction or by regular increases in venous return to the atrium due to exercise (D'Ascenzi et al., 2016). Also, atrial enlargement has been shown to be an indicator of underlying LV pathology such as hypertrophic cardiomyopathy (Elliott et al., 2014), but, considering that no pathological changes in LV structures or functions were observed in the active group in our project, our findings in LA can most likely be explained by the venous-return mechanism. In addition, data given in Paper I emphasized that the adaptation exhibited by the RA is similar to that shown by the LA, which supports the concept that long-term dynamic

training contributes to a physiological biatrial hypertrophy. Hence atrial enlargement in athletes is probably related to the sustained elevation in preload that occur during dynamic training.

McClean et al. (McClean et al., 2015) studied adult subjects and noted that the RA/LA ratio for structural dimensions was increased in both athletes and untrained controls, indicating that the RA was larger than the LA. A larger diameter of the RA than the LA was also seen in the adolescents included in our project, although this difference was not statistically significant in either the active or the control group. McClean et al. speculated that the dilatation of the RV induced by regular endurance exercise may protect the RA and the venous system from any relative elevation in afterload. Clearly, further research is needed to assess structure and pressure of the RA during exercise to identify the mechanisms involved in this process. It is important to understand the exercise-related biatrial remodeling and changes that occur in that context when considering biatrial size as a marker of cardiac anatomical and functional pathology, or to address suspicion of a pulmonary hypertension independent of tricuspid regurgitation velocity (D'Ascenzi et al., 2016).

In Paper II, the adolescents in both the active group and the control group showed increased LA strain immediately after exercise compared with the level at rest. This observation agrees with data on adults reported by Wright et al. (Wright, Esfandiari, Elmayergi, Sasson, & Goodman, 2014), who regarded these findings as indicating that improvement in relaxation characteristics during the reservoir phase may support the early diastolic LV filling during exercise, and that this is independent of immediate LV systolic adaptation. D'Ascenzi and coworkers (D'Ascenzi et al., 2011) also demonstrated a shift in the pattern of the ventricular filling period toward early diastole, which included a more rapid passive atrial emptying in athletes compared with controls. These investigators suggested that this phenomenon is probably related to an increasing flexibility and elasticity of the LV muscle and increased myocardial distensibility at

the end of diastole in athletes, which in turn leads to increased stretching of the myocardial fibers and consequently an increase in LV stroke volume that is appropriate for the athletic heart. The functional role of the LA in an athlete's heart is often neglected, and no conclusive data in that context are currently available. Research in this field is essential, because it can contribute to the differential diagnosis of cardiomyopathies, and the data obtained should be interpreted as representing a physiological adaptation to regular intensive exercise.

Peak VO_2 related to cardiac dimensions and functions

The findings reported in Paper I show strong correlations between peak VO_2 and cardiac structure indexed by BSA, including LVEDV, LVM, RV dimensions, LA volume, and RA area, as well as cardiac functions of RV. In addition, according to the results obtained immediately after exercise reported in Paper II, LA volume and LVEF were positively associated with peak VO_2, although the strongest correlations with peak VO_2 were found for LVEDV and E/e'. These findings agree with previous data suggesting that a combination of ventricular volume, mass, and heart rate reserve can explain much of the variance in $VO_{2max,}$ which implies that the extent of structural remodeling may be a major modifiable factor that affects the athletic performance (La Gerche, Burns, Taylor, et al., 2012). Furthermore, the strong correlation between peak VO_2 and atrial size demonstrated in Papers I and II suggest that the remodeling of the atria seen in the active group is at least as important as the increase in ventricular size.

In a systematic review of longitudinal studies of trained and untrained adolescents, Armstrong et al. (Armstrong et al., 2011) concluded that, in addition to chronical age, both growth and maturation positively and independently influence VO_{2max}. Therefore, it is possible that applying the approach of scaling cardiac dimensions by body size can aid comparison of cardiac size in subjects of different sex and age. However, in Paper I, the relationships of cardiac dimension and function parameters with peak VO_2 were essentially the same regardless of whether the data were or were not controlled for age and

sex. Moreover, Armstrong et al. (Armstrong et al., 2011) also confirmed that habitual physical activity rarely reaches the intensity and duration of activity associated with increased VO_{2max}, which has previously been attributed to sex differences in VO_{2max} during adolescence, when boys are generally more active than girls.

Hormones and their relationship with cardiac dimensions and functions

In the study outlined in Paper III, no differences between the groups could be found regarding resting levels of circulating hormones including cortisol, IGF-1, IGF-2, FSH, GH, LH, and TSH, but with the exception of prolactin, which was slightly increased in the active group compared with the controls. Based on the knowledge that hormones influence the regeneration phase after exercise by modulating anabolic and catabolic processes, it has been assumed that measurement of the concentrations of several hormones in the blood can aid determination of the adequate dose-response to physical stress after regeneration periods (Jurimae, Maestu, Jurimae, Mangus, & von Duvillard, 2011). However, these hormone responses are not always specific and do not closely reflect the amount of physical stress in an athlete during a period of heavy training compared to a period of reduced training. Taking this into account may amplify the results regarding undetected differences between the groups in Paper III. It has also previously been reported that basal hormone levels are not good predictors in athletes with overtraining syndrome such as decreased performance and fatigue (Cadegiani & Kater, 2017).

The mediators of pathological cardiac hypertrophy, including catecholamines and natriuretic peptides, differ markedly from the mediators of physiological cardiac hypertrophy in athletes, such as GH and growth factor IGF-1. Furthermore, chronic versus intermittent nature of the load excess may be a plausible explanation for these different mechanisms (Prior & La Gerche, 2012). In Paper III, resting levels of IGF-1 that were not controlled for any confounding factor were not correlated with greater cardiac dimensions in endurance-

trained adolescents but, notably, were associated with such extended dimensions in the untrained controls, although this relationship disappeared when controlling for BSA. Clearly, it seems that resting levels of IGF-1 are not involved in the cardiac enlargement seen in endurance-trained adolescents. The increase in cardiac dimensions despite the lack of higher levels of circulating GH and/or IGF-1 in the active group compared to controls may confirm that the increase in volume load that occurs during exercise is an important and significant stimulus. In addition, regular endurance exercise may stimulate a paracrine/autocrine hormone effect that differs from systemic effects, such as the release of locally synthesized IGF-1 from myocardial cells during exercise sessions.

Blood samples were collected from the subjects in our cohort only once, at rest at least one day after training, and thus it was not possible to evaluate the intermittent changes in hormone levels in relation to exercise. Still, our findings in subjects at rest do concur with the results of a previous study that showed no differences in IGF-1 levels between young elite athletes and sedentary controls (Ubertini et al., 2008). However, the authors of that investigation did find higher levels of GH in the athletes who performed very intense training (> 12 hours/week), which is contrary to the data given in Paper III. In our study, we did not separate the active group into subgroups according to hours of training, an aspect that may have covered increased levels of GH in the subjects who trained most extensively. It is possible that lack of differences in levels of IGF-1 between the two groups and lack of correlation between IGF-1 and GH can be attributed to autocrine/paracrine secretion of IGF-1 in muscles during exercise without modification of circulating levels of this hormone (Neri Serneri et al., 2001; Ubertini et al., 2008). Ubertini et al. (Ubertini et al., 2008) found no differences in GH between amenorrhoeic and eumenorrheic females or between subjects under and above the age of 16 years, which suggests that the GH–IGF-1-axis is active in similar ways in both prepubertal and pubertal individuals.

Reduced IGF-1 associated with exercise has been observed in highly trained adolescents involved in sports such as wrestling or gymnastics in whom the training program caused a loss of body mass that led to a negative energy balance. Even when the energy balance is maintained in weight-stable subjects, exercise training in itself may lead to small yet significant reductions in IGF-1 levels. However, longer periods of training (e.g., > 18 months) have indeed been found to be associated with stable or increasing circulating levels of GH and IGF-1. Eliakim and Nemet (Eliakim & Nemet, 2010) have suggested that this effect is an adaptation of these growth factors that is induced by regular exercise. In their review, Eliakim and Nemet also reported that IGF-1 levels in elite adolescent athletes are decreased during periods of heavy training but return to baseline upon tapering of training intensity. Those investigators also found no changes in IGF-1 levels in subjects participating in types of sports that entailed training that was not planned for a specific target, or in subjects who exercise of essentially the same intensity throughout the season, which agrees with the results regarding the active group in our project.

Discussion of the results with focus on the immune system

The effect of exercise on the immune system comprises both an acute phase response and a prolonged response (Schild et al., 2016). The data reported in Paper IV exclusively represent the endurance-trained participants and their blood samples collected at least one day after exercise, and thus we assume that these observations reflect a recovery phase and accordingly show the prolonged response to exercise.

Levels of $CD3^+$ and $CD4^+$ T cells were found to be increased in girls compared with boys. A single exercise bout normally leads to a higher level of leukocytes that can persist into exercise recovery. However, despite prolonged and/or high-intensity exercise, lymphocytes numbers usually decrease within as little as 30 minutes (Peake, Neubauer, Walsh, & Simpson, 2017). Accordingly, the sex differences

in levels of $CD3^+$ and $CD4^+$ T cells demonstrated in Paper IV may not have been related to different effects of exercise. Still, the results of that study are in contrast to the findings of an investigation of children and adolescents that showed no sex differences in lymphocyte numbers at rest or 60 minutes after 1 hour of cycling (Timmons, Tarnopolsky, Snider, & Bar-Or, 2006). In any case, our findings may be explained by a report showing that, in the general population, women have higher levels of $CD4^+$ than men (Gleeson, Bishop, Oliveira, McCauley, & Tauler, 2011). Another theory is that oral contraceptive use and/or menstrual cycle may influence the immune system in girls, because a previous review suggested that these factors can increase the magnitude of changes in the immune system, including development of lymphocytosis in response to exercise (Timmons, Hamadeh, Devries, & Tarnopolsky, 2005). However, this issue was beyond the scope of this thesis, because the questionnaire we employed did not include items about puberty or use of contraceptives, which was a shortcoming in Paper IV.

It can also be noted that level of spontaneously secreted pro-inflammatory IL-17 was higher in girls than in boys in the study outlined in Paper IV, whereas no other sex differences were found for the other cytokines and chemokines that were analyzed in our project. Research on endurance-trained adolescents is lacking with regard to sex differences in circulating levels of these proteins at rest, although, according to a review published by Gillum el al. (Gillum, Kuennen, Schneider, & Moseley, 2011), plasma levels of multiple pro- and anti-inflammatory cytokines generally increase initially in response to exercise with no differences reported between the sexes. Nonetheless, Gillum and colleagues also reported that individuals who perform long-term and regular exercise instead show decreased cytokine production during an acute bout of exercise, which may contribute to immunosuppression that lead to greater risk of infections. However, this decrease in inflammation may also constitute a key link between exercise and health by potentially reducing the risk of chronic diseases.

Timmons et al. (Timmons et al., 2006) have speculated that the cytokine response to exercise may be lower in children than in adolescents. These investigators proposed that the reason that children are relatively resistant to major inflammatory responses to exercise is to minimize the disruption of anabolic mediators such as IGF-1. This implies that growth mediators may be particularly sensitive to acute changes in inflammatory-related cytokines, which would be conductive to optimal adaptation and muscle growth. Moreover, in adolescents, Eliakim and Nemet (Eliakim & Nemet, 2010) found evidence that an exercise session may lead to a simultaneous increase in antagonistic mediators such as anabolic components of the GH–IGF-1 axis and catabolic pro-inflammatory cytokines. These researchers proposed that assessment of these changes in antagonistic circulating mediators may aid quantification of the effects of different types of prolonged exercise training and recovery modalities. The authors also suggested that the children who trained most intensively and show the greatest increase in fitness will also exhibit the largest increase in circulating levels of pro-inflammatory cytokines. Notwithstanding, few studies have examined the exercise-induced levels of these anabolic/catabolic hormones in elite athletes at different time points throughout the competitive season. Thus, further research is needed to clarify the hormonal response to different phases of training, as in "a real life" setting.

Notably, an increased immune response to the diabetes-associated autoantigen IA-2 was found in boys but not in girls in the study reported in Paper IV. Due to the design of that investigation, it is not possible to conjecture about any exact cause of this augmented response in boys. To our knowledge, there is no evidence that increased requirements for insulin or abnormal stress for the β-cells during exercise can explain our findings in this context. However, an immune response to a diabetes-related autoantigen such as IA-2 indicates that a previous immune exposure has occurred. In addition, when we compared differences in the response to IA-2 between boys and girls in the active group with such differences in the control subjects, we found that such disparities were apparent only in the

active group. This indicates that endurance-trained boys may actually have an enhanced immune response to a T1D-related autoantigen that is not seen in untrained boys. Research conducted thus far has suggested that long-term stress leads to chronically higher levels of glucocorticoids such as cortisol, which may result in abnormal function of β-cells. This in turn may lead to hyperinsulinemia that can eventually cause exhaustion of β-cell either through reduced proliferation or increased apoptosis. On the other hand, intermittent stress such as occurs during regular exercise has been demonstrated to preserve β-cell function and promote cell proliferation and thereby provide a protective effect against development of diabetes (Beaudry & Riddell, 2012). Furthermore, in a study conducted by Knip and Siljander (Knip & Siljander, 2008), it was deducted that positive results for only a single autoantibody specificity did not reflect progressive β-cell autoimmunity. In addition, our findings in Paper IV show that levels of the circulating diabetes parameters C-peptide and proinsulin were higher in girls than in boys, which suggests that any impact on the β-cells also occurs in girls. Together, the data in Paper IV indicate that an effect on β-cells may occur in endurance-trained adolescents, although this possibility must be further investigated.

Methodological considerations

The period of adolescence is difficult to define in chronological years, because it varies with both onset and termination. For most girls, adolescence ranges from age 8 to 19 years, and for most boys from 10 to 22 years (Kenney et al., 2015). The present research was performed on adolescents aged 13–19 years, which theoretically means that some of the subjects may have been in a pre-pubertal phase. Thus, a weakness of our investigations is that we did not take into account maturity status or Tanner stages. In Papers I and II, cardiac dimensions were indexed by BSA to control for differences in body size, and in Paper III the results were presented both with and without controlling for BSA. Furthermore, the study underlying Paper III showed that hormones responsible for inducing puberty (e.g., LH and

FSH) did not differ at group level between the endurance-trained and the control group, which suggests comparable pubertal status in the two groups.

The inclusion criterion for the active group was regular and intense performance of endurance exercise at an elite level for at least 2 years, and this limit was set to ensure inclusion of the younger participants. In reality, the older subjects had been practicing such exercise for much longer than 2 years, and indeed most of the participants in the active group had performed regular endurance training since childhood. However, it was difficult to define an exact time point when the training reached the elite level, although most of the active subjects began competing in their sports at the age of 9–10 years.

The selection of an appropriate exercise test protocol for assessing exercise capacity is important. We used the Bruce protocol, which is recommended when > 12 minutes of exercise is expected (Balady et al., 2010). We chose this protocol, because we wanted to use the same approach for all participants regardless of exercise history, sex, or age. Alternatively, we could have used individualized ramp protocols tailored to each individual subject to yield a fatigue-limited exercise duration of approximately 8–12 minutes. However, if the duration of exercise exceeds 12 minutes when using such protocols, the subjects may terminate exercise due to specific muscle fatigue or orthopedic factors rather than cardiopulmonary end points (Balady et al., 2010), and to avoid that possibility we chose the Bruce protocol.

We analyzed peak VO_2 in relation to whole body mass (kg^{-1}), which is the traditional and conventional approach. However, during growth and maturation, peak VO_2 increases as a function of body size, and hence it may be unclear whether the improvement in aerobic capacity depends on an increase in body size or on changes in anatomical and physiological capacity, or both. When selecting an appropriate model to interpret growth-related changes in peak VO_2, some authors argue that the data should be expressed in accordance with theoretical values such as body mass$^{0.67 \text{ or } 0.75}$ (Eisenmann, Pivarnik, & Malina, 2001). In

addition, expressing peak VO_2 in relation to fat-free mass or lean body mass in adults may reduce, but not eliminate, sex differences. Nonetheless, studies of prepubertal children have shown that peak VO_2 relative to fat-free mass is higher in boys than in girls, which indicates that cardiac functional capacity (i.e., stroke volume) and body composition account for differences in peak VO_2 (Rowland, Goff, Martel, & Ferrone, 2000; Vinet et al., 2003). Thus, it is not clear what strategy is most suitable to normalize peak VO_2 for body size and sex in adolescents. Perhaps it would be appropriate to consider the pubertal status, body composition, and body size of the subjects to explain some of the variance in our results regarding the associations between peak VO_2 and cardiac dimensions.

Assessment of LV filling pressure is required to establish an accurate diagnosis of several cardiac conditions. Invasive left and right heart catheterization has been considered to be the gold standard for confirming or refuting the presence of high LV filling pressure. However, that technique is not without risks, and therefore noninvasive estimation of LV filling pressure has been an essential step in the evaluation of diastolic function. Currently, E/e' is regarded as an instant measure of LV filling pressure, whereas the LA volume index reflects the cumulative effect of the pressure over time. A combination of these parameters is used to increase the accuracy of noninvasive assessment of the diastolic function (Cameli et al., 2016). Moreover, LA strain is strongly correlated with invasive measurement of LV filling pressure, which may be useful in addition to the conventional parameters. Unfortunately, most widespread echocardiographic indexes designed for this purpose have limitations, for example, a dedicated software for LA strain analysis has not yet been released, hence software for the LV is currently used to study the LA pattern (Cameli et al., 2016).

Scaling of cardiac parameters to BSA is influenced by body composition, which in turn is related to growth and maturation. It has been reported that allometric models are effective for partitioning the

influence of body size on physiological parameters. For instance, the association between LVM and body length varies at different stages of development, and therefore dividing LVM by body length to a power of 2.7 is a widely accepted indexing method in assessments of adolescents (Valente-dos-Santos et al., 2013). Afterward statistical analysis of cardiac parameters in our studies showed that the significance between the groups with regard to cardiac dimensions indexed by length$^{2.7}$ was similar to that noted for data indexed by BSA. Furthermore, fat-free mass, which is the metabolically active tissue in the body, is closely and positively associated with LVM in adolescents (Valente-dos-Santos et al., 2013). Body composition was not included in the assessments of our subjects, and thus it was not possible to determine whether there were any associations between fat-free mass and cardiac size or function.

Some of the blood samples from our subjects were supplemented with EDTA. It has recently been shown that EDTA is an adequate anticoagulant for blood collected for flow cytometric analysis of lymphocytes, if the analysis is performed within 24 hours of sampling (Tompa, Nilsson-Bowers, & Faresjo, 2018).

The Luminex assay has several distinct advantages over similar bioassays (Khalifian, Raimondi, & Brandacher, 2015): it provides more robust data delivery with higher throughput and density compared to traditional methods; it can multiplex up to 100 different analytes in a single well and thereby avoids inter-assay variation; it requires significantly smaller sample volumes than traditional bioassays.

Conclusions

- Compared with untrained controls, endurance-trained adolescents showed biatrial and biventricular anatomic remodeling and functional remodeling of RV at rest. These observations indicate that changes in cardiac morphology and functions have already begun to occur during adolescence as a result of intensive endurance exercise, which should be taken into consideration when performing clinical examination of the heart in endurance-trained adolescents.

- Temporal changes in the cardiac response to a maximal test were similar in both endurance-trained and untrained adolescents, which primarily suggests that the cardiac functional responses to exercise are not affected by the degree of endurance exercise that is practiced. However, after peak exercise, the diastolic function was more extensively enhanced in the active group than in the controls.

- Cardiac dimensions and functions were positively associated with peak VO_2 both at rest and immediately after exercise. This indicates that increased cardiac dimensions can be expected when peak VO_2 is increased in adolescents who perform regular endurance exercise.

- Resting levels of circulating hormones associated with growth and metabolism did not differ between endurance-trained adolescents and untrained controls. In particular, considering that regular exercise had no effect on the hormone concentrations at rest, other signaling systems and mechanisms should be taken into account in exercise-induced growth during adolescence.

- Resting level of IGF-1 was not related to increased cardiac dimensions in endurance-trained adolescents. These finding suggests that the biomechanical mechanism (i.e., receptors sensitive to changes in volume and pressure load) and/or the autocrine impact of IGF-1 locally synthesized by the myocardial cells during exercise has a greater impact on cell signaling that leads to increased cardiac size at these ages.

- In endurance-trained adolescents, the immune response differed between the sexes, and this variation included a more pronounced response to a T1D-related autoantigen in boys. On the other hand, the girls showed increased levels of proinsulin and C-peptide. These observations suggest that the β-cells are in some way affected by regular endurance exercise during adolescence, although further research is required to determine whether such an impact represents an advantage or a disadvantage for the individual.

Clinical and practical implications

- The findings presented in Papers I and II have practical implications for assessment of cardiac enlargement and function in endurance-trained adolescents, at rest and after exercise.

- The results outlined in Paper III may broaden our knowledge about factors that trigger or not trigger cardiac hypertrophy in endurance-trained adolescents.

- The data reported in Paper IV may contribute to our knowledge about possible effects of regular strenuous endurance exercise on β-cell function/stress in adolescents.

Future research

The studies included in this thesis focused on adolescents who practiced regular and intense endurance exercise for several years. Research in this field is important, because it contributes information that can aid differential diagnosis of conditions such as hypertrophic cardiomyopathy. More investigations based on trained adolescents compared with untrained controls will broaden our understanding of the growing heart and increase our knowledge of the physiological adaptation associated with cardiac conditions. This will also provide normal values to a reference interval for cardiac parameters when extensive exercise is performed in adolescence. Future research should even focus more extensively on the effects of exercise on the atria and the RV, because that topic has been poorly investigated thus far, particularly in athletic adolescents. The present studies also assessed resting levels of hormones, cytokines, and chemokines in the same subjects, but additional analyses are needed to clarify the hormonal response to different phases of training, for example, in "a real life" setting. Findings that established possible impacts on β-cells in endurance-trained adolescents must also be further evaluated. A cytokine/adipokine named visfatin may play a role in glucose metabolism, specifically by acting as an insulin mimetic, and it is largely agreed that visfatin levels appear to be elevated in childhood obesity. However, lack of data regarding the effects of acute and chronic exercise on visfatin levels precludes safe conclusions about the impact on children in that context (Jamurtas, Stavropoulos-Kalinoglou, Koutsias, Koutedakis, & Fatouros, 2015; Mellick, Feger, Oberlin, Davis, & Wideman, 2017). Further analysis of blood samples from our cohort could provide important information in this field. In addition, future research could include longitudinal studies that follow a young athletic population through puberty into adulthood with respect to cardiac size and function and the effects of IGF-1 and other hormones, and also concerning immune markers such as cytokines and possible pathophysiology.

Summary in Swedish/Svensk sammanfattning

Introduktion

Regelbunden träning har många positiva hälsoeffekter, bland annat på hjärta och kärl, och risken för övervikt och diabetes typ 2 minskar. Träning har även positiva effekter på stress, mentalt välbefinnande och sömn. Flera studier på vuxna har dock visat att regelbunden uthållighetsträning kan ge förändringar på hjärtat i form av ökade hjärtvolymer och ökad väggtjocklek. För att kunna skilja mellan förändringar på hjärtat orsakade av träning och förändringar orsakade av en allvarlig hjärtsjukdom är det viktigt med forskning och kunskap inom området. Det finns gott om studier på vuxna atleter vad gäller hjärtats anpassning till träning, men på elittränande barn och ungdomar är forskningen bristfällig.

Tillväxt av hjärtat, på grund av regelbunden träning, stimuleras via kroppens biomekaniska och hormonella mekanismer. Den biomekaniska delen styrs genom den volym- och/eller tryckökning som sker i hjärtat under ett träningspass medan den hormonella delen styrs av frisättning av olika tillväxtfaktorer, där tillväxtfaktorn ”insulin-like growth factor-1” (IGF-1) antas spela störst roll. Idag saknas det dock kunskap om hur stor del av hjärtats tillväxt som kan tillskrivas respektive faktor.

Generellt finns få studier gjorda på elittränande barn och ungdomar och effekterna på kroppen av den alltmer intensiva och i allt yngre åldrar startande högintensiva träningen är till stora delar okända.

Syften

Det övergripande syftet med avhandlingen var att undersöka hjärtats storlek och funktion, hormoner associerade till tillväxt och metabolism, samt immunförsvarets aktivitet, hos ungdomar (13-19 år) som regelbundet och sedan flera år tillbaka har tränat uthållighetsträning på elitnivå. I den första delstudien var syftet att jämföra förmakens och kamrarnas storlek och funktion mellan en

grupp tränande ungdomar och en ålders- och könsmatchad kontrollgrupp som inte tränade regelbundet. Eventuella samband mellan hjärtats storlek och den maximala syreupptagningsförmågan studerades också. Den andra delstudien syftade till att undersöka förändringar av hjärtats funktion före och efter ett maximalt arbetsprov. I delstudie tre var syftet att jämföra hormonkoncentrationen i blodet mellan de tränande och de icke tränande ungdomarna, samt undersöka eventuella samband mellan de olika hormonerna och hjärtats storlek. I den avslutande delstudien jämfördes skillnader i immunförsvarets aktivitet mellan pojkar och flickor i den uthållighetstränande gruppen.

Metoder

Alla deltagarna genomgick undersökningar av hjärtat med hjälp av ultraljud: i vila, omedelbart efter och 15 minuter efter ett maximalt arbetsprov på löpband. Ergospirometri användes för att mäta maximala syreupptagningsförmågan. Blodprov togs i vila och analyserades med avseende på hormoner, lymfocyters ytmarkörer samt cytokiner och kemokiner. Alla delstudier hade en kvantitativ ansats. Delstudierna I, III och IV var komparativa tvärsnittsstudier. Delstudie II hade en pre-post-testdesign. Olika regressionsanalyser användes för att undersöka eventuella samband mellan maximal syreupptagningsförmåga och hjärtats storlek och funktion, samt mellan olika hormoner och hjärtats storlek.

Resultat

Gruppen med ungdomar som regelbundet tränade uthållighetsträning på elitnivå hade en ökad storlek av hjärtats både förmak och kammare jämfört med ungdomar som inte tränade regelbundet. Förändringar av hjärtats funktion mellan vila och efter det maximala arbetsprovet visade i huvudsak liknande mönster i både den tränande och i den icke tränande gruppen. Starka samband mellan maximal syreupptagningsförmåga och hjärtats dimensioner och funktion kunde ses i både vila och efter det maximala arbetsprovet.

Nivåerna i blodet av hormoner relaterade till tillväxt och metabolism skilde sig inte åt mellan grupperna. Det kunde inte heller visas något samband mellan mängden tillväxtfaktor IGF-1 i blodet och hjärtats storlek hos de aktiva ungdomarna. En ökad respons i immunförsvaret mot ett typ 1 diabetes-autoantigen sågs hos de aktiva pojkarna jämfört med de aktiva flickorna. Flickorna däremot hade en ökad utsöndring av diabetesmarkören proinsulin i blodet jämfört med pojkarna.

Slutsatser

Det finns många fördelar med att vara fysiskt aktiv och att träna regelbundet, och resultaten från den här avhandlingen visade inte något som pekar på motsatsen hos ungdomar som regelbundet utövar intensiv uthållighetsträning på elitnivå. Ökade hjärtdimensioner och påverkan på hjärtats funktion i samband med ett maximalt arbetsprov kan förväntas hos dessa ungdomar med hög maximal syreupptagningsförmåga och bör därför tolkas som fysiologiska anpassningar av hjärtat och inte som patofysiologiska förändringar.

Att ökade hjärtdimensioner inte har något samband med hormoner associerade till tillväxt och metabolism i vila kan betyda att påverkan av de biomekaniska mekanismerna och/eller de lokalt producerade hormonerna i samband med träningstillfället är av större betydelse för tränings-anpassad tillväxt av hjärtat. Skillnader i immunförsvarets aktivitet i vila mellan pojkar och flickor antyder att det finns en könsskillnad i immunförsvaret hos uthållighetstränande ungdomar.

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Appendix I

Exercise studien

Studieprotokoll Ekokardiografi

Datum ……..

Tid för arbetsprovets slut ……..

Ekokardiograför ……..

Patient ID ……..

Studie ID ……..

Interventionsgrupp ☐ Kontrollgrupp ☐

I vila före arbetsprovet ska alla deltagare undersökas med ekokardiografi enligt rutin för att utesluta medfött eller förvärvat VOC. Därtill görs studiespecifika registreringar.

- Montera proberna 9L-D (carotis), M5S (2D eko) och 4V-D (4D)
- Hämta patientens ID i Query och skriv in operatörens signatur
- Patienten ska vara uppkollad med EKG av god kvalitet (tydlig p, QRS och T).
- 3 RR intervall på alla cineloopar
- Spara undersökningen även på DVD – 4G
- Logga in som adm, lösenord ulsadm vb av ändring i protokoll.
- Applikator Maria Persson på GE mariapersson@live.se 0709-609957 är behjälplig vid problem.

Vila

Blodtryck ……..

Halskärl

- Välj prob 9L-D, carotid Jkpg-carotid.

- Arteria carotis communis i längdsnitt och tvärsnitt dx och sin. Minimalt djup. Skarpa kärlgränser. Vänster och därefter höger sida.

Ekokardiografi

Välj prob M5S och program 3RRexercise-exercise
(3 R-R intervall som grundinställning)

Parasternal vy:

1. LAX cine (frame rate >40/s ändra vb width)
2. LAX M-mode LV
3. LAX M-mode Ao/LA
4. LAX färgdoppler (MI, AI)
5. SAX cine i papillarmuskelnivå (frame rate >40/s ändra vb width)
6. SAX färgDoppler Pulmonalis
7. SAX CW spektralDoppler Pulm valvulärt

Apikal vy:

1. 4Ch cine inklusive LA + RA
2. 4Ch cine fokus RV (frame rate >40/s ändra vb width)
3. 4Ch cine fokus på LV (frame rate >40/s ändra vb width)
4. 2Ch cine LV (frame rate >40/s ändra vb width)
5. 3Ch cine LV (frame rate >40/s ändra vb width)
6. 4Ch TAPSE (3 R-R, endexpiratoriskt)
7. 4Ch MAPSE septalt – lateralt (3 R-R, endexpiratoriskt)
8. 2Ch MAPSE inferiort – anteriort (3 R-R, endexpiratoriskt)
9. 5Ch spektralDoppler CW Ao valvulärt
10. 5Ch färgDoppler AoV
11. 5Ch pulsadDoppler Ao subvalv för IVRT
12. 4Ch pulsadDoppler MV inflow (justera skala och svep, 3 slag)

13. 4Ch pulsadDoppler lungvenflöde (justera skala och svep, 3 slag)
14. 4Ch färgDoppler MI
15. 4Ch färgDoppler TI
16. 4Ch spektralDoppler CW TI
17. 4Ch pulsadDoppler TV inflow
18. 4Ch färg TVI cine wide angle (Nyqvistgränsen 0,3)
19. 4Ch färg TVI cine RV (frame rate > 100/s, Nyqvistgränsen 0,3)
20. 4Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)
21. 2Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)
22. 3Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)

4D-vila : (kortversion)

Välj prob 4V-D och program 3RRexercise-exercise
(3 R-R intervall som grundinställning)

1. AFI triplan:
   2D – optimera LV
   Mulitplan
   Triplane (frame rate >40/s ändra vb width och djup)
   Store.
2. 4D 4Ch med fokus LV o LA:.
   2D - optimera LV/LA
   Large – justera ev width och djup
   Multisclize – 12 sektorer och justera ev with och djup
   Multibeat – 2 (frame rate > 12/s), be pat hålla andan och när bilden är ok
   Store – ser insamlingen ok ut
   Store
3. 4D 4Ch med fokus RV o RA: som ovan med fokus att RV o RA kommer med.

Avsluta undersökningen i EKO maskinen genom att spara ner bilderna och trycka ”End exam.

Omedelbart efter arbete och 15 minuter senare

Apikal vy:

1. 4Ch cine inklusive LA + RA
2. 4Ch cine fokus RV (frame rate >40/s ändra vb width)
3. 4Ch cine fokus på LV (frame rate >40/s ändra vb width)
4. 2Ch cine LV (frame rate >40/s ändra vb width)
5. 3Ch cine LV (frame rate >40/s ändra vb width)
6. 4Ch TAPSE (3 R-R, endexpiratoriskt)
7. 4Ch MAPSE septalt – lateralt (3 R-R, endexpiratoriskt)
8. 2Ch MAPSE inferiort – anteriort (3 R-R, endexpiratoriskt)
9. 5Ch spektralDoppler CW Ao valvulärt
10. 4Ch pulsadDoppler MV inflow (justera skala och svep, helst 6 slag)
11. 4Ch pulsadDoppler TV inflow
12. 4Ch färg TVI cine wide angle (Nyqvistgränsen 0,3)
13. 4Ch färg TVI cine RV (frame rate > 100/s, Nyqvistgränsen 0,3)
14. 4Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)
15. 2Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)
16. 3Ch färg TVI cine LV (frame rate > 100/s, Nyqvistgränsen 0,3)

4D-postex : (kortversion, se ovan)

Spara undersökningen på disk som formaterats:
(Utility/Config/Connectivity/Tools/Number disk/Format)