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Hege Wilson Landgraff

Cardiorespiratory, hematological and body composition changes in maturing girls and boys with different training backgrounds

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Errata

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line 198 Figure 1 change to Figure **2**

line 206 Figure 2 change to Figure **1**

Summary

This thesis is based on one longitudinal study, monitoring a group of children from age 12 to age 15. The main objective was to establish the association between growth, maximal oxygen uptake (VO_{2max}), hematological variables and cardiac dimensions in girls and boys during puberty. We also aimed at investigating the effects of inclusion of high volumes of endurance training in active children. Further, in a cross-sectional study, bioelectrical impedance analysis (BIA; InBody 720) was validated against dual-energy X-ray absorptiometry (DXA) for assessing body composition.

Seventy-eight children volunteered to participate in the study, and came for repeated measurements at age 12, 13 and 15. They were assigned to an endurance training group (End group; 40 girls and boys) and a non-endurance training group (non-End group; 38 girls and boys) based on the types of sport and volume of endurance training they were undertaking. Total weekly volume of training at age 14-15 was 10.7 (2.8) hours and 8.3 (3.7) hours in the End and non-End group, respectively, while weekly volume of endurance specific training was 7.3 (1.8) hours and 1.5 (1.1) hours, respectively.

VO_{2max} was determined by an incremental running test to exhaustion on a treadmill and haemoglobin mass (Hbmass) and blood volume (BV) were assessed using the optimized carbon monoxide rebreathing method. Cardiac dimension measurements were performed using two- and three-dimensional echocardiography. Anthropometric measurements included body mass (BM), height and sitting height, and body composition which was assessed using InBody 720.

There were significant sex differences in the rate of development of VO_{2max} and intravascular volumes in absolute values and relative to BM from age 12 to 15, with on average a larger increase in boys. The timing of the appearance of pronounced sex differences was aligned with significant differences in the development of body-composition, and when related to fat free mass (FFM), the age effect of sex from age 12 to 15 disappeared.

There was on average no interaction between age and training group in the development of all measures of VO_{2max}, and intravascular volumes in absolute values from age 12-15. For Hbmass and red cell volume (RCV) relative to FFM, there was no difference between boys, however for girls, there was a difference in the age effect between the training groups, with no increase in the End girls over the three years.

There were no differences between the training groups in the alteration of cardiac dimensions relative to FFM; however, the End group experienced a smaller increase in left ventricular

posterior wall thickness in diastole (LV PWd) than the non-End group. This led to a decrease in relative wall thickness (RWT) in the End group indicating eccentric remodeling, while RWT stayed unchanged in the non-End group compared to what was seen in both groups at baseline.

There were very strong to almost perfect correlations between absolute values for VO_{2maks}, intravascular volumes, and cardiac dimensions and FFM at both 12 and 15 years of age. Also, no change in FFM-relative values of VO_{2max} and no or minor changes in vascular volumes relative to FFM during the study period, indicate that these variables develop in parallel with FFM.

In boys, time to exhaustion (TTE) increased in both groups but increased significantly more in the End boys than in the non-End boys, while the development of VO_{2max} did not differ between the training groups. This suggests that endurance training may have had a significant effect on determinant factors other than VO_{2max}. This pronounced difference between the training groups was not seen in girls.

Validating InBody 720 against DXA, showed that InBody 720 underestimated FM and overestimated FFM. However, the correlation between the devices was almost perfect for both FM and FFM. Taken together, this indicate that InBody 720 is a valid method in estimating changes in FFM during growth.

In conclusion, the present longitudinal study suggests that the development of VO_{2max} and intravascular volumes was dependent on sex but not on volume of endurance training.

Using multiple linear regression analysis for developing a model for predicting changes in VO_{2max} from measurements of changes in anthropometrics, heart dimensions and intravascular volumes, indicated that a model which included changes in FFM and Hbmass was best suited. This model was able to account for 74% of the variance in the change in VO_{2max}.

Sammendrag

Denne avhandlingen er basert på en longitudinell studie som har fulgt en gruppe barn fra 12 til 15 år. Det overordnede målet var å undersøke sammenhengen mellom vekst, maksimalt oksygenopptak (VO_{2max}), hematologiske variabler og hjertedimensjoner hos jenter og gutter i puberteten. Videre ønsket vi å studere effektene av inkludering av høyt volum av utholdenhetstrening hos aktive barn. Vi gjorde også en tverrsnittsstudie, hvor bioelektrisk impedansanalyse (BIA; InBody 720) ble validert mot dual-energy X-ray absorptiometri (DXA) for estimering av kroppssammensetning.

Syttiåtte barn meldte seg frivillig til å delta i studien, og kom for gjentatte målinger ved 12, 13 og 15 år. De ble delt inn i en utholdenhetsgruppe (End gruppe; 40 jenter og gutter) og en ikke-utholdenhetsgruppe (non-End gruppe; 38 jenter og gutter) basert på hvilken idrett de var aktive i og volum av utholdenhetstrening de gjennomførte. Totalt treningsvolum per uke ved 14-15 år var 10.7 (2.8) timer End-gruppen og 8.3 (3.7) timer i non-End gruppen, mens ukentlig volum av spesifikk utholdenhetstrening var på henholdsvis 7.3 (1.8) timer og 1.5 (1.1) timer.

VO_{2max} ble målt ved løp til utmattelse på tredemølle, og hemoglobinmasse (Hbmasse) og blodvolum (BV) ble målt ved å bruke optimalisert karbonmonoksyd-gjenpustningsmetode. Målinger av hjertedimensjoner ble utført ved bruk av to- og tredimensjonal ekkokardiografi. Antropometriske målinger bestod av kroppsmasse (KM), høyde og sittehøyde, i tillegg til måling av kroppssammensetning ved hjelp av BIA.

Det var signifikante kjønnsforskjeller i utviklingen av VO_{2max} og intravaskulære volumer i absolutte verdier og relativt til KM fra 12 til 15 år, med en gjennomsnittlig større økning for gutter enn jenter. Disse kjønnsforskjellene oppstod parallelt med kjønnsforskjeller i utviklingen av kroppssammensetning, men ved å relatere variablene til fettfri masse (FFM), forsvant imidlertid alderseffekten av kjønn.

Det var i gjennomsnitt ingen interaksjoner mellom alder og treningsgruppe i utviklingen for hverken absolutte eller relative verdier av VO_{2max} eller for absolutte verdier av intravaskulære volumer fra 12-15 år. Videre var det ingen forskjell mellom guttene i utviklingen av Hbmasse og RCV (volum av røde blodceller) per kg FFM. For jenter derimot, var det en forskjell i effekten av alder mellom treningsgruppene, hvor End-jentene ikke økte disse variablene i løpet av de tre årene.

Det var ingen forskjell mellom treningsgruppene i endringer i hjertedimensjoner per kg FFM, men venstre ventrikkels bakre vegg i diastole økte mindre hos End-gruppen sammenlignet med non-End-gruppen. Dette førte til en reduksjon i relativ veggtykkelse hos End-gruppen, noe som kan tyde på eksentrisk remodellering, mens den relative veggtykkelsen forble uforandret hos non-End-gruppen.

Det var en sterk til nesten perfekt korrelasjon mellom absolutte verdier av VO_{2max}, intravaskulære volumer og hjertedimensjoner og FFM, både ved 12 og 15 år. Videre var det ingen endring i VO_{2max} per kg FFM, og ingen eller liten endring i vaskulære volumer per kg FFM i løpet av de tre årene, noe som tyder på at disse variablene utvikler seg parallelt med FFM.

Tid til utmattelse økte hos begge gruppene med gutter, men mer hos End-guttene, til tross for at utviklingen av VO_{2max} var lik mellom gruppene. Dette kan tyde på at utholdenhetstrening kan ha signifikant effekt på andre bestemmende faktorer for prestasjon enn VO_{2max}. Denne forskjellen mellom treningsgruppene var ikke til stede blant jentene.

Validering av InBody 720 mot DXA viste at InBody 720 underestimerte FM og overestimerte FFM, men at korrelasjonen mellom metodene var nesten perfekt for estimering av både FM og FFM. Samlet sett, tyder dette på at InBody 720 er en valid metode for å estimere endringer i FFM hos barn og unge som vokser.

Resultatene fra denne longitudinelle studien tyder på at utviklingen av VO_{2max} og intravaskulære volumer var avhengige av kjønn, men ikke av volumet av utholdenhetstrening.

Vi brukte multiple lineær regresjonsanalyse for å finne en modell som best kunne predikere endringer i VO_{2max} ved å inkludere mål på endringer i antropometri, hjertedimensjoner og intravaskulære volumer. Våre resultater tydet på at en modell som inkluderte endringer i FFM og Hbmasse var best egnet. Den modellen kunne forklare 74% av variasjonen i endringen av VO_{2max}.

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Oslo, July 2020

Hege Wilson Landgraff

List of papers

This dissertation is based on the following original research papers, which are referred to in the text by their Roman numerals:

I. **Landgraff HW**, Riiser A, Lihagen M, Skei M, Leirstein S, Hallén J. Longitudinal changes in maximal oxygen uptake in adolescent girls and boys with different training backgrounds. Accepted SJMSS

II. **Landgraff HW**, Hallén J. Longitudinal training-related hematological changes in boys and girls age 12 to 15. Accepted MSSE

III. Bjerring AW, **Landgraff HEW**, Stokke TM, Murbræch K, Leirstein S, Aaeng A, Brun H, Haugaa KH, Hallén J, Edvardsen T, Sarvari SI. The developing athlete's heart: a cohort study in young atheletes transitioning through adolescence. *European Journal of Preventive Cardiology*. 2019:2047487319862061

IV. **Landgraff HW**, Hallén J. Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry in the assessment of body composition in athletic adolescents. Manuscript

Abbreviations

APHV	Age at peak height velocity
a-v O2diff	Arterial venous oxygen difference
BIA	Bio electrical impedance analysis
BV	Blood volume
BM	Body mass
BMI	Body mass index
BSA	Body surface area
CO	Carbon monoxide
DXA	Dual-energy X-ray absorptiometry
EDV	End diastolic volume
ESV	End systolic volume
EV	Erythtocyte volume
FFM	Fat free mass
FM	Fat mass
HR	Heart rate
Hct	Hematocrit
[Hb]	Hemoglobin consentration
Hbmass	Hemoglobin mass
LV	Left ventricular
LVPWd	Left ventricular posterior wall thickness in diastole
VO_{2max}	Maximal oxygen uptake
MCHC	Mean hemoglobin concentration in the red blood cell
MCH	Mean hemoglobin content of the red blood cells
MCV	Mean red cell volume
O2	Oxygen
PHV	Peak height velocity
PWV	Peak weight velocity
%HbCO	Percent Carboxyhemoglobin
%FM	Percent fat mass
PV	Plasma volume
RBC	Red blood cell
RBCC	Red blood cell count
RCV	Red cell volume
RWT	Relative wall thickness
RER	Respiratory exchange ratio
RV	Right ventricular
TTE	Time to exhaustion
TBW	Total body water
2C	Two component
4C	Four component
2D	Two dimensional

3D	Three dimensional

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Background

The interaction of genes, hormones, nutrients and environmental factors plays an important role in regulating a child's growth and maturation (Malina, Bouchard, & Bar-Or, 2004). Puberty represents the transition from childhood to adulthood and is a dynamic period of development with rapid changes in body size, shape, and composition (Rogol, Roemmich, & Clark, 2002). Growth and maturation during childhood and puberty vary between children in both timing and tempo and are governed by the individual's inborn biological clock (Malina et al., 2004). Timing refers to the chronological age at which the maturational events occur, and tempo refers to the rate of maturation. Chronological age relates to calendar time and corresponds to the child's actual age, while a child's biological level of maturity is referred to as biological age. Chronological age and biological age don't run in parallel. Thus, within a group of children of the same chronological age and sex there may be large variations in biological age (Malina et al., 2004).

Anthropometry

Body height

Growth rate is greatest in the first years of life and gradually decreases until the start of the adolescent growth spurt. With this growth spurt, speed increases and girls reach peak height velocity (PHV), on average, at 12 years of age and boys at approximately 14 years of age. After this, growth gradually diminishes and ends after reaching the adult stage (Malina et al., 2004). However, there are large individual variations and sex differences regarding both the start of the growth spurt and the size of PHV (Armstrong & Welsman, 2002). Early maturing boys can start their growth spurt at the age of 10.5 and reach PHV when they are 12 years old, while late maturing boys do not start their growth spurt before they are 14.5 years old and reach PHV around 16 years of age. Similar variations are also found in girls, and there may therefore be some overlap between boys and girls (Tanner, 1989). Boys tend to demonstrate a larger PHV compared to girls (Iuliano-Burns, Mirwald, & Bailey, 2001).

Body Mass

While the increase in body height ceases with the attainment of adult height, body mass (BM) usually continues to increase into adult life (Malina et al., 2004). The peak weight velocity (PWV)

appears after PHV; however, the timing for PWV is generally less clear than it is for height (Armstrong & Fawkner, 2008). Most maximal velocities for anthropometric dimensions, except velocities for the lower limbs, occur after PHV and before or coincident with PWV (Beunen & Malina, 1988). Beunen and Malina (1988) estimated PWV to appear 0.3-0.9 years after PHV for girls and 0.2-0.4 years for boys. Body mass is often regarded as a combination of fat mass (FM) and fat free mass (FFM).

Fat Mass

During childhood, both sexes experience a rapid increase in absolute FM; however, the rate of increase varies considerably between the sexes. In girls, absolute FM increases at a relatively constant rate from an average of 5.5 kg at age 8 to about 15 kg at age 16, after which the rate of change declines (Siervogel et al., 2003). Absolute FM in boys appears to reach a plateau after PHV (Veldhuis et al., 2005). It increases from an average of 5.0 kg at age 8 to about 11 kg at age 14, after which it declines to about 9 kg at age 16 and then subsequently reaches a plateau (Siervogel et al., 2003). During the pubertal years, girls gain on average 1.14 kg FM per year, and by the end of puberty girls have on average 5-6 kg more absolute FM than boys (Loomba-Albrecht & Styne, 2009).

When total body fat is expressed as a percentage of body mass (%FM) there is a small sex difference even in early childhood, with a higher %FM in girls. %FM increases gradually with age in the years before puberty in both sexes. In boys, the increase in %FM levels off around PHV and then gradually declines until it reaches adult values at around age 17 (Veldhuis et al., 2005). In girls, on the other hand, %FM continues to increase through puberty and into early adulthood in the same manner as FM (Malina et al., 2004). PWV in girls is mainly caused by the large and continuous increase in FM during puberty and to a lesser extent by the increase in muscle mass and skeletal tissue (Baxter-Jones, 2017). The marked sex difference in the development of FM and FFM results in a net increase in %FM for girls and decline in %FM for boys throughout puberty and into adult values of approximately 25% and 13% for females and males, respectively (Loomba-Albrecht & Styne, 2009; Malina et al., 2004).

Fat free mass

BM can be divided into FM and fat free mass (FFM), where FFM represents the weight of muscles, bones, ligaments, tendons, and internal organs. FFM is mainly composed of water, protein and minerals. The chemical composition of FFM changes with age and maturation, and chemical maturity is not attained until young adulthood (Fomon, Haschke, Ziegler, & Nelson,

1982; Malina et al., 2004). The percentage of water decreases and percentages of protein and osseous minerals increase (Fomon et al., 1982). The water content of FFM varies with age and sex, and has been found to range from 68 to 81% (Moon et al., 2009; Nickerson, Tinsley, & Esco, 2019). Fomon et al. (1982) found that the water content in FFM decreased from 81% to 75% between birth and the age of 10 years. The estimated water content of FFM in 13- to 17-year-old boys is 74.7-74.2% and the percentage is 76.6-75.5% in girls (Lohman, 1986; Malina et al., 2004).

As with the development in FM, there are large sex differences in the magnitude of the increase in FFM and the differences become more pronounced during puberty. FFM increases with age in both sexes; however, boys experience a much larger rise in muscle mass and skeletal tissue than girls (Baxter-Jones, 2017). In girls, FFM increases until around the age of 15 years, and then remains relatively unchanged (Veldhuis et al., 2005). However, the rate of gain in FFM decreases over the same period (Guo, Chumlea, Roche, & Siervogel, 1997). The maximum velocity for FFM gain in girls is on average a yearly increase of 4.5 kg at around the age of 8 years and it decreases to zero at the age of 20 years (Guo et al., 1997). In boys, FFM increases steadily between the ages of 8 and 18 years, with a more rapid increase between 12 and 15 years (Siervogel et al., 2003). Contrary to girls, the velocity of FFM in boys also increases between the ages of 8 and 15 years and reaches a maximum velocity of an average of 7.0 kg per year at age 15. At the age of 20, the rate of yearly gain in FFM has decreased to an average of 3.5 kg (Guo et al., 1997).

Because the gain in FM levels off around the time of the growth spurt in boys, their PWV is primarily due to gains in muscle mass and skeletal tissue (Baxter-Jones, 2017). The marked sex difference in the development of FFM results in adult males having, on average, 20 kg more FFM compared with females (Loomba-Albrecht & Styne, 2009).

Assessment of body composition

Quantification of human body composition is increasingly used in a variety of populations, including healthy individuals, patients, athletes, the elderly and children; it is regarded as an important health and performance variable (Ackland et al., 2012; Malouf et al., 2013). The primary focus in most studies assessing body composition has been the measurement of FM or %FM. However, the close relationship between FFM and the physical demands of many sporting activities has made body composition assessment a useful tool in athletic populations (Kendall et

al., 2017). Moreover, during childhood and adolescence, FFM is significantly related to maximal oxygen uptake (Armstrong & Welsman, 2019a; Armstrong & Welsman, 2001).

There are several techniques for describing the constituent components of the body, and the choice of technique often depends on the available technology and the intended purpose for which data are to be used (Ackland et al., 2012). Dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA) are two well-known methods for estimating body composition and both are regarded as suitable for children and adults. Another well established and commonly used method to estimate percent fat, and indirectly FFM, is skinfold measurements (Lohman & Going, 2006). Previously, skinfold measurements seem to have been the preferred method in studies with children. However, BIA is now widely available, and because it requires little training and is easy to use, this makes it a good alternative to skinfold measurements.

Dual-energy X-ray absorptiometry

DXA is regarded by many as the gold standard for bone mineral measurements (Stewart & Hannan, 2000). Due to its widespread availability and ability to also measure soft tissue precisely, DXA is considered a useful reference method for body composition and thus is acknowledged by many as the standard and most precise method to assess body fat mass (Hofsteenge, Chinapaw, & Weijs, 2015; Prior et al., 1997). DXA measures three components of body composition: bone mineral content, other fat-free soft tissue and fat (lipid) (3-C model) (Ackland et al., 2012; Hofsteenge et al., 2015). It also measures regional fat distribution. Although DXA is a precise laboratory method to assess body composition, it has some limitations: it is expensive, time consuming, and involves exposure to small amounts of radiation (Kyle, Earthman, Pichard, & Coss-Bu, 2015).

Bioelectrical impedance analysis

BIA is a widely used field method for estimating body composition, and the methodassumes that the body can be described in terms of a 2-component model (2-C model) of FM and FFM. BIA is based on the principle that electric current flows at different rates through the body depending on its composition (Dehghan & Merchant, 2008) and uses body impedance to estimate total body water (TBW), which is used to predict %FM, FM and FFM from various equations (Talma et al., 2013). FFM, which is rich in water and electrolytes, has minimal impedance while the opposite is the case for FM (Dehghan & Merchant, 2008). Most BIA techniques are developed and validated against DXA, which serves as a reference method for that purpose (Kyle et al., 2015). The

method is widely used because it is cheap, non-invasive, quick, and the instrument is portable and easy to move.

When monitoring changes in FFM, the methods chosen for estimating body composition should be reliable, valid and practical to use (Jensky-Squires et al., 2008). The four component model (4-C model), which compartmentalizes the body into protein, fat, water and minerals, where BM equals the sum of FM, total body water, total body protein and total body minerals, is considered to be the most reliable technique for estimating body composition in children (Bray, DeLany, Volaufova, Harsha, & Champagne, 2002). Although DXA has not been found to be as accurate as the 4-C model, it compares well with reference methods, and is increasingly used to calibrate indirect techniques (Kriemler, 2009). Lohman and Going (2006) also regard the 4-C model as the optimal model; however, they recommend using DXA to assess body composition in children and youth because it requires less technical expertise and expense compared with the 4-C model.

Maximal oxygen uptake

Aerobic capacity is determined by maximal aerobic power (VO_{2max}) and the percentage of VO_{2max} that can be maintained for given distances or durations (Bosquet, Léger, & Legros, 2002). VO_{2max} is recognized as the gold standard of cardiorespiratory fitness, and is defined as the highest oxygen uptake an individual can attain during exercise involving large muscle masses (e.g., running, rowing, and cycling (Bassett & Howley, 2000; Åstrand, Rodahl, Dahl, & Strømme, 2003). VO_{2max} is considered the best single measure of aerobic exercise in both adults and children and is seen as one of the single most important factors in aerobic athletic performance (Åstrand et al., 2003). It is determined from maximal cardiac output (Q) and maximal arterio-venous oxygen difference (a-v O2 difference) (Bassett & Howley, 2000; Levine, 2008). During whole body work, such as running, cycling and rowing, it is generally accepted that VO_{2max} is limited by the rate of oxygen delivery to the muscles and the muscles' ability to utilize the oxygen they receive (Saltin & Strange, 1992).

VO_{2max} or VO_{2peak}

In the current pediatric exercise literature, VO_{2peak} is often used instead of VO_{2max}. One criterion in deciding whether VO_{2max} is achieved in a test to voluntary exhaustion, is reaching a plateau in oxygen uptake despite an increase in work rate (Armstrong, Welsman, & Winsley, 1996). The highest VO_2 observed, during a single incremental exercise test to voluntary exhaustion without reaching a plateau, is often termed peak VO_2 (Armstrong et al., 1996). Several studies have

reported that a minority of children demonstrate a plateau in VO_2 during treadmill or cycle ergometer exercise (Armstrong et al., 1996; Rowland, 1993), and the term VO_{2peak} is thus used to indicate a maximum test in the absence of a plateau (McManus & Armstrong, 2018). Failing to demonstrate a plateau has been suggested to be due to insufficient motivation or low tolerance for discomfort on the part of the participant (Falk & Dotan, 2019). This may result in a VO_{2max} that is not truly the participant's maximum (Day, Rossiter, Coats, Skasick, & Whipp, 2003; Falk & Dotan, 2019). A selection of secondary criteria (e.g. predicted values of respiratory exchange ratio (RER), maximal heart rate (HR_{max}) and blood lactate at VO_{2max}) have been proposed to provide assurance that VO_{2max} has been achieved (Poole, Wilkerson, & Jones, 2008).

Armstrong et al. (1996) reported that only 39% of the girls and 35% of the boys demonstrated a VO_2 plateau in a study involving forty boys and girls aged 9.9 (0.4) years to determine peak VO_2 while running on a treadmill. The test was terminated at the point of voluntary exhaustion and accepted as maximal if the child showed signs of intense effort, a heart rate near or above 200 beats·min^{-1}, or RER was at least unity. A plateau was defined as either ≤ 2 mL·kg·min^{-1} or ≤ 5% or ≤ 150 mL·min^{-1} increase from the final minute of the second to last stage until the last minute of the final stage (Armstrong et al., 1996) The authors found no evidence for differences in VO_{2peak} between those demonstrating a plateau and those who did not.

However, using secondary criteria to validate whether VO_{2max} is reached is controversial since the maximal values of these criteria may vary between populations (Poole & Jones, 2017). Poole and Jones (2017) question the use of HR_{max} and maximal RER. In children (8 to 18 years) of different ethnicity, average HR_{max} values have been shown to vary from 185 beats·min^{-1} in North American children (Cooper, Weiler-Ravell, Whipp, & Wasserman, 1984) to 205 beats·min^{-1} in Scandinavian children (Åstrand et al., 2003). Poole et al. (2008) investigated the validity of secondary criteria in establishing VO_{2max} during ramp exercise tests in eight 27(4)-year-old healthy males. Five out of eight participants did not demonstrate a plateau, and three did not meet the HR_{max} criteria. Of these three participants, two demonstrated a plateau and two achieved RER values >1.15. One participant did not meet any of the secondary criteria; however, a pronounced plateau in VO_2 was demonstrated. The authors concluded that applying secondary criteria to terminate a test can result in underestimating VO_{2max}, and in falsely rejecting participants who actually achieve VO_{2max} (Poole et al., 2008). Sufficient motivation and maximal effort seem to be key factors in a single ramp incremental test to obtain true VO_{2max} values without demonstrating a plateau. Day et al. (2003) found that VO_{2peak} (no plateau) during a maximum-effort incremental test was likely to be a valid index of VO_{2max} provided the participants exercised to the limit of their tolerance. This is in line with what Poole and Jones (2017) concluded in their review on the

topic. They advise not accepting VO_{2peak} as a maximum value measured during ramp incremental exercise unless the participants are familiar with maximal exercise testing and are highly motivated. However, even in tests with highly motivated participants, one can never be sure of having reached maximal VO_2 without evidence from additional tests (Day et al., 2003).

VO_{2max} and scaling

Physiological capacities are dependent on body and system dimensions and there is a close relationship between VO_{2max} and body size (Armstrong & Welsman, 1994). In the literature, VO_{2max} is commonly expressed in absolute values in non-weight-bearing exercise or as a simple ratio to BM in weight-bearing exercise (Loftin, Sothern, Abe, & Bonis, 2016). During childhood and adolescence there are great changes in body size and body composition, and it is therefore essential to control for differences in body size when comparing VO_{2max} in different individuals. However, simply dividing by BM will not remove the effect of body size (Welsman & Armstrong, 2019). Expressing VO_{2max} relative to BM has been questioned over the years because it assumes that $VO_{2\,max}$ changes proportionally with changes in BM (Rowland, 2005). VO_{2max} has been found to be nonlinearly related to BM (Geithner et al., 2004), and relating VO_{2max} to BM may lead to an overestimation in light individuals and penalize heavy individuals (Armstrong & Welsman, 2002). To overcome this problem, allometric scaling using a mass exponent has been suggested to be a more correct way to remove the effect of body size (Welsman & Armstrong, 2019). In allometric scaling, the size of the mass exponent is mathematically established and based on geometric rules such as "as the volume of a body is increased, its surface does not increase in the same proportion, but only in proportion to the two thirds power of the volume" (p.13) (Schmidt-Nielsen, 1984) and the theoretically computed mass exponent of 0.67 is often seen used in the literature. However, in studies with children and adolescents, several different mass exponents have been proposed (Loftin et al., 2016; Welsman & Armstrong, 2019). Welsman and Armstrong (2019) looked at a large number of cross-sectional studies and reported mass exponents ranging from 0.37 to 0.94 in boys and 0.45 to 0.76 in girls. Finding the correct mass exponent depends on factors such as sample size, BM, body composition, age and sex (Welsman & Armstrong, 2019) and therefore it is most likely sample-specific, and may vary between samples (Falk & Dotan, 2019). Because of this wide variety of mass exponents and the lack of a universal mass exponent, some investigators have recommended expressing VO_{2max} relative to FFM instead (Falk & Dotan, 2019; Loftin et al., 2016). FFM and FM are main constituents of BM and as only FFM is metabolically active and directly contributes to oxygen uptake, FM can be merely regarded as "dead weight" (Falk & Dotan, 2019). Loftin et al. (2016)

argues that scaling VO_{2max} for FFM is probably the best normalizing factor for comparing groups with different body composition.

VO_{2max} and age

When VO_{2max} is expressed in relation to age in absolute values ($l \cdot min^{-1}$), VO_{2max} increases in a near-linear manner with age in boys, while it tends to level off in girls after age 13 (Armstrong & Welsman, 2019a; Kemper & Verschuur, 1987). However, some researchers have suggested a non-linear increase in VO_{2max} with age (Geithner et al., 2004; Mirwald, Bailey, Cameron, & Rasmussen, 1981). Geithner et al. (2004) followed 105 twin pairs longitudinally from the ages of 10 to 18 years to investigate growth in peak aerobic power during adolescence. Longitudinal analyses indicated a growth spurt in VO_2 in both boys and girls, on average, at around the same time as PHV and of greater magnitude in boys (Geithner et al., 2004). In early childhood there is little difference between boys and girls. Thereafter, boys tend to have a higher VO_{2max} at all ages and the sex difference increases as children progress through puberty. At the age of 18 years, boys have a VO_{2max} about 40% higher than girls (Armstrong & Welsman, 1994). The sex difference in the development of VO_{2max} with age makes it clear that boys and girls cannot be treated as one group, and Armstrong (2019) points out the importance of not reporting VO_{2max} data from mixed sex groups of youths, whether athletes or non-athletes.

VO_{2max} and training

In healthy young adults, VO_{2max} may vary by more than 100% between a sedentary person and an athlete in a typical endurance sport (Joyner & Coyle, 2008). This variation in VO_{2max} is partly an effect of genetics and partly results from environmental factors; mainly physical activity and training.

The trainability of VO_{2max} in adolescents is still controversial, even though the question has been addressed using a variety of approaches over several decades (Dotan, 2017). Some authors conclude that proper endurance training in prepubertal and circumpubertal children affects VO_{2max} even if the effect is lower than in adults (Armstrong & Barker, 2011; Baquet, van Praagh, & Berthoin, 2003), while others claim that there is a maturational threshold below which children are not able to increase their VO_{2max} (Katch, 1983). Katch (1983) suggested that children's ability to respond to exercise was poor or not present until after puberty and that the poor response was due to low concentrations of growth hormones and sex hormones. The hypothesis is that children have a critical phase ("trigger point") that often coincides with puberty. Prior to this, children will show little or no effect due to exercise. The trigger effect suggests that the

hormones initiating puberty have a modulating effect on functional development and physiological adaptation. The hypothesis claims that certain criteria must be satisfied for physiological adaptation to take place. These may include the relationship between FM and FFM, maturation of the neuromuscular system, and certain levels of endocrine function, where sex and growth hormones play an important role. Katch (1983) emphasizes that it does not mean that there are no changes in children before puberty, but that functional changes and adaptations occur as a result of natural growth and development, and not as a result of exercise. He suggests that exercise before puberty should consist of practicing skills and techniques rather than, for instance, endurance training. The hypothesis of a potential maturational threshold for the effects of endurance training has been challenged over the years. However, evidence to refute the hypothesis is limited. Most of the evidence suggests that training does have effects on VO_{2max}, but the effect is less than in adults (Dotan, 2017). Discrepancies between studies may be due to different study designs as well as training protocols (Armstrong & Barker, 2011; Tolfrey, Campbell, & Batterham, 1998).

Endurance training in adults generally increases VO_{2max}, although trainability varies between individuals and may be zero in some (Bouchard et al., 1999). Experimental interventions, with pre- and post-training measurements, are the most common approach when investigating the effects of training in adults. In children and adolescents, several approaches have been used. Training interventions are difficult to perform in children for many reasons and relatively few randomized controlled training studies have been carried out (Armstrong & Barker, 2011; Baquet et al., 2003). In cross-sectional studies, it has been shown that endurance-trained children have higher VO_{2max} values than non-endurance-trained children (Fernhall, Kohrt, Burkett, & Walters, 1996; Maffulli, Testa, & Lancia, 1991; McNarry, Welsman, & Jones, 2012; Rusko, Rahkila, & Karvinen, 1980). However, cross-sectional studies cannot establish whether this is due to endurance training, initial selection or both.

In observational cohort studies, the development of factors of interest can be compared between training groups and non-training groups over a period of years. Again, relatively few of these cohort studies have been conducted and those that have been carried out have involved low numbers of participants, especially for girls. In Norway, a possible challenge with this approach may be recruiting inactive children to the non-training group. Reports from Statistics Norway (StatisticsNorway, 2013) showed that in 2013, the number of hours spent engaged in physical activity outside school hours varied between 8 and 9 hours per week for boys and girls aged 10-12 years.

Understanding the effect of endurance training during puberty is important for future health effects, both for coaches when designing training programs, and for developing successful athletes.

Hematologic parameters

Before puberty there are no major differences between girls and boys in hemoglobin concentration [Hb], hematocrit (Hct), or serum ferritin concentrations (Valberg, Sorbie, Ludwig, & Pelletier, 1976). Towards puberty, the sex differences become gradually more apparent and after the onset of menstruation the gap between the sexes increases. Boyadjiev and Taralov (2000) found sex-related differences in red blood cell count (RBCC), packed cell volume and Hct in athletes, as well as untrained 14-year-old boys and girls. After the age of 20, the differences are distinct, with higher values of [Hb] and Hct and significantly higher serum ferritin concentrations in males than in females (Valberg et al., 1976). Testosterone is believed to play an important role in the higher values seen in males, as testosterone is known to stimulate both the production of red blood cells (RBC) and iron incorporation into erythrocytes (Shahani, Braga-Basaria, Maggio, & Basaria, 2009). Menstruation and insufficient intake of dietary iron are the main factors responsible for inadequate or depleted iron stores during growth in young individuals (Valberg et al., 1976). Iron-deficiency anemia is associated with depleted iron stores, and the serum ferritin concentration in healthy adults reflects the size of the body's iron stores (Siimes, Addiego, & Dallman, 1974).

Hemoglobin mass and blood volume

According to Fick's equation, VO_{2max} is determined by the oxygen supply in the blood and by the oxygen consumption of skeletal muscle (Schmidt & Prommer, 2010). Total hemoglobin mass (Hbmass) in combination with total blood volume (BV) determines the [Hb] and thus the O_2 transport capacity of blood (Schmidt & Prommer, 2010).

Several studies have shown that there is a close relationship between total Hbmass, BV and VO_{2max} (Convertino, 2007; Gore, Hahn, Burge, & Telford, 1997; Heinicke et al., 2001; Åstrand, 1952). A change in total Hbmass of 1 $g \cdot kg^{-1}$ is associated with a change in VO_{2max} of 4.4 $mL \cdot kg^{-1} \cdot min^{-1}$, and there is no difference between males and females (Gore et al., 1997; Schmidt & Prommer, 2008). A change in BV of 1 $mL \cdot kg^{-1}$ is related to a change in VO_{2max} of 0.7 $mL \cdot kg^{-1} \cdot min^{-1}$, with no difference between males and females (Convertino, 1991; Schmidt & Prommer, 2008).

Hemoglobin mass and blood volume and age

Total Hbmass and BV increase with age during childhood and adolescence because of natural growth and maturation, and the growth curve for Hbmass follows the general pattern for body mass (Åstrand, 1952). The values of absolute Hbmass and BV are similar for boys and girls up to 12-13 years. After that, boys' absolute Hbmass and BV continue to rise in a linear manner with age during the pubertal years, while for girls the increase is considerably smaller and levels out at around 14-15 years of age (Åstrand, 1952). When related to BM, Hbmass and BV in boys increase, while there is no corresponding increase for girls during puberty (Åstrand, 1952). However, Åstrand emphasizes that the results should be interpreted with caution because of the small groups and low numbers of girls, in addition to large individual differences (Åstrand, 1952). The sex difference is often explained by the expansion of plasma volume (PV) and increase in RBCC that appears around the time of puberty due to the concomitant rise in testosterone levels in boys (Malina et al., 2004; Shahani et al., 2009).

Relating BV to BM is theoretically unsatisfactory, according to the International Committee for Standardization in Haematology (ICSH) ("Recommended methods for measurement of red-cell and plasma volume: International Committee for Standardization in Haematology," 1980) because the relationship between BV and BM varies according to body composition. The ICSH concluded that FFM might be a more appropriate anthropometrical reference for Hbmass than body mass. This was subsequently confirmed by Schumacher, Ahlgrim, and Pottgiesser (2008), who found a better correlation between Hbmass and FFM ($r=0.81$) compared with Hbmass and BM ($r=0.77$) in adult male athletes, and recently by Prommer et al. (2018) in endurance trained children ($r=0.959$). Prommer et al. (2018) concluded that development of FFM largely explains the development of Hbmass and BV in children.

Hemoglobin mass and blood volume and training

Whether endurance training during the pubertal years will result in further increases in Hbmass and BV is not clear. Cross-sectional studies on adults show that highly trained endurance athletes have up to 40% higher levels of Hbmass and BV than non-endurance trained athletes and untrained individuals (Heinicke et al., 2001; Schmidt & Prommer, 2008; Steiner & Wehrlin, 2011). However, we still don't know whether the higher levels are due to endurance training, genetic predisposition or both, and the mechanisms behind the higher levels of Hbmass are not yet fully understood (Convertino, 2007; Montero & Lundby, 2018).

Only a few longitudinal studies have looked at the effect of endurance training on the development of Hbmass in adolescent boys and girls (Eastwood, Bourdon, Withers, & Gore, 2009; Prommer et al., 2018; Steiner, Maier, & Wehrlin, 2019; Ulrich, Bärtsch, & Friedmann-Bette, 2011). Eastwood et al. (2009), Steiner et al. (2019) and Ulrich et al. (2011) found no effect on Hbmass related to BM with endurance training, while Prommer et al. (2018) suggested that endurance training may have an effect beyond what could be expected from growth and maturation. Some of these studies have methodological challenges, including the use of mixed sex groups (Eastwood et al., 2009; Prommer et al., 2018) and large age spans at inclusion, such as 11-15 years (Eastwood et al., 2009), and 7.6-11.5 years (Prommer et al., 2018). Since girls and boys have different physiological features during puberty, this may influence the results. Only one study had a separate group for girls (Ulrich et al., 2011) but the age at inclusion was 15 years. They concluded that changes in FFM were the main factors in increasing Hbmass over time, but that training volume had an additional effect.

Cardiac dimensions

According to the Fick equation, VO_2 can be calculated as the product of cardiac output (heart rate x stroke volume) and arterial venous oxygen difference. Maximal heart rate and the arterial venous oxygen difference at maximal exercise have been shown to be independent of aerobic fitness in healthy children, and therefore the differences in VO_{2max} between individuals (or within the same child over time) are solely determined by variations in maximal stroke volume (Rowland, 2013; Rowland, Kline, Goff, Martel, & Ferrone, 1999). Elite endurance athletes are characterized by a large, compliant heart with a compliant pericardium, which can accommodate a lot of blood to generate a large stroke volume (Levine, 2008). A large stroke volume reflects the size of the left ventricular (LV) end-diastolic filling volume (Rowland, 2013).

Cardiac dimensions and age

The development and growth of the heart during childhood and puberty follows the growth of the body (de Simone, Devereux, Daniels, Koren, et al., 1995; de Simone et al., 1998). de Simone, Devereux, Daniels, and Meyer (1995) studied 424 children and young adults of both sexes and found very small sex differences in LV mass before puberty. At the time of puberty, the sex difference in LV mass became evident, with boys experiencing a larger increase compared with girls. There was a proportional increase in chamber dimensions and wall thickness, and therefore, the no-sex difference in relative wall thickness was retained. While the relationship between body size and heart size is greatest during childhood, it gradually decreases with age during body

growth until the maturation of the body is completed. After puberty, sex differences and cardiac workload will have increasingly greater impacts on LV mass (de Simone et al., 1998). Sex differences in adult LV mass are mostly related to differences in body size because of the greater LV hypertrophy in men than in women (de Simone, Devereux, Daniels, & Meyer, 1995).

Janz, Dawson, and Mahoney (2000) found that in both boys and girls, change in height, weight and FFM were all strongly associated with change in LV mass. They also observed that change in VO_{2peak} and testosterone strongly correlated with change in LV mass in boys, but not in girls. However, increased FFM was found to be the primary body size determinant of heart growth, and the rate of change in LV mass was greater in boys than girls (Janz et al., 2000). The authors reported LV mass to increase by 62% between the ages of 10 and 14 years in boys, whereas the increase was 48% in girls. Although FFM is the strongest determinant of heart size, normalizing cardiac dimensions to body surface area is a technique widely used in the literature. Within each age distribution there is a great variety of body sizes, and it has therefore been suggested that cardiac dimensions in children should be normalized to body surface area, which incorporates both height and BM (Epstein, Goldberg, Allen, Konecke, & Wood, 1975).

Cardiac dimensions and training

'Athlete heart' is a term used to describe the morphological and functional changes in the heart after long-term conditioning observed in highly trained competitive athletes (Maron, 1986). These changes include increased LV wall thickness, LV mass, and increased LV and right ventricular (RV) chamber size (Huston, Puffer, & Rodney, 1985). This has been extensively studied in adults, and the degree of cardiac change is found to be largely dependent on the amount of endurance training (Pelliccia et al., 2018). Studies have shown that children (aged 5-17 years) engaged in endurance training have demonstrated morphological changes in the heart; however, a majority of the children in these studies experienced an increase in LV wall thickness without a significant increase in LV chamber size (Allen, Goldberg, Sahn, Schy, & Wojcik, 1977; Geenen, Gilliam, Crowley, Moorehead-Steffens, & Rosenthal, 1982), which is defined as concentric remodeling. Whether the adult-child difference in cardiac adaptation seen in these studies was due to differences in the effects of endurance training because of differences in relative training load, or differences in number of years of training, is not known.

Purpose

The overall aim of this thesis is to establish, using a longitudinal design during puberty, the association between growth, especially the development of fat-free mass (as a substitute for muscle mass) and development in heart dimensions, intravascular volumes and VO_{2max} in girls and boys and to investigate the effects of the inclusion of high volumes of endurance training in active children.

The specific aims are

1. To describe the development of VO_{2max} and intravascular volumes in girls and boys during puberty.
2. To investigate whether a high volume of endurance training affects the development of VO_{2max}, intravascular volumes and heart dimensions in girls and boys during puberty.
3. To determine whether the increase in heart size and Hbmass affect the development of VO_{2max} when controlling for growth.
4. To validate bioelectrical impedance analysis (BIA) using InBody 720 against dual-energy X-ray absorptiometry (DXA) in estimating body composition.

Methods

The present thesis is based on one study, which is presented in the thesis as four original scientific papers.

Study design

Paper I

Using a repeated-measures design, we assessed anthropometrics, VO_{2max}, sexual maturity, predicted age at peak height velocity (PHV) and the amount of training in 78 healthy children over a period of three years. All tests were performed on one day on each testing occasion, and the participants were tested three times (at ages 12.1 (0.4), 13.4 (0.3), and 15.3 (0.3) years).

Paper II

Using a repeated-measures design, we assessed anthropometrics, Hbmass, hematological variables, sexual maturity, predicted age at PHV and amount of training in 76 healthy children over a period of three years. All tests were performed on one day and the participants were tested three times (at ages 12.1 (0.4), 13.4 (0.3), and 15.3 (0.3) years).

Paper III

Using a repeated-measures design, we assessed anthropometrics, VO_{2max}, cardiac structure and morphology, sexual maturity, predicted age at PHV and amount of training in 48 healthy children over a period of three years. All tests were performed on one day, except for the cardiac measurements which were performed on a separate day. The two test occasions took place within one week. All the participants were tested twice (at ages 12.1 (0.2) and 15.3 (0.3) years).

Paper IV

This study had a cross sectional design and can be characterized as a method comparison study, including analyses of reliability (test vs. retest of BIA) and validity (BIA vs. DXA). At both Test and Retest all BIA and DXA measurements took place within 30 minutes on the same day for each participant and time between Test and Retest varied from 5 to 14 days. 18 participants (aged 15.6 (0.3) years) were tested on day one, while only 17 participants came back to be retested due to illness in one person. All measurements were performed in the afternoon without monitoring food intake or hydration status.

Participants

One hundred and twenty-six 12-year-old children engaged in endurance training (93% cross country skiers) and 48 children from other sports (95% team sports) volunteered to participate in the study. All these children were invited to come back for follow-ups at ages 13 and 15. Only participants for whom complete and valid data were available over the three years of the study were considered for papers I, II and III, respectively. This yielded a pure longitudinal sample of 78 children in paper I, 76 children in paper II and 48 children in paper III. The children were assigned to an endurance training group (End group) and a non-endurance training group (non-End group) based on the type of sport and the number of weekly hours of endurance training they practiced. Table 1 gives the number of subjects per group and per sex for each paper. Paper IV was a cross-sectional study with a different cohort of athletes, including 18 cross-country skiers (7 boys) at age 15.6 (0.3).

Table 1: Design and number of participants of the four included papers.

Paper		Participants		Design
		End	non-End	
I	N (boys)	40 (23)	38 (29)	Longitudinal (age 12, 13 and 15)
II	N (boys)	42 (24)	34 (23)	Longitudinal (age 12, 13 and 15)
III	N*	31	17	Longitudinal (age 12 and 15)
IV	N (boys)	18 (7)	0	Cross sectional (age 15)

** Girls and boys together.*

Table 2 shows the subject characteristics of those from each group who only participated at the age of 12. There were no differences in anthropometrics between those who came back for repeated measures and those who participated only once, except for the End-girls. The End-girls who dropped out had higher %FM than those who stayed on ($p=0.03$). VO_{2max} was lower among those who dropped out ($p \leq 0.034$), except for the non-End boys ($p=0.441$)

Table 2: Subject characteristics at age 12 for those who did not come back for repeated measures at age 13 and 15.

	BM (kg)	Height (cm)	Fat mass (%)	VO2max ($mL \cdot min^{-1}$)	VO2max ($mL \cdot kg^{-1} \cdot min^{-1}$)
End-girls	44.2 (5.6)	155.8 (6.5)	14.1 (3.0)	2380 (304)	54.0 (5.5)
End-boys	41.1 (6.8)	153.2 (6.9)	11.5 (6.9)	2353 (318)	57.9 (6.7)
non-End girls	47.0 (11.4)	154.0 (8.4)	21.9 (7.2)	2274 (501)	48.4 (4.3)
non-End boys	44.6 (10.3)	153.3 (6.1)	18.6 (11.7)	2407 (385)	54.4 (9.4)

Ethics

Written parental consent was obtained prior to any testing. All experimental procedures were approved by the Norwegian Regional Committee for Medical Research Ethics and conformed to the standards set by the Declaration of Helsinki. All participants received age-appropriate information in writing ahead of participation, which was repeated orally by the test leader upon arrival and prior to testing.

Methods and test procedures

Anthropometry and sexual maturity

Anthropometry: In all four papers all measurements were conducted with the participants wearing shorts, t-shirt and no shoes. Stature and sitting height were measured to the nearest 0.1cm using a stadiometer (Seca, Hamburg, Germany) and BM to the nearest 0.1 kg using a digital scale (Seca, Hamburg, Germany). Sitting height was used to predict years from PHV (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002).

Sexual maturity: All participants underwent a brief health check by a medical doctor. In girls, breast development was assessed according to Tanner (Tanner, 1989) and they were asked about menarche. In boys, blood samples were analyzed for testosterone. Chronological age was calculated as the difference between date of birth and date of testing.

Body composition

Bioelectrical impedance analysis (BIA)

In papers I, II and IV, body composition was assessed by BIA using InBody 720 (InBody, 720, Biospace Co, Ltd, Seoul, Korea) according to the manufacturer's guidelines. The participants were standing barefoot with the whole of the soles of the feet in contact with the foot electrodes. The hand electrodes were held with the thumb placed lightly on top of the thumb electrode and the other fingers touching the other electrode. Arms were held at angle of approximately 15 degrees between the arms and the sides of the body. This position was maintained until measurements were completed (~2 minutes). The manufacturer's built-in equation was used for the calculation of body composition.

Dual-energy X-ray absorptiometry (DXA)

In paper IV body composition was also assessed by DXA and was carried out using a Lunar iDXA (GE Healthcare, Madison, Wisconsin, USA) according to the manufacturer's guidelines. Each day prior to testing, the DXA was calibrated according to the manufacterer's instructions using a standard calibration block. Participants were scanned from head to toe in a supine position, providing values for total lean tissue and fat mass. The DXA machine automatically chose the scanning mode, with all the participants scanned in the standard mode. All Lunar DXA scan files were automatically analyzed with enCORE software version 14.10.022 (GE).

Description of training groups for papers I, II and III

In papers I, II and III, each year the participants completed a questionnaire to assess the types of sports participation, number of years participating in each sport and the number of weekly training hours. At 15 years of age, we also interviewed the participants in order to get a more detailed picture of weekly training content. The total training volume (number of training hours) was divided into endurance training and other types of training. The endurance training was continuous or interval workouts using running, skiing or biking. Based on the type of sport they practiced, and the volume of endurance training reported at age 15, we assigned the participants into an End group and a non-End group, (called *Athletes* and *Former* athletes, respectively, in paper III, all of whom were recruited from cross-country ski clubs). Those who performed more than 5 hours endurance training per week became the End group. None of the children doing other sports (mainly team sports) reached this volume of endurance training. Thus, these children and the former cross-country skiers who were no longer endurance athletes served as the non-End group.

Training volume, papers I, II and III

Volumes of total training and endurance training are presented in Table 3. Both training groups participated in organized sport. In the non-End group, most of the training was focused on technical and tactical skill development, whereas in the End group, the training became gradually more focused on endurance training over the years. At age 14-15, the End group performed typical endurance training (continuous or interval workouts using running, skiing or biking) for more than 5 hours per week.

Table 3: Volume of total training and endurance training in papers I, II and III.

		Total volume of organized sport (hours)		Volume of endurance training (hours)	
		End-group	non-End group	End-group	non-End group
Paper I	Age 12	7.0 (2.3)	6.3 (2.0)		
	Age 15	10.6 (2.9)*	8.1 (4.0)	7.2 (1.8)*	1.5 (1.2)
Paper II	Age 12	7.1 (2.3)*	5.8 (2.1)		
	Age 15	10.7 (2.7)*	7.6 (3.6)	7.3 (1.9)*	1.0 (0.8)
Paper III	Age 12	6.6 (2.1)	7.4 (1.7)		
	Age 15	10.3 (2.2)	8.5 (5.6)	7.4 (1.9)*	1.9 (1.7)

**denotes significant difference between groups (p<0.05). End, endurance; non-End, non-endurance.*

Description of participants, paper IV

In this cross-sectional study, 18 cross-country skiers (11 girls) underwent body composition assessment at mean (SD) age 15.7 (0.3) and 15.5 (0.3) years for boys and girls, respectively ($p = 0.213$). All participants were included in the validity analyses. One boy did not complete the second BIA assessment; thus, 17 participants were included in the reliability analyses. The physical characteristics of the participants are reported in Table 1, Paper I. Fat mass (in kg and percent) was significantly higher in girls compared to boys. The other physical characteristics did not differ significantly between the sexes.

Venous blood samples

Emla cream (AstraZeneca 55, Lidocain 25 mg/g, Prilocain 25 mg/g) was used as a topical anesthetic before venipuncture to reduce pain and distress for the participants. Blood samples were drawn from an antecubital vein into 4 mL EDTA glass tubes (EDTA glass, BD vacutainer K2E 7,2 mg) and 5 mL serum gel tubes (VACUETTE® TUBE 5 mL Z Serum Separator Clot Activator). The EDTA coated tubes were sent to a medical laboratory, (Fürst, Oslo, Norway) the following morning and analyzed for [Hb], RBCC, Hct and mean red cell volume (MCV) (Sysmex XN-9000 Automation, Sysmex Corporation, Kobe, Japan). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing [Hb] by Hct. The serum tubes were left to rest for at least 30 min before centrifuging at 3500 G for 10 minutes at 4°C. The serum was then transferred to Eppendorf tubes and frozen. All samples were stored at -80°C until analysis. When all the samples had been collected, the serum tubes were sent to a medical laboratory, (Fürst, Oslo, Norway) and analyzed for serum ferritin (Advia Chemistry XPT, Siemens Medical Solutions Diagnostics, Japan) and testosterone (Advia Centaur XPT, Siemens Healthcare

Diagnostic Inc., USA). In four out of 88 participants, venous blood samples were missing either due to technical problems analyzing the samples or the participants' aversion to blood tests.

Exercise testing

VO_{2max} was determined by an incremental running test to exhaustion on a treadmill (Woodway Elg 70 or PPS 55, Weil am Rhein, Germany). The protocol was the same for all three years and each participant was tested with the same equipment each time and by the same experienced test leader. Before the incremental test, the participants warmed up for 5 minutes at an incline of 5.3% and at a speed of 8 $km \cdot h^{-1}$. In all three years and for all participants, the incremental test started at incline 6.3% and speed 7 $km \cdot h^{-1}$ and both incline and speed were increased by 1% and 1 $km \cdot h^{-1}$ every minute until a speed of 11 $km \cdot h^{-1}$ was reached. For further increases in intensity, only the incline was increased (1% per minute). The test was terminated when the participant could no longer complete the desired workload despite vigorous verbal encouragement. A facemask (Hans Rudolph Instr., USA) was used during the test and oxygen uptake was measured continuously with an automated system (Oxycon Pro, Jaeger-Toennis, Hochberg, Germany or Moxus Modular Metabolic System, AEI Technologies Inc., Pittsburgh, USA). Heart rate was measured continuously (Polar RS800; Polar Electro Oy, Kempele, Finland). The exercise test was considered maximal if clear signs of maximal effort such as sweating, facial flushing and unsteady gait were demonstrated and, despite strong verbal encouragement, the participant was unwilling or unable to continue. In addition this was supported by a RER greater than 1.0 (McManus & Armstrong, 2018). The highest 60-s averaged oxygen uptake achieved on the test was accepted as VO_{2max}. Time to exhaustion (TTE) was defined as the total number of minutes the participants ran during the maximal test (measured from the start of the incremental test to the time at which the test was terminated).

Assessment of total hemoglobin mass and blood volume parameters

Hbmass was assessed using the optimized carbon monoxide rebreathing method as described in detail by Prommer and Schmidt (2007). In brief, a bolus of carbon monoxide (CO) was inhaled and rebreathed for 2 min through a closed circuit consisting of a glass spirometer (Blood Tec Gbr, Bayreuth, Germany) and a 3-litre anesthetic bag containing 100% oxygen (O_2). The administered amount of CO was individually calculated to 1.0 and 0.8 $mL \cdot kg^{-1}$ of body mass for boys and girls, respectively. During CO rebreathing, CO leakage was checked at the mouthpiece, nose clip and spirometer using a portable CO-gas analyzer (Dräger-Pac 7000, Dräger Safety AG

Co, Lübeck, Germany). Arterialized capillary blood samples (125µl) were taken from a pre-warmed fingertip before and at 6 and 8 minutes after commencing the rebreathing and immediately analyzed twice for percent carboxyhemoglobin (%HbCO) using a diode array spectrophotometer (ABL80 FLEX CO-OX, Radiometer, Copenhagen, Denmark). From the difference in %HbCO before and after the CO application, the Hbmass was calculated as described by Schmidt and Prommer (2005). BV, PV and RCV were calculated from Hbmass using venous [Hb] and venous Hct according to Burge and Skinner (1995) and Heinicke et al. (2001).

Before any actual measurements, all participants were familiarized with the rebreathing procedure, with air applied instead of CO, until they fully mastered the technique.

All measurements were done in the same order, i.e. always VO_{2max} before the Hbmass measurement. This was done to avoid reductions in maximal performance due to blocking of the oxygen binding sites by CO (Prommer et al., 2018).

Echocardiography

In paper III both at baseline and at follow-up all participants underwent an echocardiographic study (Vivid E9, GE, Vingmed, Horten, Norway). Using two-dimensional (2D) echocardiography, LV dimensions were assessed. Parameters were measured according to the recommendations of the European Association of Cardiovascular Imaging (EACVI) (Lang et al., 2015), including indexing all chamber dimensions to body surface area. LV mass was calculated using the Devereux formula (Devereux et al., 1986). LV geometry was assessed by calculating relative wall thickness (RWT) as two times left ventricular posterior wall thickness in diastole divided by left ventricular diastolic diameter (2*LVPWd)/LVIDd). LV volumes, EF and mass were also calculated from three-dimensional (3D) assessments.

Statistical analyses

Papers I and II

A three-way mixed ANOVA was run to examine the effects of sex, training group (group) and age on the different variables. Data are mean (standard deviation) unless otherwise stated. A Shapiro-Wilk test ($p > 0.05$) was used to test whether the variables for the different groups and time points (78 dataset) were normally distributed. In 73 out of 78 datasets, the variables were normally distributed. Testosterone at age 12 and 13 was not normally distributed. For unpaired

comparisons, the Student's *t*-test was run when data were normally distributed, and a Mann-Whitney U Test was used when data were not normally distributed. GraphPad Prism 8.2.1 (GraphPad Software Inc., La Jolla, CA) and Microsoft Excel 2013 were used for statistical analyses.

Paper III

Analyses were carried out using standard statistical software (SPSS version 21, SPSS Inc., Chicago, IL, USA) and Stata 15.0 (StataCorp, LLC, Texas, USA). Data were presented as mean (SD), and numbers and percentages, respectively. The Student's *t*-test were used to determine differences between two groups at baseline and follow-up. Linear mixed models were used to assess the impact of training group on VO_{2max}, LV end-diastolic volume (EDV), LV end-systolic volume (ESV) and RWT. For intra-individual changes from baseline to follow-up, a paired *t*-test was used.

Paper IV

Data are mean (standard deviation) unless otherwise stated. GraphPad Prism 8.2.1 (GraphPad Software Inc., La Jolla, CA), Microsoft Excel 2013 and SPSS (Version 25.0; IBM Corporation, Armonk, New York) were used for statistical analyses.

Between-method differences for FFM, FM and %FM were calculated by subtracting InBody 720 from DXA (DXA-InBody 720). Test-retest reliability was examined by using mean difference ±95% limits of agreement (LoA) including Bland-Altman plots. Intraclass correlation coefficients (ICC 3,1-single measures) with 95% confidence intervals (CI) were calculated, and the following criteria were adopted for interpreting the strength of the correlation (ICC) between the measures:, < 0.5, poor; 0,5 - 0.75, moderate; 0.75 - 0.90, good ; 0.90-1.0, excellent (Koo & Li, 2016). The coefficient of variation (CV= SD of the differences between test and retest divided by √2 divided by the pooled mean of the variable times 100%) was also calculated. Validity was examined using mean difference ±95% LoA including Bland-Altman plots and Pearson's correlation coefficient (*r*). The following criteria were adopted for interpreting the strength of correlation (r) between the measures: <0.1, trivial; 0.1– 0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very large; and 0.9–1.0, almost perfect (Hopkins, Marshall, Batterham, & Hanin, 2009). For paired comparisons between test-retest and mean difference between BIA and DXA, a paired Student's *t*-test was run. To test for differences in age and anthropometrics between boys and girls, an unpaired Student's *t*-test was run. Statistical significance was set at $p<0.05$.

Additional analysis

Multiple regression analyses were performed to assess the effects of FFM, LV EDV and Hbmass on VO_{2max}. Multicollinearity was evaluated by a variance inflation factor (VIF) and a value of VIF > 5 indicated excessive multicollinearity. GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA) was used for statistical analyses.

Results and discussion

Detailed results and discussion of how VO_{2max}, intravascular volumes and heart dimensions change during puberty, differences between active girls and boys, and the effects of including high volume of endurance training in their weekly physical training, are presented in papers I, II and III. In paper IV, the estimation of fat-free mass based on bioelectrical impedance analysis with InBody 720 is validated against dual-energy X-ray absorptiometry.

In this chapter, the main findings of the four papers are presented and discussed according to the aims of this thesis. One main broad finding of the included papers is that structural changes (i.e. changes in hematological variables and heart dimensions) as well as changes in VO_{2max} are highly associated with growth; specifically, with changes in FFM. Therefore, this thesis includes additional analyses to those presented in the papers to analyze whether the increase in heart size and Hbmass affect the development of VO_{2max} when controlling for growth.

One main aim was to investigate whether a high volume of endurance training affects the development of VO_{2max}, intravascular volumes and heart dimensions in girls and boys during puberty. It turned out that the training groups differed to some degree, with regard to the main anthropometrics, and for boys with regard to maturation. Before the discussion related to the aims, the training groups will therefore be described, and the differences discussed.

Subject characteristics: papers I and II

Anthropometrics

The participants' anthropometric variables at ages 12, 13 and 15 from both papers I and II (n=88, 55 boys) are presented in Figure 1. On average, at age 12 there were no sex differences in any of the anthropometrical measures. Sex differences became apparent with increasing age, and at age 15, boys were taller, had more FFM, less %FM ($p<0.001$ for all) and were heavier ($p=0.036$) than girls, while there was no sex difference in BMI (body mass index) at any age. BM increased on average by ~ 5.1 kg and ~6.1 kg per year in girls and boys respectively, which was accompanied by a yearly increase in FFM of ~3.6 kg and ~6.0 kg in girls and boys, respectively. The average yearly increase in FM was ~1.5 kg in girls ($p<0.001$) and a non-significant increase of ~0.2 kg in boys ($p=0.087$). The marked sex difference in the development of FM and FFM resulted in a net increase of on average ~1.4% in %FM for girls and a decline of ~0.8% per year

in %FM for boys. In general, the sex differences are in line with previous studies (Loomba-Albrecht & Styne, 2009; Veldhuis et al., 2005). However, in both girls and boys in the current study the %FM was lower than that reported for this age group by Veldhuis et al. (2005).

The End- and non-End groups had, on average, similar heights ($p=0.899$) and FFM ($p=0.545$) at all ages, while BM was lower in the End group at age 12 ($p=0.022$) and tended to be lower at age 13 ($p=0.056$). On average the End group had a larger increase in BM compared with the non-End group ($p=0.033$). This group difference was only statistically significant when comparing the two groups of boys ($p=0.031$).

The End group had, on average, a lower %FM over the three years ($p=0.003$). There was a significant difference between End- and non-End boys in the change in %FM from age 12 to age 15, with a larger decrease in the non-End boys ($p=0.042$), while there was no such difference between the two groups of girls.

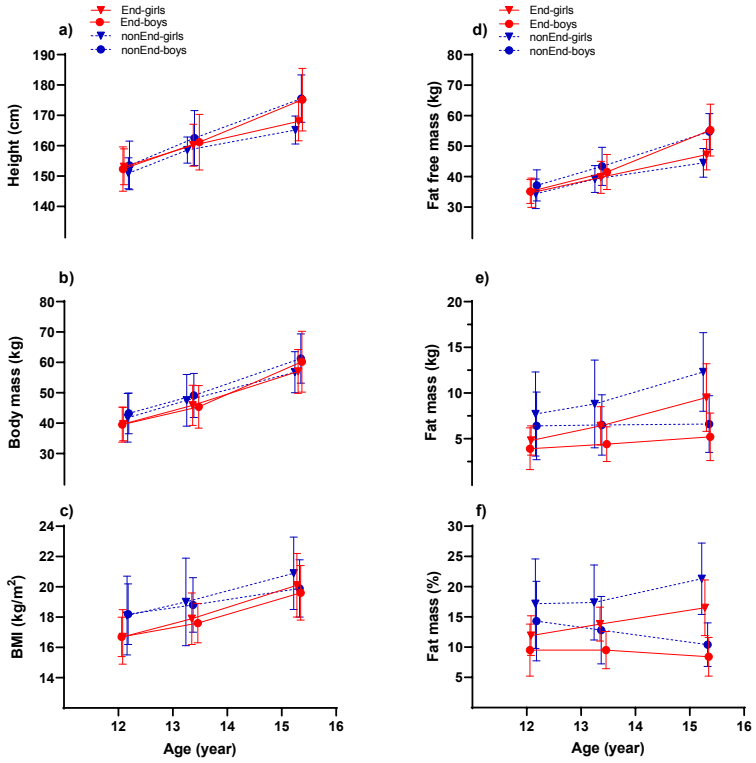

Figure 1: Anthropometric variables at age 12, 13 and 15 in boys and girls undertaking high (End girls and boys) and low (non-End boys and girls) volumes of endurance training, a) height, b) body mass, c) BMI (body mass index), d) fat free mass, e) fat mass and f) fat mass %.

Biological age

The girls were on average at the predicted age for PHV (APHV) (0.0 (0.5)) at the first examination, whereas the boys were 1.6 (0.5) years before predicted APHV. Seventeen out of 33 girls had passed their predicted APHV, and four had done so by more than 6 months (7 months) while none of the boys had reached their predicted APHV at the start of the study. At age 15, all the girls had passed their predicted APHV, and 96 % of the boys had done so. There was no difference between the training groups at any ages, in either APHV or in predicted years from APHV for either girls or boys.

At the start of the study, menarche had not occurred in any of the girls. Based on breast development, the Tanner stage was 1 or 2 in 29 out of 33 girls and 4 girls were at Tanner stage 3. At age 15 years, 93% of the non-End girls and 72% of the End girls had begun menstruation. Forty-seven out of 54 boys had S-Testosterone levels below 3.5 nmol·L^{-1} (100 ng·dL^{-1}) at age 12.

Levels of S-Testosterone were higher in non-End boys than in End boys at age 12 (2.1 (2.6) vs 0.7 (1.2) nmol·L^{-1}; p=0.019) at age 13 (5.6 (5.6) vs 2.6 (3.5) nmol·L^{-1}; p=0.025) and age 15 (12.2 (5.4) vs 7.7 (4.5) nmol·L^{-1}; p=0.002).

Although there was no difference in chronological age between any of the groups at any time during the study period, there seemed to be a difference in biological age between the End- and non-End boys. The non-End boys had higher levels of testosterone at all ages, which together with the differences in anthropometrics suggest that the End boys were on average later maturers compared with the non-End boys. The significantly greater BM in the non-End boys was mainly due to significantly greater FM. It has previously been reported that at the same chronological age, rapidly maturing children have larger amounts of FM, %FM and FFM (Guo et al., 1997). This supports our data indicating that the non-End boys were early maturers compared with the End boys; however, there was no difference between the groups in FFM at any age.

Both groups of boys showed increased BM over the three years, but only the End boys experienced a significant increase in FM ($p<0.001$), whereas FM on average stayed constant in the non-End boys (p=0.863). This may explain the 3 times higher rate of decline in %FM in the non-End boys compared with the End boys. In boys, it is expected that %FM levels off around PHV and then gradually declines (Veldhuis et al., 2005) which supports our result for both groups of boys. However, our results showed there was a difference between the groups and may suggest that our End boys were late maturers and possibly also a selected group.

There was no difference between the End- and non-End girls in BM, but the non-End girls had more FM at all ages, and thus also significantly higher %FM at all ages. However, there is no reason to believe that this difference in %FM between the groups was due to differences in biological age. In both groups of girls, none of the girls had begun menstruation and there was no difference between the groups in stage of breast development (on average stage 2), hence there did not seem to be a difference in biological age between the End- and non-End girls. The lower %FM in the End girls could indicate insufficient nutritional status, which was also supported by the low levels of serum ferritin (discussed later).

Significant differences between girls and boys in APHV at all ages ($p<0.001$) indicate that girls were more mature than boys throughout the entire study period. At age 12, girls were 0.0 (0.4) years from APHV and boys -1.6 (0.5) years from APHV. The sex difference of ~1.6 years in APHV agrees well with the literature (Malina et al., 2004).

Aim 1: Description of the development of VO_{2max} and intravascular volumes in girls and boys during puberty

VO_{2max} and the various hematological variables at ages 12, 13 and 15 are summarized in absolute values and relative to BM in Table 4 and relative to FFM in Figure 2.

Table 4: VO_{2max}, TTE and blood variables and in absolute values and relative to BM at age 12 (Test 1), 13 (Test 2) and 15 (Test 3) in girls and boys undertaking high (End boys and girls) and low (non-End boys and girls) volumes of endurance training.

	End girls (n=17-18)						non-End girls (n=9-11)					
	Test 1		Test 2		Test 3		Test 1		Test 2		Test 3	
VO_{2max} (mL·min^{-1})	2318	(341)#	2722	(360)	3099	(295)#	2293	(289)	2599	(254)#	3089	(408)#
VO_{2max} (mL·kgBM^{-1}·min^{-1})	59	(5.6)#	60	(4.4)#	55	(5.2)#	56	(6.5)	57	(4.7)	55	(4.0)#
TTE (min)	6.2	(1.6)	7.1	(1.3)	6.8	(1.5)	5.5	(0.9)	5.5	(0.9)	5.9	(0.9)
Hbmass (g)	398	(67)	474	(89)	558	(74)#	412	(62)	484	(67)	559	(72)#
Hbmass (g·kgBM^{-1})	10.0	(0.8)	10.3	(1.2)#	9.8	(1.2)#	9.9	(1.3)	10.2	(1.2)	9.9	(0.8)#
RCV (mL)	1160	(187)	1420	(261)	1623	(214)#	1196	(137)	1426	(201)	1660	(219)#
RCV (mL·kgBM^{-1})	29.2	(2.5)	30.8	(3.9)	28.5	(3.3)#	30.2	(4.6)	30.6	(3.9)	29.9	(2.6)#
PV (mL)	2167	(397)	2533	(445)	3039	(351)*#	2013	(312)	2416	(410)	2659	(432)#
PV (mL·kgBM^{-1})	54	(5.0)	55	(6.1)	53	(6.0)*#	50	(7.8)	52	(7.7)	49	(5.4)#
BV (mL)	3326	(576)	3954	(695)	4662	(538)#	3209	(433)	3842	(605)	4319	(632)#
BV (mL·kgBM^{-1})	83	(7.0)	86	(9.7)	82	(8.8)#	81	(11.9)	82	(11.5)	78	(7.5)#

	End boys (n=23-24)						non-End boys (n=23-29)					
	Test 1		Test 2		Test 3		Test 1		Test 2		Test 3	
VO_{2max} (mL·min^{-1})	2543	(290)	2967	(410)	3903	(618)	2524	(395)	2935	(451)	3665	(455)
VO_{2max} (mL·kgBM^{-1}·min^{-1})	66	(7.2)*	66	(4.9)*	66	(5.4)*	59	(7.9)	60	(5.1)	60	(4.5)
TTE (min)	6.5	(1.2)	8.2	(1.4)	9.3	(1.4)	5.6	(1.4)	6.2	(1.5)	6.6	(1.2)
Hbmass (g)	407	(59)	501	(82)	717	(120)	439	(66)	542	(99)	719	(94)
Hbmass (g·kgBM^{-1})	10.4	(0.6)	11.1	(0.8)	12.0	(0.9)	10.1	(0.8)	10.9	(1.1)	11.9	(0.9)
RCV (mL)	1197	(208)	1472	(265)	2087	(381)	1314	(201)	1600	(298)	2203	(333)
RCV (mL·kgBM^{-1})	30.4	(2.0)	32.7	(2.2)	34.7	(2.5)	29.9	(2.2)	32.2	(3.1)	36.0	(2.6)
PV (mL)	2172	(316)	2543	(410)	3482	(581)	2227	(318)	2621	(437)	3294	(405)
PV (mL·kgBM^{-1})	55	(4.8)*	57	(3.8)*	58	(5.3)*	51	(5.2)	53	(5.5)	54	(5.5)
BV (mL)	3369	(501)	4015	(668)	5569	(941)	3541	(495)	4221	(710)	5497	(672)
BV (mL·kgBM^{-1})	86	(5.9)*	89	(5.6)*	93	(7.1)	81	(6.6)	85	(7.4)	90	(6.2)

*Values are mean (SD).VO_{2max}, maximal oxygen uptake; TTE, time to exhaustion; Hbmass, hemoglobin mass; RCV, red cell volume; PV, plasma volume; BV, blood volume; BM, body mass. * denotes significant difference between training groups p<0.005. # denotes significant sex difference within the same training group p<0.005.*

VO_{2max}

At age 12, boys, compared with girls, had higher VO_{2max} in absolute values (2532 (350) $mL \cdot min^{-1}$ vs 2310 (318) $mL \cdot min^{-1}$, p=0.008) and relative to BM (62 (8.2) $mL \cdot kg^{-1} \cdot min^{-1}$ vs 58 (5.9) $mL \cdot kg^{-1} \cdot min^{-1}$, (p=0.046) and tended to have higher VO_{2max} when related to FFM (71 (6.9) $mL \cdot kg^{-1} \cdot min^{-1}$ vs 68(5.9) $mL \cdot kg^{-1} \cdot min^{-1}$, p=0.063). At age 15, the sex differences in VO_{2max} relative to BM were more evident (62 (8.2) $mL \cdot kg^{-1} \cdot min^{-1}$ vs 55 (4.7) $mL \cdot kg^{-1} \cdot min^{-1}$, p<0.001), while the sex differences for VO_{2max} relative to FFM were the same as at age 12 (69 (4.7) $mL \cdot kg^{-1} \cdot min^{-1}$ vs 67 (5.3) $mL \cdot kg^{-1} \cdot min^{-1}$, p=0.068). These sex differences, especially for VO_{2max} relative to FFM, were influenced by the exceptionally high values for End boys (Table 4, see above). For the non-End group, there were no sex differences for VO_{2max} relative to BM before age 15, and for VO_{2max} relative to FFM, there were no sex differences at any age (Figure 2). The average yearly increase in absolute VO_{2max} was ~240 and ~390 $mL \cdot min^{-1}$ in girls and boys, respectively (p<0.001 for both). Relative to BM, girls experienced an average decrease of ~1.1 $mL \cdot kg^{-1} \cdot min^{-1}$ (p=0.003), while there was no change over the years in boys (p=0.972) (Table 4). VO_{2max} relative to FFM decreased slightly with age for the boys (0.6 mL per year; p=0.059), while there was no change with age for the girls (p=0.342) (Figure 2). The sex difference in the development of VO_{2max} was significant for both absolute values and relative to BM (p≤0.006), but not relative to FFM (p=0.652).

Except for the high values in the endurance boys, it seems likely that sex differences seen in the changes in body composition can largely explain the sex differences in the development of VO_{2max}. %FM was not different between sexes at age 12. From age 12 to 15, %FM increased significantly in girls and decreased significantly in boys (p<0.001 for both). Together this means that in boys, relative FFM was on average higher and increased more over the years than in girls. This seems to explain boys' larger increase in VO_{2max} in absolute values, and girls' decrease in VO_{2max} relative to BM, since when related to FFM, the age effect of sex from age 12 to 15 disappeared. Also, for the non-End group alone, there were no sex differences for VO_{2max} relative to FFM at any age. Hence, the development of VO_{2max} appears to parallel the development of FFM. This is in accordance with what other studies have observed, and it seems to be independent of age, sex, body size and body composition (Ekelund, Franks, Wareham, & Åman, 2004; Goran, Fields, Hunter, Herd, & Weinsier, 2000; Maciejczyk et al., 2014).

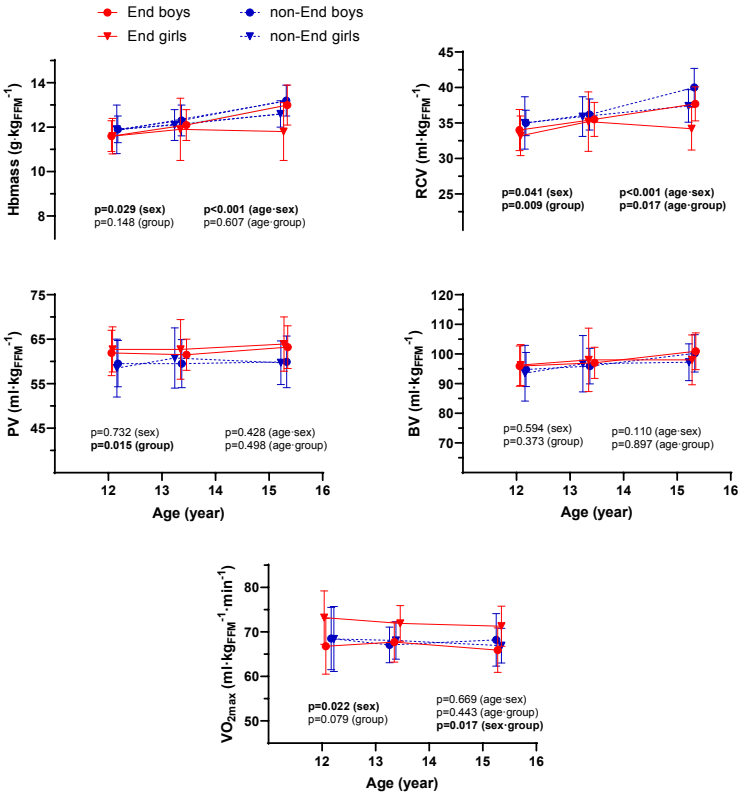

Figure 2: FFM-relative hematological variables and VO_{2max} in relation to age.

Intravascular volumes

At age 12 years, there was no sex difference for any of the measures of intravascular volumes (Hbmass, PV, BV or RCV).

In absolute values, intravascular volumes increased for both sexes, but more so for boys than girls ($p<0.001$ for all). Relative to BM, all variables increased for boys, but not for girls (sex differences, $p<0.001$ for all variables). Furthermore, from age 13 to 15 all these variables decreased for girls ($0.002<p<0.02$). These changes and sex differences were very similar to what we observed for VO_{2max}. However, FFM-normalized values differed somewhat between the two sets of parameters. While the age effect on FFM-normalized PV showed similar patterns to VO_{2max} with no age effect and no sex differences, Hb-mass and RCV increased with age, and the age effect interacted with sex (<0.001 for both). However, these interactions with sex were mainly caused by a sex difference in the End group ($p\leq0.006$) with no increase in the End girls

for these variables, while Hbmass relative to FFM increased in the non-End girls (Figure 2). As discussed in paper II, we suspect this sex difference to be affected by malnutrition. This will be discussed with reference to the effect of training type later. Based on the data from the boys and non-End girls, it seems likely that the development of VO_{2max} and Hbmass are both linked to the development of FFM, but for Hbmass there is an additional effect, not related to FFM. However, this additional effect may be less pronounced in girls.

Between the ages 12 and 15 years, we observed that FFM-related Hbmass and BV increased in boys while it stayed constant in girls. This is in accordance with a small cross-sectional study by (Åstrand, 1952). He observed an increase for males between the ages of 14 and 30 years, with no corresponding increase noted for any of the groups of females under the age of 20 years. Our results also agreed well with a longitudinal study by Prommer et al. (2018), who found a similar increase in both sexes until the onset of puberty, followed by an almost exponential increase in boys and considerably less increase in girls in the years following puberty.

Summary Aim 1

In conclusion, there were significant sex differences in the rate of development of VO_{2max} and intravascular volumes in absolute values and relative to BM from age 12 to 15. At age 12 the sex differences between variables were minor, which indicate that, although girls are more physically mature at this age, this had a minor effect on these physiological variables. The timing of the appearance of pronounced sex differences was aligned with significant differences in the development of body composition. FFM seemed to be the main determining factor for the development of VO_{2max} and intravascular volumes independent of age and sex; however, for Hbmass and RCV, there seemed to be an additional increase independent of FFM, which was less clear in girls.

Aim 2: The effect of high volumes of endurance training on the development of VO_{2max}, intravascular volumes and heart dimensions in girls and boys during puberty

As discussed above, we found that the changes in both VO_{2max} and intravascular volumes were largely determined by the increase in FFM, in both girls and boys. Also discussed above, there were some differences in the development of the anthropometry between training groups, meaning that some of the potential differences between training groups in the variables in question may have been influenced by the differences in growth and anthropometry. Therefore,

to investigate whether there were any differences between the training groups, analysis was mainly based on the differences in the development of the variables relative to FFM (Figure 2). Absolute values as well as BM-relative values are presented in Table 4.

VO_{2max}

For girls, there were no differences in VO_{2max} at any ages, independent of scaling. However, End boys had a higher VO_{2max} at age all ages compared to non-End boys, independent of scaling. The existence of higher values even at age 12 could be due either to prior training or to a selection bias. Since both groups were engaged in similar volumes of mainly play-based activities, we do not consider it likely that the higher VO_{2max} in our End boys at age 12 was training-induced, even if we cannot exclude this possibility.

The End boys were on average later maturers compared with the non-End boys. This cannot, by itself, explain the difference in BM-relative VO_{2max} since this measure changed minimally with age. Neither can the lower %FM in the End boys alone explain their higher BM-relative VO_{2max}, since VO_{2max} relative to FFM also was higher in the End boys. If we exclude the possibility that the difference in VO_{2max} relative to FFM between the groups was training-induced, other factors must play a role and these factors may be inherited, indicating that the End group was a selected group compared to the non-End group.

While VO_{2max} was higher in the End boys, the development of VO_{2max} over the years was similar in the two groups of boys and the two groups of girls, respectively, both in absolute values and relative to BM and FFM. Hence, the significant difference in the volume of endurance training (7.3 (1.8) hours vs 1.5 (1.2) hours, including continuous and interval workouts) had no additional effect on the development of VO_{2max}. In both training groups and both sexes, VO_{2max} relative to FFM stayed relatively constant over the years. Together, these findings indicate that the development of VO_{2max} was proportional to the growth of FFM in both girls and boys and was independent of training type. The finding that FFM is the most powerful determining factor for VO_{2max} in adolescents agrees with the conclusion of Armstrong and Welsman (2019b) who studied more than 300 teenagers aged 12 – 18 years. It is therefore possible that during pubertal growth, as long as children are exercising, adding more training for specifically developing VO_{2max} has no additive effect compared to being active in sports with more focus on developing fundamental and sport-specific motor skills. This was supported by the linear regression analysis with yearly change in VO_{2max} relative to FFM from age 13-15 as the dependent variable and weekly hours of *total training* as the independent variable, which showed that there was a very

weak to almost zero relationship between these variables for both training groups (p>0.05 for both) (Figure 3a) and there was no difference between the groups (p=0.597).

Using the volume of *endurance training* as the independent variable, the linear regression analysis showed that there was a very weak relationship between the weekly volume of endurance training and the yearly change in VO_{2max} relative to FFM for both training groups (p>0.05 for both) (Figure 3b). There was no statistically significant difference between the training groups (p=0.099).

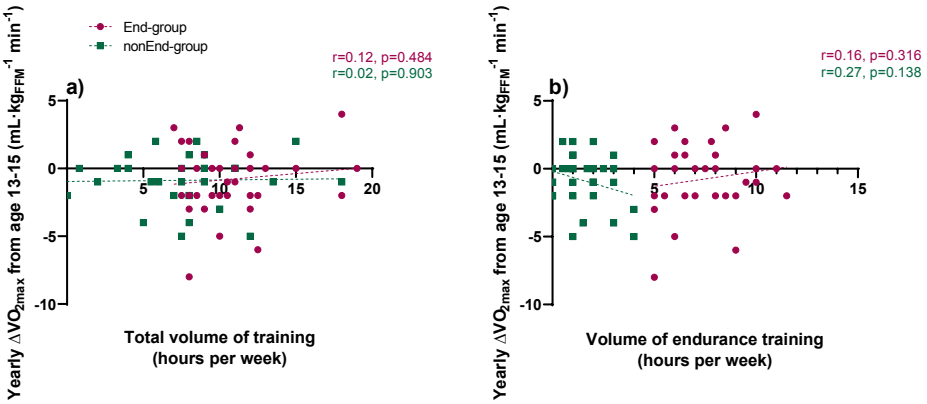

Figure 3: Relationship between ***a)*** *volume of weekly* ***total training*** *and yearly change in VO_{2max} relative to FFM from age 13-15; and* ***b)*** *volume of weekly* ***endurance training*** *and yearly change in VO_{2max} relative to FFM from age 13-15.*

The trainability of VO_{2max} in adolescents is still controversial, even though the question has been addressed using a variety of approaches over several decades (Dotan, 2017). Some authors conclude that proper endurance training in prepubertal and circumpubertal children affects VO_{2max} even if the effect is lower than in adults (Armstrong & Barker, 2011; Baquet et al., 2003), while others claim that there is a maturational threshold below which children are not able to increase their VO_{2max} (Katch, 1983). Discrepancies between studies may be due to different study designs as well as training protocols (Armstrong & Barker, 2011; Tolfrey et al., 1998). The present study supports the "maturational threshold hypothesis" but also has some limitations, which will be discussed below.

Intravascular volumes

At age 12, only BV relative to BM differed between the training groups, due to a higher PV in the End boys compared with the non-End boys ($p=0.004$). Otherwise, there were no differences between the training groups for any of the intravascular volumes for any scaling. At age 15, the difference in BV between training groups had disappeared, while PV relative to BM was still higher in End boys ($p=0.017$) and had also become higher in End girls compared with non-End girls ($p=0.035$). FFM-relative PV tended to be higher both for End girls ($p=0.081$) and End boys ($p=0.068$) compared with the non-End groups (for both sexes together, $p=0.008$). At this age, RCV relative to FFM was lower in the End groups both for girls ($p=0.014$) and boys ($p=0.005$). Hence, BV relative to FFM was similar between the training groups, while PV was higher and RCV was lower in the End groups. Hbmass relative to FFM was also similar in the two groups of boys, but as mentioned, lower in the End girls compared with the non-End girls. We speculate whether the development of intravascular volumes during the study period was influenced by malnutrition in the girls, and therefore the effects of training groups will be discussed separately for girls and boys.

For the boys, Hbmass and RCV relative to FFM increased from age 12 to 15 (all $p<0.001$) with a similar increase in both training groups ($p=0.644$ and $p=0.163$, respectively), while PV stayed constant in both groups. Our data support the conclusion of Prommer et al. (2018) that the increase in absolute Hbmass at these ages is mainly determined by the increase in FFM. However, contradicting the conclusion of Prommer et al. (2018), we did not find any effect due to the volume of endurance training on Hbmass in our boys. Our results also showed that there was an additional increase, independent of FFM. Since Prommer et al. (2018) did not have a control group, the increase in FFM-relative Hbmass could be an effect of time rather than training (Steiner et al., 2019).

Unlike the boys, for the girls, the development of Hbmass and RCV relative to FFM during the study period was different between End- and non-End girls. While there was no increase in FFM-relative Hbmass in the End girls, there was an increase in non-End girls, indicating an FFM-independent mechanism also exists in girls, although with a slower rate than for boys. It can be speculated that the lack of increase in End girls, and the slower rate of increase in FFM-relative Hbmass in the non-End girls compared with boys, may be caused by malnutrition and low iron stores. Eight out of 18 (~45%) End girls and 2 non-End girls experienced a yearly change in %FM of less than 0.7 percentage points compared with the average yearly increase of 1.4 percent points in the rest of the girls. The lack of increase in Hbmass relative to FFM in the

End girls may be explained by some of these girls' very low increase in %FM. This was supported by the fact that a very small or negative yearly change in %FM (<0.7 percent points) was strongly associated with a correspondingly small or negative yearly change in Hbmass relative to FFM (r=0.90), whereas there was no relationship between these variables in girls with "normal" development of %FM (yearly increase >0.7 percent points; r=0.01) (Figure 4). This strikingly high association may be coincidental, and it should be pointed out that several girls with higher than average increases in %FM also had negative or minor change in Hbmass relative to FFM.

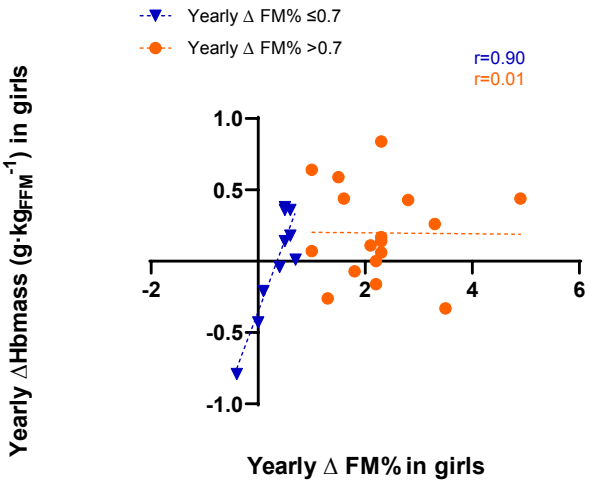

Figure 4: Relationship between yearly change in %FM and yearly change in Hbmass relative to FFM in girls with a yearly change in %FM ≤0.7 (triangles) and >0.7 (circles).

To check whether Hbmass was affected by maturation, we calculated simple correlations between years from PHV and absolute Hbmass values, separately for each year and each sex. For both girls and boys, there were strong correlations between years from PHV and Hbmass in absolute values for all 3 years (0.64<r<0.085; p<0.001). However, for FFM-relative values, there were no correlations with years from PHV. This supports FFM being the main factor determining Hbmass. However, the fact that there was an additional increase, independent of FFM (i.e. FFM-dependent Hbmass increase), indicates that maturational factors independent of growth exist. Whether these factors include testosterone, other hormones or other factors related to maturation requires further investigation.

Contrary to the findings for BV and Hbmass, a group effect was evident for PV and RCV relative to FFM, with higher values in the End groups for PV ($p=0.015$) and lower values for RCV ($p=0.009$) on average over the three years. One explanation for the lower RCV in the End groups is that the End groups had lower RBCC (per liter of blood). This is in line with the findings of Boyadjiev and Taralov (2000), who compared red blood cell variables in highly trained male and female pubescent athletes from different sports disciplines (age 14.0 (0.06) years) and untrained controls (age 14.6 (0.09) years). They found that the highly trained group had lower RBCC, Hct and [Hb] than the untrained group, which is in line with our results for both girls and boys (Table 5). In the present study, there was also a group effect on the change in MCV, which increased more with age in the non-End groups compared with the End groups. There were also group effects on MCHC (Hb-concentration in the red blood cells) and MCH (Hb-content of the red blood cells). Taken together, these findings indicate that the higher volume of endurance training induced lower RBCC and lower MCV, resulting in a lower RCV. However, a higher PV compensated for this possible training effect, resulting in the same BV between the training groups.

Table 5: Hematological variables at age 12 (Test 1), 13 (Test 2) and 15 (Test 3) in girls and boys undertaking high (End boys and girls) and low (non-End boys and girls) volumes of endurance training.

	End girls (n=17)			non-End girls (n=9)		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
[Hb] ($g \cdot dL^{-1}$)	13.2 (0.5)*	13.2 (0.6)*#	13.1 (0.7)*#	13.8 (0.8)	13.8 (0.7)	14.0 (0.8)
Hct (%)	38.4 (1.6)*	39.5 (1.6)*	38.2 (1.9)*#	41.1 (2.4)	40.9 (1.2)	42.4 (2.2)
RBCC ($10^{12} \cdot L^{-1}$)	4.6 (0.2)*	4.6 (0.2)	4.5 (0.2)*#	4.8 (0.3)	4.8 (0.2)	4.9 (0.4)
MCH (pg)	28.8 (1.1)	28.7 (1.2)	29.2 (1.0)	28.6 (1.4)	28.9 (1.7)	28.9 (1.4)
MCHC ($g \cdot dL^{-1}$)	34.4 (1.0)	33.6 (1.1)	34.1 (0.8)*	33.7 (0.9)	33.8 (1.2)	33.1 (1.6)
MCV (fL)	84 (2.6)	85 (2.8)	85 (2.9)	85 (5.7)	85 (3.9)	87 (6.2)

	End boys (n=23)			non-End boys (n=23)		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
[Hb] ($g \cdot dL^{-1}$)	13.3 (0.7)*	13.7 (0.5)	14.1 (0.6)	13.7 (0.8)	14.1 (1)	14.4 (0.9)
Hct (%)	39.0 (2.3)*	40.2 (1.3)*	41.1 (2.1)*	40.8 (2.4)	41.6 (2.6)	44.0 (3.2)
RBCC ($10^{12} \cdot L^{-1}$)	4.6 (0.2)*	4.8 (0.2)	4.9 (0.2)*	4.8 (0.3)	4.9 (0.3)	5.1 (0.4)
MCH (pg)	28.8 (1.3)	28.9 (1.1)	29.4 (0.8)*	28.6 (1.0)	28.9 (1.0)	28.5 (1.1)
MCHC ($g \cdot dL^{-1}$)	34.1 (1.4)	34.1 (0.8)	34.4 (0.9)*	33.7 (0.9)	33.9 (0.8)	32.8 (1.4)
MCV (fL)	84 (2.7)	85 (2.8)	85 (2.8)*	85 (3.4)	85 (2.6)	87 (3.4)

*Values are mean (SD).[Hb], hemoglobin concentration; Hct, hematocrit; RBBC, red blood cell count; MCH, Hb-content of the cell; MCHC Hb-concentration in the red blood cells; MCV, mean cell volume. * denotes significant difference between training groups $p<0.005$. # denotes significant sex difference within the same training group $p<0.005$.*

Hct was already lower in the End group at age 12 and remained lower at age 15, more so in girls than boys. In boys, the age-related increase in Hct was not different between training groups, due to similar age effects on RBCC in the two groups. This is in line with the changes found in a larger reference cohort (Pluncevic Gligoroska et al., 2019). Also, in line with this reference group, MCV increased with age in the non-End group (both sexes). However, in the present study, MCV did not increase with age in the End group (both sexes). Hence, it may be that endurance training blunts the normal increase in MCV during puberty, and that this may be one mechanism causing the reduced Hct. In non-End girls, RBCC did not change with age, again in line with the reference cohort (Pluncevic Gligoroska et al., 2019), while the RBCC in the End girls decreased. Since the age effect on RBCC was similar in the two groups of boys, but not the two groups of girls, and endurance training seems to affect MCV in both sexes, it may be hypothesized that the reduction of RBCC in the End girls is due to low ferritin levels, while the lack of increase in

MCV is more sensitive to endurance training. These two mechanisms together may result in the very low Hct and [Hb] in the End girls relative to non-End girls.

The higher PV relative to FFM in the End groups compared with the non-End groups, and the lack of change with age, are in line with Steiner et al. (2019) and the values for both End boys and non-End boys are very similar in the two studies, regardless of the age difference. Based on the two studies, it seems that FFM-relative PV does not change from age 12 to 19 but tends to be higher in endurance-trained populations. In addition, we found that PV relative to FFM was the same for boys and girls both in the End and non-End groups, respectively. The two studies together indicate that during adolescence, Hbmass is dependent on sex and independent of the volume of endurance training; RCV is dependent on both sex and volume of endurance training, and PV is independent of sex and dependent on the volume of endurance training.

Cardiac dimensions

Cardiac dimensions (both volumes and masses) were assessed by both two-dimensional (2D) and three-dimensional (3D) echocardiography. In paper III, LV-mass was reported from the both 2D and 3D assessments, while EDV and ESV were reported from 3D assessments only. To assess the impact of training group on the variables studied, linear mixed models were used. Throughout this thesis we used a three-way mixed ANOVA, which does not accept missing values (as opposed to linear mixed models). There are fewer complete 3D data sets compared with 2D measurements, therefore 2D results are reported in the following discussion. The statistics for between-group differences in absolute values and changes were the same when using 3D compared with 2D measurements.

In paper III, the variables are indexed to body surface area (BSA), while throughout this thesis we have normalized to BM and FFM. Therefore, in the following discussion, the variables will be reported both for BSA and FFM.

The 2D assessments of EDV, ESV, LV mass and RWT are reported in Table 6 as absolute values, relative to BSA, and relative to FFM. These variables will be discussed below. There was no difference in any of the morphological parameters between the two groups at age 12. Between the ages of 12 and 15 years, the chamber volumes and LV mass increased in absolute values. For EDV and ESV, the changes tended to be larger in the End group (called *active* in paper III) than in the non-End group (called *former* in paper III), while there were no differences between groups for changes in LV mass. Also relative to BSA, the change in EDV and ESV tended to be larger in the athletes, while relative to FFM, there were no differences for any of the variables. Left

ventricular posterior wall thickness in diastole (LV PWd) tended to increase (p=0.075) in the End group, whereas the non-End group experienced a significant increase in LV PWd (p=0.012). Taken together, these changes led to a decrease in RWT in the End group (-0.05 (0.07), p=0.001), whereas there was no significant change in RWT in the non-End group (0.00 (0.06), p=0.575).

Athlete's heart, which can be described as training-induced changes in cardiac morphology, is quite a common finding among professional athletes (Baggish & Wood, 2011; Lewicka-Potocka et al., 2020). These changes include the heart increasing in size while maintaining compliance and contractility, such that the larger heart can fill and empty larger volumes more efficiently (D'Andrea et al., 2015). Regular endurance training has been seen to cause a gradual decrease in in systematic arterial resistance and an increase in venous return. This exercise-induced pressure and volume overload causes cardiac remodeling (eccentric hypertrophy) with higher LV EDV, LV mass and LV wall thickness (D'Andrea et al., 2015).

The difference between the groups in the alterations of these dimensions, and especially the difference in ventricular volumes relative to BSA, indicates that the hearts of the active endurance athletes had undergone eccentric remodeling compared with the non-End group. However, relative to FFM, ventricular volumes did not vary between the the groups.

Interestingly, BSA-relative LV mass increased by 16 % (p=0.01) and 21 % (p=0.09) in the End group and the non-End group, respectively, while FFM-relative LV mass did not change in any of the groups (p>0.6). This also indicates that heart dimensions increase in parallel to FFM during growth. Since BSA is a product of both height and BM, and BM increases relatively more than height, this may question the use of BSA as an indexing factor for heart dimensions. Because of this, the tendencies to larger increases in EDV and ESV relative to BSA in the End group compared with the non-End group should be interpreted with caution.

Table 6: Cardiac dimensions in End and non-End athletes at baseline and follow-up.

		End-group		non-End group		
	n (End/non-End)	Baseline	Follow up	Baseline	Follow up	*p*-value
Cardiac morpholgogy						
LV EDV (mL)	*29/16*	103 (16)	145 (30.4)*	105 (16.7)	133 (23.7)*	0.068
LV EDV/BSA (mL/m2)	*29/16*	79 (9)	85 (11.4)*	80 (6)	80 (12.9)	0.097
LV EDV/FFM (mL/kg)	*29/15*	3.0 (0.3)	2.7 (0.2)*	3.1 (0.2)	2.7 (0.4)*	0.256
LV ESV (mL)	*29/16*	42 (7)	62 (14.2)*	44 (8)	57 (9.2)*	0.057
LV ESV/BSA (mL/m2)	*29/16*	33 (4.5)	36 (5.9)*	34 (3.9)	34 (5.1)	0.084
LV ESV/FFM (mL/kg)	*29/15*	1.2 (0.2)	1.2 (0.1)	1.3 (0.1)	1.1 (0.2)*	0.183
LV Mass (g)	*30/16*	93 (18.5)	140 (41.8)*	87 (19.2)	130 (30.9)*	0.739
LV Mass/BSA (g/m2)	*30/16*	72 (12.2)	82 (19.7)*	67 (14.9)	77 (15.4)	0.979
LV Mass/FFM (g/kg)	*30/15*	2.7 (0.5)	2.6 (0.5)	2.6 (0.6)	2.6 (0.4)	0.652
LV IDd (cm)	*29/16*	4.1 (0.4)	5.0 (0.5)	4.1 (0.3)	4.9 (0.4)	0.355
LV PWd (cm)	*29/16*	0.73 (0.09)	0.78 (0.12)	0.68 (0.09)	0.81 (0.12)*	0.100
RWT	*29/16*	0.35 (0.05)	0.31 (0.04)*	0.33 (0.05)	0.33 (0.05)	0.030

**; significant change from baseline to follow up within each group (p<0.05), p-value; difference between groups in changes from baseline to follow up; End, endurance; non-End, non-Endurance; n (End/non-End), number of participants in End-group (End) and non-End group (non-End); LV, left ventricular; EDV, end diastolic volume; BSA, body surface area; FFM, fat free mass; ESV, end systolic volume; IDd, internal diameter in diastole; PWd; posterior wall thickness in diastole; RWT, relative wall thickness.*

Summary Aim 2

In general, there were no differences in $VO_{2max,}$ hematological variables and heart dimensions between the training groups at age 12, and only minor differences between the training groups at age 15. This indicates that the differences between high volumes of systematic endurance training and similar volumes of training mainly aimed at developing motor skills had no or small effects on the development of the variables studied. However, while the development of VO_{2max} and BV were similar for both groups, there were some differences between the training groups for PV and RCV, relative to BM, with higher PV and lower RCV in the End group at age 15. Relative to FFM there were no differences between the groups in the development of cardiac dimensions; however, the End group experienced a smaller increase in LV PWd than the non-End group. This led to a decrease in RWT in the End group, while RWT stayed unchanged in the non-End group. Taken together, these results indicate that the main differences between the training groups were that endurance training led to a decrease in RWT, had a positive effect on PV, and blunted the increase in RCV seen in the non-End group.

Aim 3: Do changes in Hbmass and heart size affect changes in VO_{2max} when controlling for growth?

In the following chapter, analysises of the data from paper I, II and III are combined and not presented in any of the papers. Multiple linear regression analysis was used to develop a model for predicting change in VO_{2max} from measurements of changes in anthropometrics, heart dimensions and intravascular volumes. The participants in paper III were included and the change scores from age 12 to 15 were used in the analyses. As discussed above, VO_{2max}, heart dimensions and intravascular volumes in our subjects were all highly associated with FFM at all ages. Hence, these variables were all associated with each other and FFM was used as the marker of change in anthropometrics (growth). To represent the heart dimension, change in EDV was selected because the variable had the highest correlation to the change in VO_{2max}. To represent changes in intravascular volumes, change in Hbmass was selected as a marker because this variable has been shown in many studies to be highly correlated with VO_{2max} and changes in VO_{2max} (Gore et al., 1997; Prommer et al., 2018; Schmidt & Prommer, 2010). In the multiple regression analyses, multicollinearity was evaluated using a variance inflation factor (VIF) and a value of VIF > 5 indicated excessive multicollinearity.

Basic descriptive statistics for the changes in the different variables and regression coefficients are shown in Table 7. Each of the independent variables had a significant correlation with VO_{2max} ($r>0.70$, $p < 0.001$) and with each other ($r> 0.57$, $p< 0.001$). The multiple regression analysis showed that the changes in FFM and Hbmass had significant ($p<0.05$) partial effect on changes in VO_{2max}, while EDV did not (Table 8, Model 1). The model was able to account for 75% of the variance in the change in VO2max ($p<0.0001$). VIF for FFM was 4.8; close to, but below, the cut-off defined for excessive multicollinearity.

Table 7: Correlation between change in predictor (independent) variables and change in the dependent variable VO_{2max}.

	Zero-order r			
Variable	FFM	EDV	Hbmass	VO_{2max}*
FFM (kg)		0.718	0.840	0.839
EDV (mL)			0.575	0.727
Hbmass (g)				0.803
mean	17.5	37	248	983
SD	6.1	24	108	504

**Unit for VO2max is mL/min*

Because of the obvious dependence on growth for all these variables, different two-predictor models including FFM and EDV and FFM and Hbmass were also tested (Table 8). These models were able to account for 71% and 74% of the variance in the change in VO_{2max} ($p<0.0001$), respectively. The model including FFM and Hbmass was close to and not statistically different from the full three-predictor model. Moreover, the model without FFM (with changes in EDV and Hbmass as predictors) gave high predictions of change in VO_{2max} (71%, not shown). Together, these models indicate that EDV and Hbmass are highly associated with growth of FFM; therefore, growth is embedded in these two predictors. Finally, the model with only FFM as the predictor accounted for 70% of the variance in the change in VO_{2max} ($p<0.0001$).

Table 8: Multiple regression predicting VO_{2max} ($mL \cdot min^{-1}$) from fat-free mass (FFM), end diastolic volume (EDV) and total hemoglobin mass (Hbmass) using predictor models including three (Model 1) or two predictors (Model 2 and 3).

	Model 1				Model 2				Model 3			
Predictor variable	β	SE	p	95%CI	β	SE	p	95%CI	β	SE	p	95%CI
Delta FFM	37.2	15	0.018	6.9 to 67	55.2	11	<0.0001	33.9 to 76	48	12	0.0004	23.2 to 73
Delta EDV	4.16	2.6	0.125	-1.2 to 9.5	5.31	2.6	0.05	0.01 to 10.6				
Delta Hbm	1.48	0.7	0.04	0.07 to 2.9					1.47	0.7	0.043	0.05 to 2.9
R^2	0.766				0.726				0.754			
Adjusted R^2	0.746				0.711				0.741			
P	<0.0001				<0.0001				<0.0001			

Obviously, VO_{2max} increases with growth and it seemed that growth of FFM was a better predictor than BM. The two-predictor models indicated that change in heart dimensions did not add significantly to the prediction of change in VO_{2max}, while change in Hbmass did, but only by 4% compared with FFM as the predictor alone. Theoretically, two subjects with the same growth of FFM may differ somewhat with regard to growth of the heart and the one with the largest increase in heart dimensions also will show the largest increase in VO_{2max}. The same logic would apply for Hbmass. If this is the case, adding these factors should improve the model. When this does not happen to a great extent, it indicates that VO_{2max} follows the variations in FFM. The increased precision of the two- and three-predictor models could also be caused by the fact that adding assessment of variables strongly associated with FFM will give a more precise estimate of the true increase in FFM and in reality, FFM is the only predictor with no additional effect of including of the other two variables. Our analysis cannot exclude this possibility. However, a

more robust conclusion is that FFM is by far the strongest predictor, with minor but significant increased precicion of the estimate of the change in VO_{2max} when Hbmass is added to the model.

Figure 5 gives the correlation between the actual increase in VO_{2max} and the predicted increase in VO_{2max} from the three-predictor model. One subject requires extra explanation because the subject experienced a decrease in FFM from age 12 to 15 due to growth reduction therapy. Interestingly, inclusion of this subject did not change the model significantly, as indicated by the fact that the model predicted the actual decrease in VO_{2max} rather well. This was the case regardless of which model we used, indicating that the therapy affected all the structural variables – FFM, heart dimensions and intravascular volumes – approximately equally. Furthermore, it also affected VO_{2max} to the same extent. This example strengthened our conclusion that VO_{2max} is determined by structural variables but including heart dimensions or Hbmass does not give any extra information or add precision to the model.

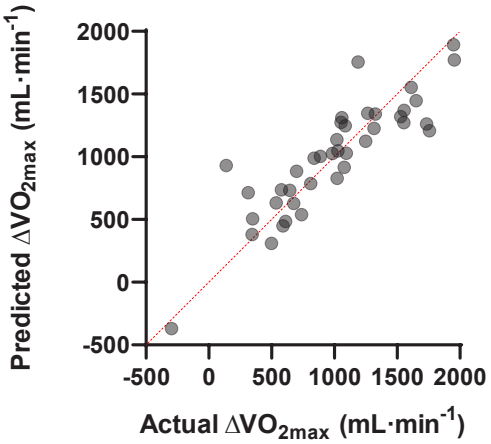

Figure 5: Relationship between actual change in FFM-related VO_{2max} and predicted VO_{2max} using Model 1 (including FFM, Hbmass and EDV).

Summary Aim 3

The two-predictor model (Model 3), using FFM and Hbmass as predictor variables, was able to account for 74% of the variance in the change in VO_{2max} ($p<0.0001$). This is only 1% less than the three-predictor model (Model 1), which also included EDV. Since including EDV in the model seemed to add very little to the precision, we regarded Model 3 to be a better alternative

for predicting change in VO_{2max} than Model 1. Model 3 has the advantage of involving assessments from only two predictors, and it is almost as precise as model 1. In conclusion, FFM is by far the strongest predictor, with minor but significant increased precicion of the estimate of change in VO_{2max} when Hbmass is added to the model, which is not significantly improved further by including EDV.

Performance

The previous discussion has addressed structural and physiological changes with age, differences between sexes and the impact of high volume of endurance training on these changes. The VO_{2max} test was performed to exhaustion and time to exhaustion (TTE) is a measure of performance. In boys, but not in girls, TTE increased with age ($p<0.001$) and more so in End boys than non-End boys ($p<0.001$). There was also a tendency towards increased TTE in the non-End girls ($p=0.058$) but not in the End girls. Performance, measured as TTE in the VO_{2max} test, was superior in the End boys compared to the non-End boys at all ages. End girls also performed better than the non-End girls, but the difference was only statistically significant at age 13.

In boys, TTE on the treadmill increased in both groups, but the increase was nearly 3 times larger in the End boys than in the non-End boys, while the development of VO_{2max} did not differ between the training groups. In girls, the performance did not change with time, which fitted with the fact that VO_{2max} relative to BM decreased, while VO_{2max} relative to FFM did not change. Performance in aerobic exercise is closely related to VO_{2max} and VO_{2max} has been regarded the single best measure of an individual's aerobic fitness. However, VO_{2max} is not the only factor that determine performance (Åstrand et al., 2003). Running economy and the ability to utilize a high percentage of the VO_{2max}, as well as anaerobic capacity, will affect performance (Joyner & Coyle, 2008). This suggests that endurance training may have had a significant effect on determinant factors other than VO_{2max}. In a subset of our participants (boys only) oxygen cost at a given submaximal running speed was reduced similarly in End boys and non-End boys (data not shown). The superior improvement in performance in the End boys may therefore be explained by a superior utilization of their VO_{2max} and/or superior anaerobic capacity.

Aim 4: Validation of bioelectrical impedance analyses (BIA) against dual-energy X-ray absorptiometry (DXA).

In papers I, II and III, FFM was estimated using BIA (InBody 720). Because of the importance of FFM in the longitudinal study, we wanted to validate the method used against a reference method; i.e. InBody 720 against dual-energy X-ray absorptiometry (DXA), and to evaluate the reliability.

Reliability of InBody 720 and DXA

There were no sex differences for any of the test-retest mean differences with either InBody 720 or DXA (Supplemental Table 1, Paper IV). The test-retest results showed an excellent correlation for all the assessments (ICC≥0.990, both devices), and test-retest mean differences did not vary between InBody and DXA for any of the body composition estimates. The high test-retest reliability is in accordance with several previous studies (Anderson, Erceg, & Schroeder, 2012; Gibson, Holmes, Desautels, Edmonds, & Nuudi, 2008; McLester, Nickerson, Kliszczewicz, & McLester, 2018; Talma et al., 2013; Volgyi et al., 2008).

Both devices measured FFM more precisely than FM, as shown by the lower CV for FFM (0.95% and 1.14% for InBody and DXA, respectively, p=0.474) compared with the CVs for FM (4.0% and 2.0%, respectively, p=0.008) and %FM (4.5% and 2.5%, respectively, p=0.025). Whereas there was no difference between the devices in CV for FFM, CV for FM was significantly lower for DXA compared with InBody 720. These results suggest that InBody 720 may provide precise estimations of FFM but less precise estimations of FM when compared with DXA. This is supported by Wang et al. (2013), who compared two different BIA devices with DXA and MRI in 200 healthy men and women. They found that BIA provided accurate and reliable estimations of skeletal muscle mass, but less accurate estimations of FM in relation to DXA or MRI.

The very small mean difference between test and retest and the narrow LoA for both FFM and FM (kg and %) (Table 2, Paper IV), together with the high ICC, suggest that both InBody 720 and DXA have excellent reliability for all the body composition estimates, especially for estimating FFM.

Validity of InBody 720

The current data showed an almost perfect correlation (Pearson's r) between InBody 720 and DXA for all the body composition estimates (r≥0.966). However, InBody 720 overestimated FFM by 2.7 kg and underestimated FM by 4.0 kg and 7.4% compared with DXA, which agrees well with other studies on adult athletic populations (Esco et al., 2015; Raymond, Dengel, & Bosch, 2018).

The young male and female athletes in the current study were a very lean and homogenous group, with an average BMI for the whole group of 19.5. Since many previous studies on children have involved overweight and obese individuals and no studies, to the best of my knowledge, have included adolescent athletes, our participants cannot always be directly compared with participants in other studies. Ling et al. (2011) found that InBody 720 underestimated FM in individuals with low FM and overestimated FM at high FM. The participants in Ling et al.'s study exhibited a wide range of BMIs from normal (BMI 18.5-24.9) to obese (BMI>30). In terms of body composition, our participants can be compared with the leanest group in this study, and our results therefore agree well their results for participants with the lowest BMI.

In the present study we evaluated InBody 720 against DXA as a reference method. All reference methods are based on assumptions to convert raw data measures of FFM (total water, protein and minerals) into measures of %FM, and the constants needed for these calculations can vary from person to person (i.e. they are age- and sex-specific) (Talma et al., 2013). It is therefore important that the assumptions by which the raw data are converted into final values are correct; e.g. the hydration factor of FFM. During growth, the relative proportions of the three main components of FFM (water, protein and minerals) change with age and pubertal status (Wells & Fewtrell, 2006). Neither InBody 720 nor DXA have published their prediction equations and the devices cannot be adjusted for sex, age or special populations. We can therefore speculate whether the constants used in the algorithms by InBody 720 and DXA were unsuitable for the population in the current study and thus produced different results in estimating FFM and consequently FM.

Although the mean difference between InBody 720 and DXA was significant for all the body composition measures, the relationship between the devices was almost perfect (Pearson's r≥0.966 boys and girls combined) for both FFM and FM. This indicates that InBody 720, in relation to DXA, is a valid method in estimating body composition.

Summary Aim 4

Validating BIA against DXA showed that the mean difference between the devices was significant for all the body composition estimates. However, the relationship between BIA and DXA was almost perfect (Pearson's r≥0.966) for both FFM and FM (kg and %), and the reliability for FFM was similar between the devices (CV<1.1). We, therefore, concluded that BIA is a suitable method for estimating changes in FFM during growth as it is reproducible, easy to use, and valid in relation to DXA, however the two devices are not interchangeable.

Strengths and limitations

Strengths

A major strength of the study is the longitudinal design and the inclusion of both girls and boys. All the participants were tested by the same experienced test leaders throughout the entire study period and the same equipment was used on all three test occasions.

When involving athletes in a study, the quality of coaches and the training programs are important. A strength of this study is that the coaches in the participants' ski clubs are mainly recruited from our own students at Norwegian School of Sport Sciences. We know that their endurance training programs satisfy the criteria for effective training to develop elite endurance athletes. This is also supported by the fact that the ski clubs in question regularly recruit skiers to the Norwegian national teams in cross country skiing. In 2018, four skiers from these clubs competed in the winter Olympics.

Limitations

The hypothesis that there may be a maturational threshold for the effects of endurance training has been challenged over the years. However, evidence to refute the hypothesis is limited. The majority of the evidence suggests that training does have effects on VO_{2max}, but the effect is less than in adults (Dotan, 2017). The present study supports the "maturational threshold hypothesis" but has also some limitations. Specifically, the End group was a selected group and had higher VO_{2max} at the onset of the study. This may be part of the reason why these children did not increase their VO_{2max} more than the non-End group, since it has been shown that the response to training is related to the initial VO_{2max} in children (Armstrong & Barker, 2011; Mandigout, Lecoq, Courteix, Guenon, & Obert, 2001; Tolfrey et al., 1998). Furthermore, both training groups in the present study were physically active, with participation in organized team and endurance sports

averaging from 6.7 hours per week at age 12 to 9.5 hours per week at age 15, with no significant differences between groups. Children with a more sedentary lifestyle may of course have responded positively to systematic endurance training. Hence, the present study may indicate that in growing active children, a specific focus on endurance training may not have an additional effect on VO_{2max} compared with a similar volume of general physical training. Furthermore, we cannot refute the possibility that even higher intensity and/or higher volumes than those used in our End group may affect VO_{2max}. Another limitation with our study is that the volumes of training are estimates based on recall questionnaires handed in on each test occasion; thus, we do not have an exact and detailed overview of the training content and volume for each of the three years of the study.

The lack of an inactive control group of boys and girls is also a limitation. Therefore, we cannot exclude the possibility that we would have seen differences between a group performing no physical exercise and the training groups in the present study, and that the inclusion of an inactive group would have given novel information. On the other hand, asking a group of children at the age of 12 to stay inactive for three years would be both challenging and ethically problematic.

Another limitation that needs to be addressed is the relatively small number of participants, especially non-End girls. Also, some of the variables seem to be influenced by nutrition. Hence, identified differences and no differences between End and non-End groups must be regarded with caution. However, our results stand in agreement with other related studies.

Perspectives

Participation in organized sports is a popular leisure time activity and contributes significantly to physical activity levels in children in many countries. In Norway and Finland, participation in organized sports clubs has increased over the last 30 years and the association between participation in sport clubs and volume of physical activity was stronger in 2014 than in 1985 (Mathisen, Kokko, Tynjala, Torsheim, & Wold, 2019). Furthermore, participation in organized sport in youth may contribute to a physically active lifestyle in adulthood (Tammelin, Nayha, Hills, & Jarvelin, 2003). Some of the children are aiming at becoming adult athletes. Preparation for a specific sport includes developing fundamental movement competence, training of specific motor skills, and training for developing physical capacities such as maximal strength and VO_{2max}. The present thesis indicates that during pubertal growth, provided that children are physically active and execising systematically, adding more training for specifically affecting $VO_{2max,}$ vascular volumes and cardiac dimensions has no additive effects compared to just being active in sport with more focus on developing fundamental movement competence and specific motor skills. More controlled studies are needed to explore the effects of training on physical capacities during puberty.

Conclusion

1. There were significant sex differences in the development of VO_{2max} and intravascular volumes, both in absolute values and relative to BM from age 12 to 15, with a larger increase in boys, on average. However, when related to FFM, the age effect of sex from age 12 to 15 disappeared. Hence, during puberty, increases in VO_{2max} and intravascular volumes are mainly determined by increases in FFM, and this was also the case for cardiac dimensions.

2. There were minor differences between the training groups, indicating that high volumes of systematic endurance training and high volumes of general physical training with little inclusion of systematic endurance training had similar effects on the variables studied.

3. Using multiple linear regression analysis for developing a model for predicting change in VO_{2max} from measurements of changes in anthropometrics, heart dimensions and intravascular volumes indicated that a model which included FFM and Hbmass was best suited and it was able to account for 74% of the variance in the change in VO_{2max}.

4. BIA seemed to be a reliable method for estimating FFM. When validated against DXA there was a significant but constant difference between the devices. However, the correlation between the devices was almost perfect for both FM and FFM. Taken together, this indicates that InBody 720 is a valid method in estimating changes in FFM during growth.

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Paper I

Wang, J. G., Zhang, Y., Chen, H. E., Li, Y., Cheng, X. G., Xu, L., . . . Li, B. (2013). Comparison of two bioelectrical impedance analysis devices with dual energy X-ray absorptiometry and magnetic resonance imaging in the estimation of body composition. *Journal of Strength and Conditioning Research, 27*(1), 236-243. doi:10.1519/JSC.0b013e31824f2040

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Paper I

Landgraff, HW, Riiser A, Lihagen M, Skei M, Leirstein S, Hallén J. Longitudinal changes in maximal oxygen uptake in adolescent girls and boys with different training backgrounds. Accepted SJMSS.

Paper II

Landgraff, HW, Hallén J. Longitudinal training-related hematological changes in boys and girls age 12 to 15. Accepted MSSE.

Paper III

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The developing athlete's heart: a cohort study in young athletes transitioning through adolescence

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Abstract

Background: Athlete's heart is a term used to describe physiological changes in the hearts of athletes, but its early development has not been described in longitudinal studies. This study aims to improve our understanding of the effects of endurance training on the developing heart.

Methods: Cardiac morphology and function in 48 cross-country skiers were assessed at age 12 years (12.1 ± 0.2 years) and then again at age 15 years (15.3 ± 0.3 years). Echocardiography was performed in all subjects including two-dimensional speckle-tracking strain echocardiography and three-dimensional echocardiography. All participants underwent cardiopulmonary exercise testing at both ages 12 and 15 years to assess maximal oxygen uptake and exercise capacity.

Results: Thirty-one (65%) were still active endurance athletes at age 15 years and 17 (35%) were not. The active endurance athletes had greater indexed maximal oxygen uptake (62 ± 8 vs. 57 ± 6 mL/kg/min, $P < 0.05$) at follow-up. There were no differences in cardiac morphology at baseline. At follow-up the active endurance athletes had greater three-dimensional indexed left ventricular end-diastolic (84 ± 11 mL/m^2 vs. 79 ± 10 mL/m^2, $P < 0.05$) and end-systolic volumes (36 ± 6 mL/m^2 vs. 32 ± 3 mL/m^2, $P < 0.05$). Relative wall thickness fell in the active endurance athletes, but not in those who had quit (–0.05 ΔmL/m^2 vs. 0.00 mL/m^2, $P = 0.01$). Four active endurance athletes had relative wall thickness above the upper reference values at baseline; all had normalised at follow-up.

Conclusion: After an initial concentric remodelling in the pre-adolescent athletes, those who continued their endurance training developed eccentric changes with chamber dilatation and little change in wall thickness. Those who ceased endurance training maintained a comparable wall thickness, but did not develop chamber dilatation.

Keywords

Exercise-induced (D059267) cardiomegaly, three-dimensional (D019560) echocardiography, exercise test (D005080)

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Introduction

Increased cardiac dimensions and changes in haemodynamics in those engaging in endurance sports have been described as early as the beginning of the 20th century.[1] These changes include increased left ventricular (LV) wall thickness, LV mass and increased LV and right ventricular (RV) chamber size, and are generally referred to as the 'athlete's heart'.[2] Although the precise mechanisms have proved elusive, they are believed to be physiological adaptions to normalise wall stress after altered loading conditions.[3] The amount of aerobic

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endurance training is thought to be the strongest predictor for the degree of cardiac changes observed.[4,5] A meta-analysis found the greatest athlete's heart-associated cardiac changes in bicyclists and cross-country skiers.[6]

Altered cardiac morphology and function have been found even in very young athletes,[7,8] and with increased competitiveness and professionalism there has been an increasing demand to improve our understanding of how intense endurance training affects the developing heart.[9]

Our group recently published a cross-sectional study on the hearts of pre-adolescent cross-country skiers, in which we found all the hallmark features of the athlete's heart.[8] As these elite athletes transition into adolescence, their hearts are undergoing the concurrent processes of development into maturity and athletic remodelling.

Success in endurance sports is closely linked to cardiac performance, thus it may be that a lack of progression is due to the inability of an athlete's heart to adapt further. With a cohort of promising, pre-adolescent athletes, we aimed to describe the cardiac features of both those who continued regular endurance training and those who did not.

In our baseline study, we found a concentric form of remodelling in pre-adolescent endurance athletes. We hypothesise that with continued endurance exercise, further cardiac changes will be primarily of an eccentric nature, similar to those found in adult elite endurance athletes.[6]

Methods

Participants were recruited from skiing clubs in the southeast of Norway in April to May 2013 and underwent baseline examinations from May to June the same year. The baseline examinations were performed at 12 years of age, one year after the athletes were allowed to participate in organised national competitions by the Norwegian Olympic and Paralympic Committee and Confederation of Sports provisions on children's sport.[10] All participants were invited to the follow-up in 2016. At both baseline and follow-up, the participants filled out self-reported questionnaires on training intensity and duration as well as prior illnesses.

Written informed consent was given by the legal guardians of all study participants. The study complies with the Declaration of Helsinki and was approved by the regional committee for medical research ethics (ref. 2011/659 S-08702d).

Transthoracic echocardiography

Both at baseline and at follow-up all participants underwent an echocardiographic study (Vivid E9; GE, Vingmed, Horten, Norway). Using greyscale harmonic imaging, standard echocardiographic views were obtained. Data were digitally stored for post hoc analysis (EchoPac; GE, Vingmed). All measurements were performed by a single, blinded observer. Using two-dimensional (2D) echocardiography LV dimensions, ejection fraction (EF), ad modum Simpson and LV diastolic function parameters were assessed. Left atrial (LA) volume was measured using the biplane method. Right atrial (RA) area, RV basal and mid-ventricular diameter and RV fractional area change were assessed in the four-chamber view. Parameters were measured according to the recommendations of the European Association of Cardiovascular Imaging (EACVI),[11] including indexing all chamber dimensions to body surface area. LV mass was calculated using the Devereux' formula.[12] LV geometry was assessed by calculating relative wall thickness (RWT) as (2*LVPWT)/LVEDD. LV volumes, EF and mass were also calculated from the three-dimensional (3D) datasets.

2D strain echocardiography

Strain analysis was performed using 2D echocardiography. For the assessment of longitudinal strain, the endocardial borders were traced in the end-systolic frame of the 2D images from the apical four, two-chamber and apical long axis views. Strain was evaluated on a frame-by-frame basis by automatic tracking of acoustic markers throughout the cardiac cycle. Segments that failed to track properly were manually adjusted by the operator. Any segment that subsequently failed to track was excluded. Peak systolic LV longitudinal strain was assessed in 16 LV segments and averaged to LV global longitudinal strain (GLS). Similarly, by tracing the endocardial borders in the parasternal short-axis view at the papillary muscle level, circumferential strain was evaluated. Peak systolic LV global circumferential strain was assessed by averaging six LV segments. RV GLS was calculated by averaging three RV free wall segments in the apical four-chamber view. The frame rate at baseline was 65 ± 12 Hz and at follow-up 63 ± 14 Hz.

Cardiopulmonary exercise testing

Maximal oxygen uptake (VO_{2max}) was determined by an incremental exercise test to exhaustion on a treadmill (Woodway Elg 70, Weil am Rhein, Germany). Speed and inclination were set to 7 km/hour and 6.3%, respectively, and the participants walked/ran at this intensity for one minute. Subsequently, both speed and inclination were increased by one km/hour and 1% every minute until a speed of 11 km/hour was reached. A further increase in intensity was achieved by

increasing the inclination. When the participant could no longer complete the desired workload, the test was terminated. Oxygen uptake was measured continuously with an automated system (Oxycon Pro; Jaeger-Toennis, Hochberg, Germany). The exercise test was accepted as maximal if the majority of the following termination criteria were met: respiratory exchange ratio greater than 1.0, heart rate greater than 200 beats/minute, display of indicators of a maximal effort such as sweating and, despite strong verbal encouragement, the participant was unable or unwilling to continue.

Statistical analysis

Analyses were carried out using SPSS version 21 (SPSS Inc., Chicago, IL, USA) and Stata 15.0 (StataCorp LLC, Texas, USA). Data were presented as mean±SD and numbers and percentages, respectively. The χ^2 test (categorical variables) and the Student's *t*-test (continuous variables) were used to determine differences between two groups at baseline and follow-up. Linear mixed models were used to assess the impact of training group on VO_{2max}, LV end-diastolic volume (EDV), LV end-systolic volume (ESV) and RWT. Training group, time point and the interaction of these were added as fixed effects and the individual athlete as a random effect. The models were adjusted for sex. For intra-individual changes from baseline to follow-up, the paired *t*-test was used. Reproducibility was expressed as intraclass correlation coefficient.

Results

Out of 76 participants in the baseline study, 48 (63%) took part in the follow-up. Those who attended the follow-up had a higher indexed VO_{2max} (64 ± 7 vs. 59 ± 5mL/kg/min, $P < 0.01$) at baseline, but did not differ in regard to other basic characteristics or echocardiographic data compared to those who did not return for follow-up. Two athletes failed to complete cardiopulmonary exercise testing (CPX) at baseline due to issues with the mask. At follow-up, one former and one active endurance athlete failed to complete CPX due to injuries. One active endurance athlete was diagnosed with asthma. No other chronic illnesses were reported. All participants in the study were Caucasian.

Of the 48 athletes, 31 (65%) reported more than 5 hours of weekly endurance exercise and were classified as active endurance athletes, while 17 athletes (35%) reported less than 5 hours of weekly endurance exercise and were classified as former endurance athletes. In the active group, 29 participated in cross-country skiing (94%), one in competitive rowing (3%) and one in orienteering (3%).

The active endurance athletes engaged in 10.3 ± 2.2 hours of organised training per week, of which 7.4 ± 1.9 hours were endurance training. The former endurance athletes reported 8.5 ± 5.6 hours of organised training per week, of which 1.9 ± 1.7 hours were endurance training. Of the 17 former endurance athletes, three (15%) had quit cross-country skiing after or during the previous season (0.5–1 years earlier), the rest at least 1.5 years earlier. All participants reported less than 3 hours of weekly strength training.

A comparison of basic characteristics between the active and former endurance athletes is summarised in Table 1. There were no significant differences in anthropometric data at baseline. At follow-up, the active endurance athletes had a lower resting heart rate.

Cardiopulmonary exercise testing

CPX data, comparing the two groups at baseline and follow-up, are summarised in Table 1. With no difference at baseline, the active endurance athletes had greater indexed and absolute VO_{2max} and time to exhaustion at follow-up. The respiratory exchange ratio and maximal heart rate did not differ at any point of measurement.

With a mixed linear regression model with VO_{2max} as the dependent variable, we found a greater increase in VO_{2max} from baseline to follow-up in the active endurance athlete group (1101 ΔmL/min vs. 683 ΔmL/min, $P < 0.005$). Both groups experienced a reduction in indexed VO_{2max}, but the reduction was less pronounced in the active endurance athletes (–3 ΔmL/min/kg vs. –6 ΔmL/min/kg, $P < 0.05$).

Cardiac morphology and function

There was no difference in any morphological parameter between the two groups at baseline (Table 2). At follow-up the active endurance athletes had greater 3D indexed LV EDV and ESV. There was also a trend towards greater indexed RV end-diastolic and end-systolic areas in the active endurance athletes at follow-up.

With a mixed linear regression model, we found a greater increase in indexed LV EDV from baseline to follow-up in the active endurance athletes (11 $\Delta mL/m^2$ vs. $4\,mL/m^2$, $P < 0.05$). For LV ESV there was a similar non-significant trend (3 $\Delta mL/m^2$ vs. $0\,mL/m^2$, $P = 0.05$). RWT fell in the active endurance athletes, but not in those who had quit (–0.05 $\Delta mL/m^2$ vs. $0.00\,mL/m^2$, $P = 0.01$).

There were moderate, positive correlations between weekly hours of endurance training and changes in VO_{2max} ($R = 0.55$, $P < 0.001$), indexed LV EDV ($R = 0.45$, $P < 0.01$), LV ESV ($R = 0.35$, $P < 0.05$),

Table 1. Comparison of basic characteristics and cardiopulmonary exercise test results between active and former endurance athletes at baseline and at follow-up.

	Baseline			Follow-up		
	Active athletes ($n=31$)	Former athletes ($n=17$)	*P* value	Active athletes ($n=31$)	Former athletes ($n=17$)	*P* value
Characteristics						
Age, years	12.1 ± 0.2	12.0 ± 0.2	0.06	15.3 ± 0.3	15.2 ± 0.3	0.81
Male, *n* (%)	22 (71)	12 (71)	0.98			
Height, cm	152 ± 7	152 ± 9	0.99	173 ± 10	172 ± 8	0.79
Weight, kg	40 ± 6	40 ± 6	0.82	59 ± 9	57 ± 6	0.53
BSA, m^2	1.31 ± 0.12	1.31 ± 0.14	0.91	1.70 ± 0.18	1.67 ± 0.13	0.62
BMI, kg/m^2	17.2 ± 1.5	17.4 ± 1.8	0.71	19.5 ± 1.8	19.3 ± 1.7	0.61
Resting HR, bpm	70 ± 11	72 ± 16	0.65	59 ± 8	66 ± 10	<0.05
Cardiopulmonary exercise test						
VO_{2max}, mL/min	2527 ± 344	2511 ± 430	0.89	3617 ± 692	3202 ± 531	<0.05
VO_{2max}, indexed	65 ± 7	63 ± 7	0.33	62 ± 8	57 ± 6	<0.05
RER	1.06 ± 0.04	1.05 ± 0.04	0.16	1.11 ± 0.05	1.12 ± 0.08	0.42
HR max, bpm	201 ± 8	199 ± 7	0.39	199 ± 9	200 ± 8	0.66
TtE, min:sec	6:14 ± 1:12	5:39 ± 1:12	0.13	8:21 ± 2:07	6:23 ± 1:32	<0.01

BSA: body surface area; BMI: body mass index; HR: heart rate; VO_{2max}: maximal oxygen uptake per minute; RER: peak respiratory exchange ratio; TtE: time to exhaustion.
Data are expressed as mean±SD.
P values are calculated using the Student's *t*-test and χ^2 tests.

RV end-diastolic area ($R=0.39$, $P<0.01$) and RV end-systolic area ($R=0.44$, $P<0.01$). No correlation was found for weekly hours of non-specified exercise.

There was no significant intergroup difference in any functional parameter at either baseline or follow-up (Table 2).

Intra and inter-observer intraclass correlations were performed in 10 of the 12-year-old athletes and were 0.99 and 0.95, respectively, for 3D LV EDV, 0.97 and 0.93 for 3D LV ESV, 0.93 and 0.94 for 3D LV mass, and 0.77 and 0.73 for 3D LV EF. For LV and RV strain, we have performed intra and inter-observer variability analysis in earlier studies.[13]

Discussion

Cardiac morphology

In this longitudinal follow-up study, we found no difference in either wall thickness or cardiac mass between those who continued competitive endurance sports and those who quit. However, ventricular volumes did differ. Those who continued to engage in competitive endurance exercise no longer experienced a concentric remodelling, as they did at baseline, but underwent balanced or even eccentric remodelling. The morphological changes found at follow-up are in contrast to those found at baseline. At 12 years of age, we found the greatest differences in wall thickness and cardiac mass; not chamber dimensions when comparing our pre-adolescent athletes with age-matched controls. This led to higher RWT, with a subsection of young athletes even exceeding the normal range.[8] As a consequence of chamber dilatation, the RWT of the active endurance athletes at 15 years of age normalised (Figure 1). Of the four active endurance athletes in our cohort with RWT exceeding the reference value (RWT > 0.42) at baseline, none did so at follow-up.

This early concentric remodelling is not predicted by the Morganroth hypothesis, named after the hallmark paper by Morganroth and colleagues in 1975.[4,14] Morganroth proposed that endurance athletes develop eccentric remodelling due to volume overload, while power athletes develop concentric remodelling due to increased afterload. If we were to look at the athletes at 15 years of age in isolation, ignoring the cardiac morphology at 12 years of age, it would fit well with this hypothesis, as would earlier studies in adult endurance athletes. Conversely, concentric remodelling in pre-adolescent endurance athletes would not fit into the same narrative.

In a recent cardiac magnetic resonance study, Barczuk-Falecka et al.[15] have found the same phenomenon in pre-adolescent Polish footballers. The authors

Table 2. Comparison of cardiac morphology and function between active and former endurance athletes at baseline and at follow-up.

	Baseline			Follow-up		
	Active athletes ($n=31$)	Former athletes ($n=17$)	*P* value	Active athletes ($n=31$)	Former athletes ($n=17$)	*P* value
Cardiac morphology						
2D LA volume/BSA, ml/m^2	27.3 ± 6.2	29.4 ± 6.3	0.39	31.7 ± 7.0	33.0 ± 7.0	0.54
IVSd, mm	7.9 ± 0.8	7.8 ± 1.0	0.54	8.1 ± 1.2	7.8 ± 0.9	0.41
LVIDd/BSA, mm/m^2	2.1 ± 0.3	2.0 ± 0.3	0.60	3.0 ± 0.2	2.9 ± 0.2	0.34
LVPWd, mm	7.3 ± 0.9	6.8 ± 0.9	0.07	7.8 ± 1.2	8.1 ± 1.2	0.42
2D LV mass/BSA, g/m^2	72 ± 12	67 ± 15	0.23	82 ± 20	77 ± 15	0.45
3D LV EDV/BSA, mL/m^2	76 ± 8	74 ± 8	0.89	84 ± 11	79 ± 10	<0.05
3D LV ESV/BSA, mL/m^2	33 ± 4	33 ± 4	0.99	36 ± 6	32 ± 3	<0.05
3D LV mass/BSA, g/m^2	69 ± 7	71 ± 4	0.57	76 ± 11	74 ± 6	0.19
Relative wall thickness	0.35 ± 0.05	0.33 ± 0.05	0.12	0.31 ± 0.04	0.33 ± 0.05	0.05
RA area/BSA, cm^2/m^2	10.9 ± 1.3	10.6 ± 1.1	0.56	9.5 ± 1.2	9.1 ± 1.5	0.32
RVD basal, mm	38 ± 3	38 ± 4	0.72	42 ± 5	41 ± 4	0.79
RVD mid-cavity, mm	31 ± 3	33 ± 3	0.22	37 ± 5	34 ± 5	0.08
RV EDA/BSA, cm^2/m^2	16 ± 3	16 ± 3	0.50	14 ± 2	12 ± 2	0.07
RV ESA/BSA, cm^2/m^2	9 ± 2	10 ± 2	0.07	8 ± 1	8 ± 2	0.08
LV and RV systolic function						
3D LV EF, %	56 ± 3	56 ± 3	0.93	58 ± 3	59 ± 2	0.52
3D LV SI, mL/m^2	42 ± 5	42 ± 5	0.80	48 ± 6	47 ± 8	0.14
LV GLS, %	22.7 ± 1.7	22.5 ± 2.0	0.64	22.5 ± 1.9	21.7 ± 2.3	0.22
LV GCS, %	24.0 ± 3.0	25.9 ± 4.4	0.28	24.5 ± 2.1	24.2 ± 2.1	0.66
RV FAC, %	40 ± 7	39 ± 8	0.35	39 ± 4	39 ± 4	0.96
TAPSE, mm	2.5 ± 0.3	2.5 ± 0.3	0.44	2.3 ± 0.3	2.3 ± 0.4	0.76
RV GLS, %	28.9 ± 4.2	27.1 ± 2.4	0.23	25.7 ± 2.9	24.9 ± 3.1	0.43
LV diastolic function						
Mitral E velocity, cm/sec	0.94 ± 0.09	0.99 ± 0.17	0.27	0.99 ± 0.15	0.99 ± 0.17	0.89
Mitral A velocity, cm/sec	0.45 ± 0.11	0.49 ± 0.13	0.55	0.44 ± 0.10	0.44 ± 0.09	0.99
Mitral E/A ratio	2.2 ± 0.5	2.2 ± 0.6	0.98	2.3 ± 0.6	2.4 ± 0.7	0.92
E/e′ ratio	6.9 ± 1.1	7.3 ± 1.6	0.53	6.9 ± 1.6	7.3 ± 1.4	0.54

LA: left atrium; BSA: body surface area; IVSd: interventricular septum thickness in end-diastole; LVIDd: left ventricular internal diameter in end-diastole; LVPWd: left ventricular posterior wall thickness in end-diastole; LV: left ventricle; EDV: end-diastolic volume; ESV: end-systolic volume; RA: right atrium; RV: right ventricle; EDA: end-diastolic area; ESA: end-systolic area; EF: ejection fraction; SI: stroke index; GLS: global longitudinal strain; GCS: global circumferential strain; FAC: fractional area change; TAPSE: tricuspid annular plane systolic excursion.
Data are expressed as mean±SD.
P values are calculated using the Student's paired *t*-test and χ^2 tests.

found no differences with regard to chamber dimensions, but both LV mass and wall thickness were significantly greater in pre-adolescent athletes.[15] Similarly, Pela et al. found an average RWT of 0.35 in 13-year-old athletes, the same high value as the 12-year-old athletes in our baseline study.[16]

This dynamic does not seem to be isolated to young athletes. In a recent experimental study, Arbab-Zadeh et al.[17] demonstrated the same initial dynamic in the development of athlete's heart in adults. Previously untrained, healthy adults were exposed to intensive endurance training, and the initial cardiac response was concentric wall thickening. There was no eccentric chamber dilatation until after 6–9 months.[17]

Combined, these studies suggest a different mechanism in the development of athlete's heart in endurance athletes than a purely eccentric response to volume overload. It could very well be that the initial feature in the development of athlete's heart is concentric remodelling, and that the dilated ventricles seen in the fully developed athlete's heart is in fact a late result of continued endurance training. Such a two-phased

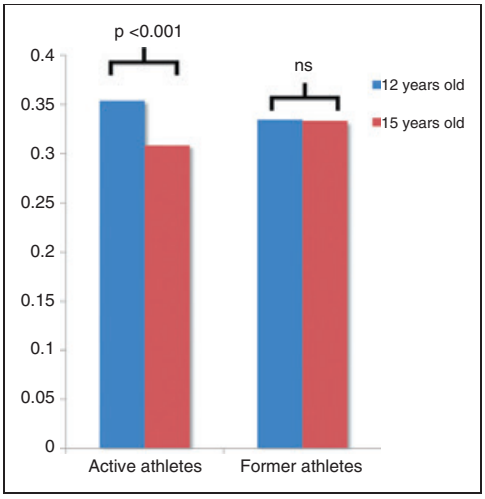

Figure 1. Change in relative wall thickness from baseline to follow-up among those who continued endurance training and those who did not.

dynamic might explain the observed heterogeneity of athlete's heart morphology in young endurance athletes.

Cardiac function

Neither group saw any significant changes in LV deformation parameters, which is in accordance with the findings of a recent meta-analysis on LV function in athlete's heart.[18] However, both groups saw a similar decline in RV GLS and tricuspid annular plane systolic excursion (TAPSE). While data on the subject are quite sparse, studies on RV GLS in pre-adolescent athletes have generally found greater deformation values than similar studies in adult athletes.[19,20] The dynamics of RV remodelling might well be different in the developing heart, which could help explain both the findings from this study and the discrepancies in RV GLS between pre-adolescent and adult athletes seen in other studies. Furthermore, exercise has been found to be inversely correlated with RV GLS even in adult athletes.[21] It should be noted, however, that both RV GLS and TAPSE remained well within reference values and the changes from baseline to follow-up are small.

Clinical implications

With increasing professionalism and with more and more pre-adolescent athletes engaging in intense exercise, distinguishing physiological changes of athlete's heart from pathological cardiac changes is becoming increasingly clinically relevant. There is evidence that intense endurance exercise not only hastens the onset and increases the burden of symptoms in hereditary heart disease such as arrhythmogenic right ventricular cardiomyopathy, but that overtraining in itself may induce arrhythmias through irreversible cardiac changes.[20,22,23] Identifying athletes at risk of cardiac disease and sudden cardiac death is of vital importance, and is the focal point of the recently published pre-participation guidelines from the European Heart Rhythm Association and the European Association of Preventive Cardiology.[24] However, in addition to predict rare events accurately in a large population, correctly identifying those not at risk might be an equally important task. Concentric remodelling might alarm a clinician expecting only eccentric changes in young endurance athletes. Our study suggests that concentric remodelling in conjunction with chamber dilatation can be considered normal in the early development of athlete's heart, and that it is likely to normalise with continuing exercise.

Limitations

While the former endurance athletes engaged in less endurance exercise than the active endurance athletes, the group was far from sedentary. Everyone in this cohort was engaged in regular endurance training at 12 years of age, and most of the former endurance athletes were still exercising regularly. Some also pursued other non-endurance sports competitively. This could potentially mask cardiac changes that would be visible in comparison to a sedentary population. Interestingly, however, only weekly hours of endurance exercise correlated with increases in VO_{2max} and changes in cardiac morphology. As this study does not have a sedentary control arm, the cardiac changes can not be controlled for cardiac maturation. The differences between the two groups may have been greater had the former endurance athletes ceased exercising altogether. A third of the athletes from the baseline study were lost to follow-up, adversely affecting statistical power.

We estimated LV mass using 3D echocardiography, and while there is evidence that utilising 3D echocardiography for assessing LV mass is more precise than traditional echocardiographic techniques, cardiac magnetic resonance is still the gold standard.[25]

Conclusion

In young athletes who performed high volume endurance training from the age of 12 to 15 years, after an initial concentric remodelling, the cardiac chambers started to dilate, and the RWT decreased and normalised. In contrast, those who ceased high volume

endurance training did not see this dilation and did not experience a drop in RWT. Our results support the notion that the morphological changes described in the power athlete's and the endurance athlete's heart might be incorrect. According to our data, an early concentric remodelling in pre-adolescents followed by an eccentric chamber dilatation in adolescents should be expected in young endurance athletes.

Author contribution

Sebastian I Sarvari, Thor Edvardsen, Hege EW Landgraff, Jostein Hallén and Svein Leirstein contributed to the conception or design of the work. All co-authors contributed to the acquisition, analysis, or interpretation of data for the work. Anders W Bjerring drafted the manuscript. All co-authors critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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Paper IV

Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry in the assessment of body composition in athletic adolescents

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Abstract

The purpose of the present study was to examine and compare the reliability of multifrequency bioelectrical impedance analysis (BIA) with dual-energy X-ray absorptiometry (DXA) for predicting body composition in athletic adolescents, and to evaluate BIA against a reference method; DXA.

Fat-free mass (FFM), fat mass (FM) and percentage fat (FM%) were measured by BIA (InBody 720) and DXA in 18 (11 girls) healthy adolescent cross-country skiers (aged 15.6 (0.3). The BIA and DXA measurements took place within 30 minutes on the same day for each participant, and the same procedure was repeated within 5 to 14 days for all participants, except for one participant.

There were no significant differences between test and retest for any of the variables assessed by InBody 720 or DXA. The test-retest results showed an excellent correlation for both InBody 720 and DXA for all the assessments (ICC≥0.990). Precision of repeated measurements of FFM and FM (kg and %) expressed as coefficient of variation (CV) was lowest for FFM with both InBody 720 and DXA (0.95% and 1.14%, respectively) and the corresponding CVs for FM (kg and %) were 2.5% and 4.4%, and 2.1% and 4.0% with InBody 720 and DXA, respectively. There was no difference between the devices in CV for FFM (p=0.474), whereas for FM and FM% the CVs between the devices were significantly different (p=0.008 and p=0.025, respectively).

There were almost prefect correlations (Pearson's r) between InBody 720 and DXA for all variables examined (FFM, FM and %FM) (r≥0.966). However, InBody 720 assessed systematically higher total FFM and lower total FM (kg and %) compared to DXA. The mean differences between InBody 720 and DXA for FFM, FM (kg and %) and their limits of agreement (LoA) (±1.96SD) were -2.7 (1.8) kg, 4.0 (1.8) kg and 7.4% (3.3%), respectively.

Conclusion: The very small mean difference between test and retest, and the narrow LoA for both FFM and FM (kg and %) together with the high ICC, suggest that both InBody 720 and DXA have excellent reliability for all the body composition estimates, especially for estimating FFM, as indicated by the very low CV. Further, the methods provided estimates of FFM, body fat mass and body fatness that were highly correlated in a population of healthy adolescent athletes. However, the significant mean differences between the InBody and DXA results suggest that the methods should not be used interchangeably.

Introduction

Quantifying human body composition is an increasingly common procedure in various populations including healthy individuals, patients, athletes, the elderly and children; it is regarded an important health and performance variable (Ackland et al., 2012; Malouf et al., 2013). The primary focus in most studies assessing body composition has been the measurement of fat mass (FM) or %fat mass (FM%). However, the close relationship between fat-free mass (FFM) and the physical demands of many sporting activities has made body composition assessment a useful tool in athletic populations (Kendall et al., 2017). Moreover, during childhood and adolescence, FFM is significantly related maximal oxygen uptake (Armstrong & Welsman, 2019). Monitoring body composition is of great interest to coaches and researchers in both young and adult athletes and requires reliable and easy to use methods.

There are several techniques or models for describing the constituent components of the body, and the choice of technique often depends on the available technology and the intended application of the data (Ackland et al., 2012). The available techniques can be divided into three categories: reference, laboratory and field techniques (Ackland et al., 2012).

The 'gold standard' for estimating percentage body fat (% BF) is cadaver analysis (Ackland et al., 2012; Talma et al., 2013). However, since this method is not normally feasible and cannot be used for individual analyses, other methods have been accepted as reference methods (Ackland et al., 2012).

The simplest model for describing the composition of the body is the two-component model (2-C) which divides body mass (BM) into two components; FM and FFM. All models include FM, and FFM is simply calculated by subtracting FM from BM. FFM is composed of various constituents, (water, protein, mineral (bone and soft tissue)), and the ability to further divide FFM into two, three, or more components is what characterizes and distinguishes the multicomponent models (e.g. 3-C, 4-C) from the 2-C model (Ackland et al., 2012; Wells & Fewtrell, 2006).

MRI and CT scanning are multicomponent models (4-C), which divide the body into FM, bone, muscle and other tissue. These techniques are considered sufficiently accurate for estimating %BF in living humans and are thus regarded as the best reference methods for estimation of body fat (Ackland et al., 2012; Kyle, Earthman, Pichard, & Coss-Bu, 2015; Talma et al., 2013). The use of 3- or 4-component models to assess body composition in children represents the optimal approach, since the need for assumptions regarding FFM chemical compositions, and thus model error, are minimized (Lohman & Going, 2006). However, multicomponent models are time consuming, may involve unnecessary radiation exposure, and are generally not available to clinicians due to the expensive technology involved. They are therefore not very practical in large epidemiological and field studies (Ackland et al., 2012; Kyle et al., 2015; Talma et al., 2013).

Dual-energy X-ray absorptiometry (DXA) has become the gold standard for bone mineral measurements (Stewart & Hannan, 2000). Due to its widespread availability and ability to also measure soft tissue precisely, DXA is considered a useful reference method for body composition and is thus acknowledged by many as the standard and most precise method to assess body fat mass (Hofsteenge, Chinapaw, & Weijs, 2015; Prior et al., 1997). DXA measures three components of body composition: bone mineral content, fat (lipid), and other fat-free soft tissue (3-C model) (Ackland et al., 2012; Hofsteenge et al., 2015). It also measures regional fat distribution. Although DXA is a precise laboratory method to assess body composition it has some limitations, such as high cost, time requirements, and exposure to small amounts of radiation (Kyle et al., 2015).

Bioelectrical impedance analysis (BIA) is a widely used field method for estimating body composition. The BIA method assumes that the body can be described in terms of a 2-component model (2-C model) of FM and FFM. The method uses body impedance and total body water (TBW) as input variables to predict %FM, FM and FFM from various equations (Talma et al., 2013). Most BIA techniques are developed and validated against DXA, which serves as a reference method (Kyle et al., 2015). The method is widely used because it is cheap, non-invasive, quick and the instrument is portable and easy to move.

Thus, the purpose of the present study was to examine the reliability of the BIA method for predicting body composition in athletic adolescents and to evaluate BIA against a reference method, DXA. Validating BIA against DXA has been done on a range of populations in several previous studies (e.g. Esco et al., 2015; Hofsteenge et al., 2015; Shafer, Siders, Johnson, & Lukaski, 2009; Volgyi et al., 2008; Wang et al., 2013). Most studies conducted on children have focused on obesity and only a few have examined healthy children and adolescents (Chiplonkar et al., 2017; Fors, Gelander, Bjarnason, Albertsson-Wikland, & Bosaeus, 2002; Lim et al., 2009; Sluyter, Schaaf, Scragg, & Plank, 2010). However, to the best of our knowledge, no study has been conducted on lean, athletic adolescents.

Materials and methods

Study design

This study can be characterized as a method comparison study and includes analyses of reliability (test vs. retest of BIA) and validity (BIA vs. DXA). At both Test and Retest, BIA and DXA measurements took place within 30 minutes on the same day for each participant, and the time between Test and Retest varied from 5 to 14 days. All measurements were performed in the afternoon without monitoring food intake or hydration status.

Participants

Eighteen cross country skiers (11 girls) volunteered to participate in the study. Mean (SD) age was 15.7 (0.3) and 15.5 (0.3) years for boys and girls, respectively ($p = 0.213$). All subjects were included in the validity analyses. One boy did not complete the second BIA assessment; thus, 17 subjects were included in the reliability analyses. Written parental consent was obtained prior to testing. All experimental procedures were approved by the Norwegian Regional Committee for Medical Research Ethics and conformed to the standards set by the Declaration of Helsinki.

Measurements

Height and body mass (BM) were measured to the nearest 0.1cm and 0.1 kg respectively, using a combined stadiometer and digital scale (Seca, Hamburg, Germany). The participants wore shorts, t-shirt and no shoes during measurements.

BIA measurements were carried out using InBody 720 (Biospace Co, Ltd, Seoul, Korea) according to the manufacturer's guidelines. The participants stood barefoot with the whole of the sole of the foot in contact with the foot electrodes. The hand electrodes were held with the thumb placed lightly on top of the thumb electrode and the other fingers touching the other electrode. Arms were held at an angle of approximately 15 degrees between the arms and the side of the body. This position was maintained until measurements were completed (~2minutes). The manufacturer's built-in equation was used for calculation of body composition.

DXA measurements were carried out using Lunar iDXA (GE Healthcare, Madison, Wisconsin, USA) according to the manufacturer's guidelines. Each day prior to testing, the iDXA was calibrated according to the manufacturer's instructions using a standard calibration block. Participants were scanned from head to toe in a supine position, providing values for total lean tissue and fat mass. The iDXA machine automatically chose the scanning mode, with all subjects scanned in the standard mode. All Lunar iDXA scan files were automatically analyzed with enCORE software version 14.10.022 (GE).

Statistical analyses

Data are mean (standard deviation) unless otherwise stated. GraphPad Prism 8.2.1 (GraphPad Software Inc., La Jolla, CA), Microsoft Excel 2013 and SPSS (Version 25.0; IBM Corporation, Armonk, New York) were used for statistical analyses.

Between-method differences for FFM, FM and %FM were calculated by subtracting InBody 720 from DXA (DXA-InBody 720). Test-retest reliability was examined by using mean difference ±95% limits of agreement (LoA) including Bland-Altman plots. Intraclass correlation coefficients (ICC 3,1-single measures) with 95% confidence intervals (CI) were calculated, and the following criteria were adopted for interpreting the strength of the correlation (ICC) between the measures:, < 0.5, poor; 0,5 - 0.75, moderate; 0.75 - 0.90, good ; 0.90-1.0, excellent (Koo & Li, 2016). The coefficient of variation (CV=

SD of the differences between test and retest divided by √2 divided by the pooled mean of the variable times 100%) was also calculated. Validity was examined using mean difference ±95% LoA including Bland-Altman plots and Pearson's correlation coefficient (*r*). The following criteria were adopted for interpreting the strength of correlation (r) between the measures: <0.1, trivial; 0.1– 0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very large; and 0.9–1.0, almost perfect (Hopkins, Marshall, Batterham, & Hanin, 2009). For paired comparisons between test-retest and mean difference between BIA and DXA, a paired Student's t-test was run. To test for difference in age and anthropometrics between boys and girls, an unpaired Student's t-test was run. Statistical significance was set at $p<0.05$.

Results

The physical characteristics of the participants are reported in Table 1, measured both with InBody 720 and DXA. Fat mass was significantly higher in girls compared to boys in both kg and percentages, and with both methods. The other physical characteristics were not statistically significantly different between the sexes, but the boys tended to be taller and have a higher FFM.

Reliabilty of InBody 720 and DXA

The test-retest mean differences were similar for InBody 720 and DXA for all the body composition measurements. (Table 2 and Supplemental Figure 1). There were no sex differences for any of the test-retest mean differences with either InBody 720 or DXA (Supplemental Table 1).

Test-retest ICCs were ≥ 0.99 for all variables examined (FFM, FM kg and FM%) with both InBody 720 and DXA (Table 2).

The largest CV was seen for FM (kg and %) with both devices, and it was larger with InBody 720 compared with DXA (2.1%) for both FM (p=0.008) and FM% (p=0.025) (Table 2). CV for FFM did not differ between InBody 720 and DXA (p=0.474) (Table 2).

The CV for FM (kg and %) was higher for boys than girls (Supplemental Table 1). The opposite was the case for FFM, with higher CV for girls (Supplemental Table 1).

Validity of InBody 720

The correlation (Pearson's r) for InBody 720 vs DXA comparisons for all variables examined (FFM, FM and %FM) was ≥0.966.

InBody 720 assessed systematically higher total FFM and lower total FM (kg and %) compared to DXA (Table 1 and Fig. 1). The mean difference between InBody 720 and DXA for FFM, FM (kg and %) and their LoA (±1.96SD) were -2.7 (1.8) kg, 4.0 (1.8) kg and 7.4% (3.3%), respectively (Figure 1).

There was no significant difference between boys and girls in mean difference between InBody 720 and DXA for any of the body composition estimates (Supplemental Table 2).

Discussion

In this study we evaluated the reliability of two devices for estimating body composition: DXA and InBody 720, and validated InBody 720 against DXA. The main findings were: 1) for both devices the level of reliability was found to be excellent for all the body composition estimates (FFM, FM and FM%) and there was no difference in reliability between the devices; and 2) InBody 720 assessed systematically higher FFM and lower FM compared with DXA.

Reliability

In the whole sample, there was no significant difference between test and retest for any of the variables assessed by InBody 720 or DXA. The test-retest results showed an excellent correlation for both InBody 720 and DXA for all the assessments (ICC≥0.990). Precision of repeated measurements of FFM and FM (kg and%) expressed as CV (Table 2) was lowest for FFM with both InBody 720 and DXA (0.95% and 1.14%, respectively), with no difference between the devices (p=0.474). For FM, CV was 4.0-4.5 % for InBody 720 and 2.0-2.5 % for DXA (p=0.008 for FM and p=0.025 for %FM). Based on these results we regarded the level of reliability to be excellent for both InBody 720 and DXA in assessing body composition, especially for FFM. The high test-retest reliability is in accordance with several previous studies (Anderson, Erceg, & Schroeder, 2012; Gibson, Holmes, Desautels, Edmonds, & Nuudi, 2008; McLester, Nickerson, Kliszczewicz, & McLester, 2018; Talma et al., 2013; Volgyi et al., 2008).

The CV for FFM was more reproducible than for FM with both devices. This is only because FFM is higher, since the typical error for FFM was ~0.5 kg and for FM ~0.3 kg. Measures of FFM compared with FM for both InBody 720 and DXA (Table 2) showed that FM was smaller than FFM, which could explain why measuring FM accurately is more difficult than FFM, as also pointed out by Tompuri et al. (2015). Boys had significantly less FM (kg and %) than girls (p≤0.002 with both devices) which may explain the larger CV for boys in estimating FM in the test-retest for both InBody and DXA.

Whereas there was no difference between the devices in CV for FFM, CV for FM was significantly lower for DXA compared with InBody 720. These results suggest that InBody 720 may provide precise estimation of FFM but less precise estimation of FM when compared with DXA. This is supported by Wang et al. (2013), who compared two different BIA devices with DXA and MRI in 200 healthy men and women. They found that BIA provided accurate and reliable estimation of skeletal muscle mass, but less accurate estimation of FM in relation to DXA or MRI.

The very small mean difference between test and retest and the narrow LoA for both FFM and FM (kg and %) (Table 2), together with the high ICC, suggest that both InBody 720 and DXA have excellent reliability for all the body composition estimates, especially for estimating FFM.

Validity

Results from the current study showed that InBody 720 overestimated FFM by 2.7 kg, and underestimated FM by 4.0 kg and 7.4% compared with DXA (Figure 1). The difference between InBody 720 and DXA was similar for boys and girls for all the body composition estimates. There were almost prefect correlations (Pearson's r) between InBody 720 and DXA for all variables examined (FFM, FM and %FM) ($r \geq 0.966$), which agrees with other studies on children (Fors et al., 2002; Okasora et al., 1999).

The significant underestimation of FM (and overestimation of FFM) by InBody 720 appears to be consistent across the range of FM and FFM values. Only a few studies have included an athletic population when comparing InBody with DXA (Esco et al., 2015; Raymond, Dengel, & Bosch, 2018). Esco et al. (2015) and Raymond et al. (2018) studied female and male collegiate athletes, respectively, and the results from the current study agree well with both these studies with adult athletes.

Other previous studies with non-athletic populations have also reported similar results to those of the current study when comparing BIA with DXA, but often with a proportional bias for FM and FFM (Esco et al., 2015; McLester et al., 2018; Raymond et al., 2018; Tompuri et al., 2015). Ling et al. (2011) found that InBody 720 underestimated FFM and overestimated FM compared with DXA in a group of 284 middle aged men and women. However, the authors also observed that BIA underestimated FM in individuals with low FM and overestimated at high FM. They concluded that the overestimation of FM appeared to increase with increasing BMI. The participants in Ling et al.'s study exhibited a wide range of BMI from normal (BMI 18.5-24.9) to obese (BMI>30), which was also the case in the study by Shafer et al. (2009). Okasora et al. (1999) studied 104 children, both healthy and sick, ranging from severely underweight to severely overweight. They found that %FM was greater by BIA compared with DXA in the extremely underweight, whereas the opposite was the case in the extremely overweight. Further, Tompuri et al. (2015) found that BIA particularly overestimated percent FFM in children with high %FM, and tended to underestimate percent FFM in children with low %FM; they concluded that higher body fat content increased the methodological offsets. The participants in the current study were a very homogenous group and the BMI of the boys and girls varied from 16.6 to 23.8 with an average BMI for the whole group of 19.5. Thus, the underestimation of FM and overestimation of FFM by BIA in relation to DXA in the current study is in line with the results from the leanest groups in the studies by Ling et al. (2011), Shafer et al. (2009) and Tompuri et al. (2015).

Although DXA is considered to provide accurate estimates of body composition and is used by many as a reference method as in the current study, DXA also has some limitations. The multi-component models (e.g. 4C or 5C) are regarded as the best reference methods for estimation of body fat in living humans (Ackland et al., 2012; Kyle et al., 2015; Talma et al., 2013). In a validation study between

DXA and a 5C-model for predicting FM% in a group of female athletes, Moon et al. (2009) found that DXA systematically overestimated FM% by 3.71±6.39%. This was later confirmed by Watson, Venables, and Murgatroyd (2017), who validated a 4-C model against DXA in a group of healthy men and women. They found a systematic bias between the two methods in estimating FM, with DXA overestimating FM up to an average FM of ~32 kg. Above ~32 kg, DXA shifted to underestimating FM (Watson et al., 2017). All the participants in the current study had an FM far below 32 kg (on average 8.5 kg (InBody) and 12.5 kg (DXA)). Thus, using DXA as our reference method may explain part of the difference, we found between InBody 720 and DXA. The true values in the lean athletes in the current study may lie between the results of the two methods.

All reference methods are based on assumptions to convert raw data measures of FFM (of which water, protein and minerals are main components) into measures of %FM, and the constants needed for these calculations can vary from person to person (i.e. they are age- and sex-specific) (Talma et al., 2013). There are physiological differences between children and adults and it has been shown that the water and bone mineral content of the FFM changes with age (Kyle et al., 2015). During childhood, a change in the chemical composition of FFM takes place, with a decrease in the percentage of water and an increase in percentage of protein and osseus mineral (Fomon, Haschke, Ziegler, & Nelson, 1982). Fomon et al. (1982) observed that the water content of FFM between birth and the age of 10 decreased from 81-75% in boys and from 81-77% in girls. Estimated water content of FFM in 13-17-year-old boys has been reported to be ~74% and the value is ~76% in girls of the same age (Lohman, 1986; Malina, 2004). BIA measures the water content of the body, and FFM is calculated from TBW using an assumption that FFM contains 73% water in adults (Wang et al., 1999). The 73% reference factor may thus result in an overestimation of FFM and an underestimation of FM in children and others who have a higher FFM water content than the reference factor of 73% (Kyle et al., 2015).

The hydration factor should be sex- and age-specific when applied to children (Lohman, 1986). Neither InBody 720 nor DXA have published their prediction equations and the devices cannot be adjusted for sex, age or special populations. Lack of information on, for example, subject characteristics used to develop the manufacturer's equation makes it difficult to decide whether it is appropriate for the population in the current study. We can therefore speculate whether the constants used in the algorithms by InBody 720 and DXA were unsuitable for the population in the current study and thus produced different results in estimating FFM and consequently in FM.

Although the mean difference between InBody 720 and DXA was significant for all the body composition measures, the relationship between the devices was almost perfect (Pearson's $r \geq 0.966$ boys and girls combined) for both FFM and FM. We conclude that InBody 720 is reliable and valid in relation to DXA; however, the two devices are not interchangeable.

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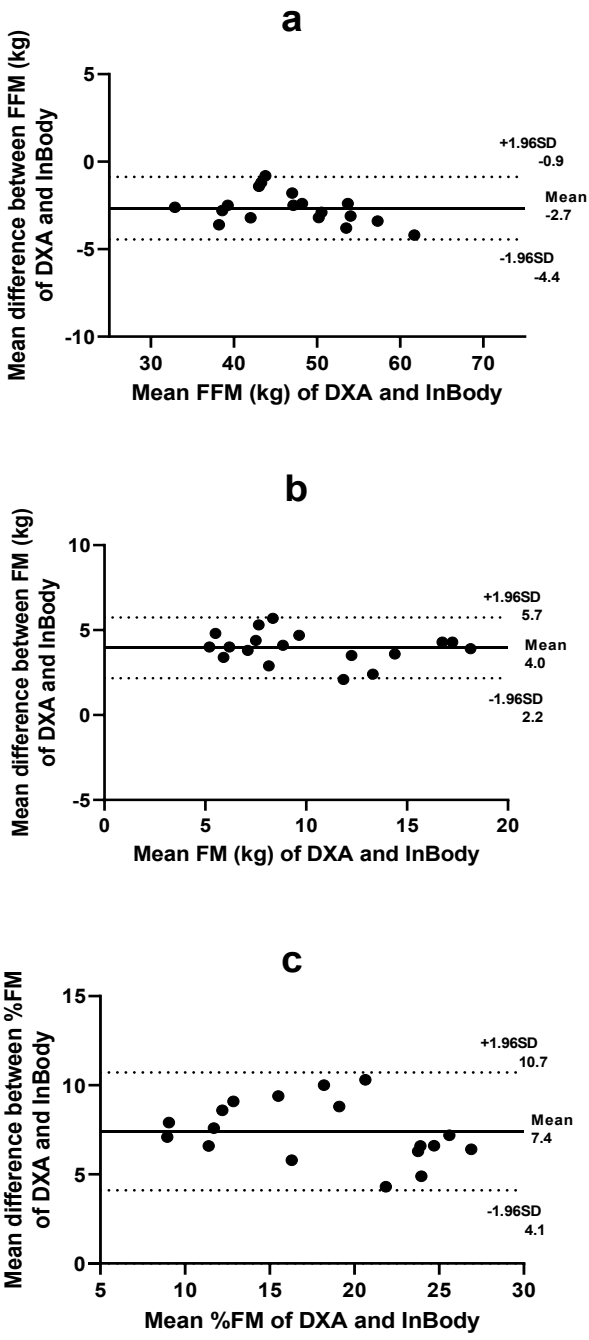


Figure 1: Bland-Altman plot with mean difference ±95% LoA for validity of InBody 720 vs DXA for predicting a) FFM, b) FM and c) %FM using data from test 1 (*n*=18).

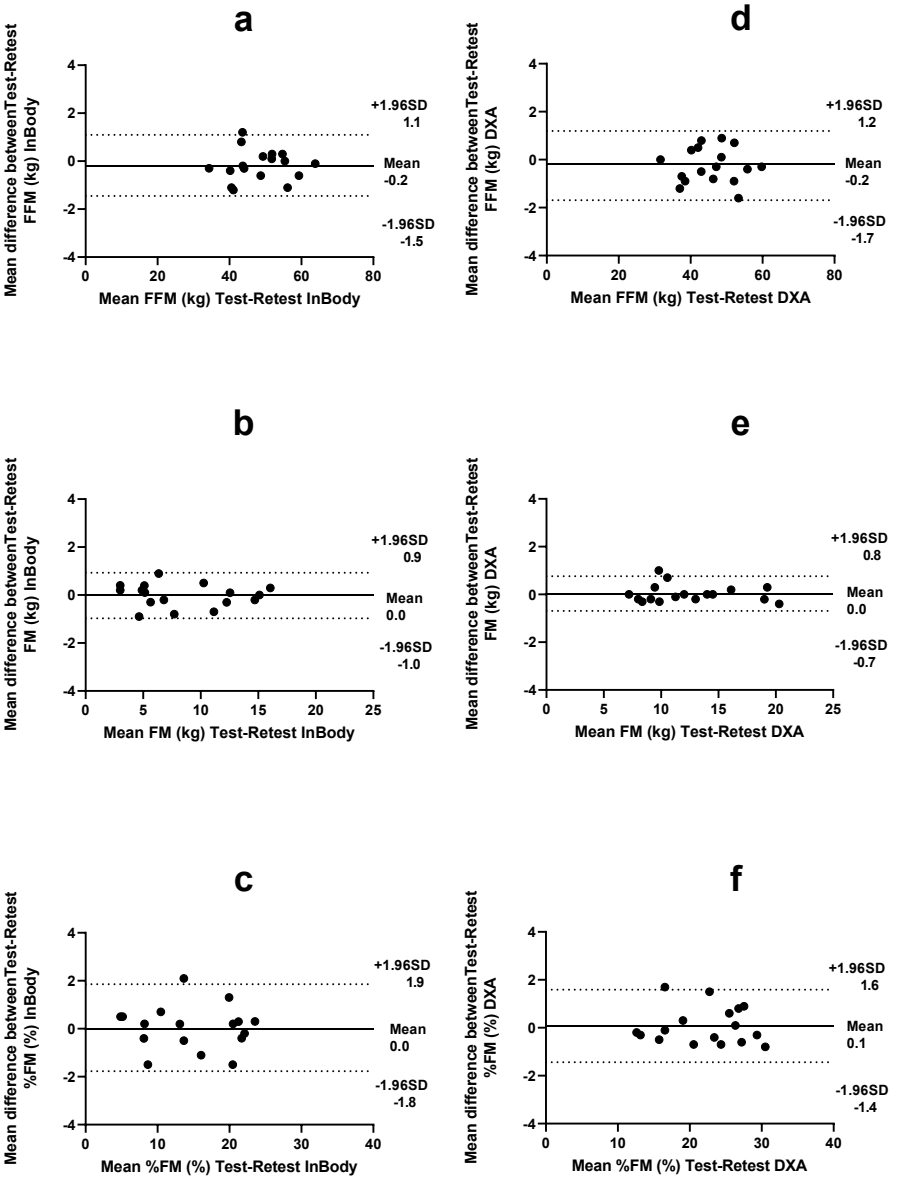
Table 1: Anthropometrics and body composition characteristics of the participants. Results presented as means (SD).

	All (n=18)		Boys (n=7)		Girls (n=11)		p-values
Height (cm)	171.0	(8.5)	176.5	(11.4)	168.0	(5.4)	0.054
Weight (kg)							
InBody 720	57.3	(8.7)	57.3	(9.4)	57.3	(8.7)	0.213
DXA	57.8	(8.7)	57.9	(9.5)	57.7	(8.7)	0.962
Fat free mass (kg)							
InBody 720	48.2	(7.7)*	52.1	(9.6)*	45.8	(5.4)*	0.091
DXA	45.6	(7.3)	49.1	(9.0)	43.3	(5.3)	0.101
Fat mass (kg)							
InBody 720	8.2	(4.4)*	4.3	(0.9)*	10.7	(3.8)*	0.001
DXA	12.2	(4.2)	8.8	(1.5)	14.4	(3.8)	0.002
Fat mass (%)							
InBody 720	14.4	(6.4)*	8.0	(2.7)*	18.5	(4.2)*	<0.001
DXA	21.9	(5.8)	16.1	(3.6)	25.5	(3.5)	<0.001

*Mean values represent test results (no retest values). p-values represent differences between boys and girls. *denotes significant difference between InBody 720 and DXA.*

Table 2: Reliability statistics (Test-Retest) for fat free mass, fat mass and %fat mass from InBody 720 and DXA measurements in 17 boys and girls.

	InBody 720			DXA		
	Mean difference ±95% LoA	ICC (95%CI)	CV	Mean difference ±95% LoA	ICC (95%CI)	CV
FFM (kg)	-0.2 ± 1.3	0.997 (0.991, 0.999)	0.95	-0.2 ± 1.4	0.995 (0.987, 0.998)	1.1
FM (kg)	-0.02 ± 1.0	0.994 (0983, 0.998)	4.0	0.0 4± 0.7	0.996 (0.989, 0.999)	2.1
FM (%)	0.04 ± 1.8	0.990 (0.972, 0.996)	4.4	0.08 ± 1.5	0.991 (0.975, 0.997)	2.5



Supplement Figure 1. Bland-Altman plot with mean difference ± 95% LoA for Test-Retest reliability for predicting FFM, FM, %FM (a, b, c) using InBody 720 and DXA (d, e, f) (n=17)

Supplemental table 1: Reliability statistics (Test-Retest) for fat free mass, fat mass and %fat mass from InBody 720 and DXA.

	Boys (n=6)			Girls (n=11)		
	Mean difference ±95% LoA	ICC (95%CI)	CV	Mean difference ±95% LoA	ICC (95%CI)	CV
InBody 720						
FFM (kg)	-0.1 ± 0.6	0.999 (0.996, 1,000)	0.44	-0.2 ± 1.5	0.989 (0.961, 0.997)	1.21
FM (kg)	-0.1 ± 0.9	0.915 (0.516, 0.988)	7.67	0.0 ± 1.0	0.991 (0.967, 0.998)	3.37
FM (%)	-0.1 ± 1.5	0.967 (0.788, 0.995)	6.78	0.1 ± 2.0	0.972 (0.898, 0.992)	3.94
DXA						
FFM (kg)	-0.2 ± 1.0	0.999 (0.990, 1,000)	0.75	-0.3 ± 1.7	0.987 (0.952, 0.996)	1.38
FM (kg)	0.0 ± 0.9	0.945 (0.663, 0.992)	3.83	0.0 ± 0.6	0.997 (0.987, 0.999)	1.58
FM (%)	0.0 ± 1.6	0.977 (0.848, 0.997)	3.59	0.1 ± 1.5	0.976 (0.912, 0.993)	2.17

Supplemental table 2: Validity statistics for predicted fat free mass, fat mass and % fat mass from InBody 720 measurements against DXA.

	Boys (n=7)		Girls (n=11)	
Variable	Mean difference ±95% LoA	Pearson's *r* (95% CI)	Mean difference ±95% LoA	Pearson's *r* (95% CI)
FFM (kg)	-2.9 ± 1.7*	0.997 (0.980, 1.000)	-2.5 ± 1.8*	0.985 (0.941, 0.996)
FM (kg)	4.4 ± 1.7*	0.845 (0.254, 0.977)	3.7 ± 1.7*	0.975 (0.903, 0.994)
FM (%)	8.1 ± 2.3*	0.972 (0.817, 0.996)	7.0 ± 3.6*	0.900 (0.653, 0.974)

Mean difference represents DXA-InBody 720.

**p<0.001 for mean difference between InBody 720 and DXA*

Appendix I

Approval from the Regional Committee for Medical and Health Research Ethics

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

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Dato: 24.03.09
Deres ref.:
Vår ref.: S-08702d, 2008/18361

Vedr. svar på merknader for studien "Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere"
Søknad om opprettelse av forskningsbiobank

Vi viser til svar på merknader av 26.02.09 med følgende vedlegg: Revidert informasjonsskriv av 13.03.09.

Komiteen behandlet svar på merknader 18.03.09. Prosjektet er vurdert etter lov om behandling av etikk og redelighet i forskning av 30. juni 2006, jfr. Kunnskapsdepartementets forskrift av 8. juni 2007 og retningslinjer av 27. juni 2007 for de regionale komiteer for medisinsk og helsefaglig forskningsetikk.

Komiteen finner svarene tilfredsstillende

Komiteen har følgende merknader til søknad om opprettelse av forskningsbiobank:
Komiteen har ingen innvendinger mot opprettelse av forskningsbiobank og videresender søknaden om opprettelse av denne sammen med kopi av dette vedtaket til Helsedirektoratet for endelig godkjenning.

Vedtak:
Prosjektet godkjennes slik det nå foreligger.

Med vennlig hilsen

Stein A. Evensen (sign.)
Professor dr.med.
leder

Ingrid Middelthon
komitésekretær

Kopi:
- Helsedirektoratet

Helsedirektoratet

Professor Jostein Hallén
Norges Idrettshøyskole, Pb 4014
0806 Oslo

Deres ref.:
Saksbehandler: VDA
Vår ref.: 09/2098
Dato: 29.04.2009

Melding om opprettelse av forskningsbiobank - Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere

Vi viser til brev fra REK Sør-Øst D av 25. mars 2009 med melding 2558 av 16. september 2008 om forskningsbiobank om ovenstående og til kopi av brev fra REK Sør-Øst D av 24. mars 2009 der det framgår at prosjektet godkjennes og opprettelse av forskningsbiobanken tilrås.

Helsedirektoratet er delegert å vurdere meldinger om opprettelse av forskningsbiobanker i hht. biobankloven § 4.

Direktoratet har ingen innsigelser til at forskningsbiobanken opprettes i henhold til biobankloven.

Direktoratet forutsetter at opprettelsen av den planlagte forskningsbiobanken oppfyller nødvendige krav til godkjenning, konsesjon m.v. i henhold til annet relevant regelverk, herunder bioteknologiloven, helseregisterloven og legemiddelloven.

Direktoratet har registrert at meldingen om forskningsbiobanken er sendt til Nasjonalt folkehelseinstitutt som har fått ansvaret for å føre et offentlig tilgjengelig register over landets biobanker, jf. biobankloven § 6.

Vennlig hilsen

Ragnhild Castberg e.f.
avdelingsdirektør

Dokumentet er godkjent elektronisk

for Vibeke Dalen
seniorrådgiver

Kopi: REK Sør-Øst D (S-08702d, 2008/18361)
Biobankregisteret Melding 2558

Helsedirektoratet • Divisjon spesialisthelsetjenester
Avd. bioteknologi og generelle helselover
Vibeke Dalen, tlf.: 24 16 31 95
Postboks 7000 St. Olavs plass, 0130 Oslo • Besøksadresse: Universitetsgata 2, Oslo • Tlf.: 810 20 050
Faks: 24 16 30 01 • Org. nr.: 983 544 622 • postmottak@helsedir.no • www.helsedirektoratet.no

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES

Harald Hårfagres gate 29
N-5007 Bergen
Norway
Tel: +47-55 58 21 17
Fax: +47-55 58 96 50
nsd@nsd.uib.no
www.nsd.uib.no
Org.nr: 985 321 884

Jostein Hallén
Seksjon for fysisk prestasjonsevne
Norges idrettshøgskole
Postboks 4014
0806 OSLO

Vår dato: 31.03.2009	Vår ref: 20165 / 2 / IB	Deres dato:	Deres ref:

TILRÅDING AV BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 07.10.2008. All nødvendig informasjon om prosjektet forelå i sin helhet 02.03.2009. Meldingen gjelder prosjektet:

20165	*Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere*
Behandlingsansvarlig	*Norges idrettshøgskole, ved institusjonens øverste leder*
Daglig ansvarlig	*Jostein Hallén*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, eventuelle kommentarer samt personopplysningsloven/-helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/forsk_stud/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://www.nsd.uib.no/personvern/prosjektoversikt.jsp.

Personvernombudet vil ved prosjektets avslutning, 31.12.2014, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Bjørn Henrichsen

Inga Brautaset

Kontaktperson: Inga Brautaset tlf: 55 58 26 35
Vedlegg: Prosjektvurdering

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst D	Ingrid Middelthon	22845515	01.04.2011	2011/659/REK sør-øst D
			Deres dato:	Deres referanse:
			09.03.2011	

Vår referanse må oppgis ved alle henvendelser

Professor Jostein Hallén
Pb. 4014 Ullevål stadion
NIH
0896 Oslo

2011/659 D Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere

Vi viser til prosjektendringsskjema for ovennevnte studie mottatt 09.03.2011.

Forskningsansvarlig: Norges idrettshøgskole ved øverste administrative ledelse.

Prosjektleder: Professor Jostein Hallén

Endringene innebærer:
Det søkes om å øke antall prosjektdeltakere og å få rekruttere via skoler.

REKs Forskningsetisk vurdering
REK har vurdert endringssøknaden og har ingen forskningsetiske innvendinger mot endringen av prosjektet.

Vedtak
REK godkjenner prosjektet slik det nå foreligger, jfr. helseforskningsloven § 11.

Tillatelsen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i endringssøknaden, protokollen, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK. Vi gjør oppmerksom på at dersom endringene er vesentlige må prosjektleder sende ny søknad, eller REK kan pålegge at så gjøres.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren», http://www.norsk-helsenett.no/informasjonssikkerhet/bransjenormen/Personvern%20og%20informasjonssikkerhet%20i%20forskningsprosjekter%20v1.pdf

Prosjektet skal sende sluttmelding til REK Sør-Øst D, se helseforskningsloven § 12

For øvrig gjelder de vilkår som er satt i forbindelse med tidligere godkjenning av prosjektet.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no eller på e-post til: post@helseforskning.etikkom.no.
Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen,

Stein A. Evensen (sign.)
professor dr. med.
leder REK Sør-Øst D

Ingrid Middelthon
seniorrådgiver

Besøksadresse: Postboks 1130 Blindern 0318 Oslo
Telefon: 22850548
E-post: rek-sorost@medisin.uio.no
Web: http://helseforskning.etikkom.no/

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Silje U. Lauvrak	22845520	22.04.2013	2011/659/REK sør-øst D
			Deres dato:	**Deres referanse:**
			20.03.2013	

Vår referanse må oppgis ved alle henvendelser

Til Jostein Hallen

2011/659 S-08702d Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere

Forskningsansvarlig: Norges Idrettshøyskole
Prosjektleder: Jostein Hallen

Vi viser til søknad om prosjektendring datert 20.03.2013 for ovennevnte forskningsprosjekt. Søknaden er behandlet av leder for REK sør-øst på fullmakt, med hjemmel i helseforskningsloven § 11.

Endringene innebærer:
- Ny prosjektmedarbeider er knyttet til prosjektet.
- Endring av prosjektstart til 20.04.13 og prosjektslutt til 31.12.17
- Økning av antall forskningsdeltakere med 100 nye
- Inklusjon av nye tester:
  1) Måling av styrke, spenst og hurtighet.
  2) Hjertedimensjoner måles med ultralyd.
  3) Muskeltykkelse i en benmuskel og en armmuskel blir målt ved ultralyd.
  4) Kroppssammensetning måles ved impedansmåling.
- Informasjonsskriv og protokoll er revidert i henhold til endringene.

Vurdering
REK har vurdert endringssøknaden og har ingen forskningsetiske innvendinger mot endringen av prosjektet.

Vedtak
REK godkjenner prosjektet slik det nå foreligger, jfr. helseforskningsloven § 11, annet ledd.

Tillatelsen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden, endringssøknad, oppdatert protokoll og de bestemmelser som følger av helseforskningsloven med forskrifter.

REKs vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jfr. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst. Klagefristen er tre uker fra mottak av dette brevet, jfr. forvaltningsloven § 29.

Vi ber om at alle henvendelser sendes inn med korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Besøksadresse:
Gullhaugveien 1-3, 0484 Oslo

Telefon: 22845511
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

Med vennlig hilsen

Stein A. Evensen
Professor dr. med.
Leder

Silje U. Lauvrak
Rådgiver

Kopi til: *postmottak@nih.no*

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Hege Cathrine Finholt, PhD	22857547	02.10.2017	2011/659/REK sør-øst D
			Deres dato:	Deres referanse:
			08.09.2017	

Vår referanse må oppgis ved alle henvendelser

Jostein Hallen
Norges Idrettshøyskole

2011/659 Hemoglobinmasse og blodvolum hos unge utholdenhetsutøvere

Forskningsansvarlig: Norges Idrettshøyskole
Prosjektleder: Jostein Hallen

Vi viser til søknad om prosjektendring datert 08.09.2017 for ovennevnte forskningsprosjekt. Søknaden er behandlet av leder for REK sør-øst D på fullmakt, med hjemmel i helseforskningsloven § 11.

Endringene innebærer:

- Ny Prosjektmedarbeider Mari Lihagen, Vitenskapelig assistent
- Ny prosjektslutt: 31.12.2023
- Økning i antall forskningsdeltakere
- Innhenting av nye data fra samme utvalgsgruppe
- To nye tester

Vurdering
REK har vurdert de omsøkte endringene, og har ingen forskningsetiske innvendinger til endringene slik de er beskrevet i skjema for prosjektendring.

Vedtak
REK godkjenner prosjektet slik det nå foreligger, jfr. helseforskningsloven § 11, annet ledd.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknad, endringssøknad, oppdatert protokoll og de bestemmelser som følger av helseforskningsloven med forskrifter.

Klageadgang
REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Eventuell klage sendes til REK sør-øst D. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst D, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn på korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Hege Cathrine Finholt, PhD
Rådgiver

Kopi til: *postmottak@nih.no*

Appendix II

Questionnaire regarding volume of training and type of sport

Kartlegging av idrettsaktivitet/fysisk aktivitet ***Navn:***

DEL A

1. Driver du med noen form for fysisk aktivitet/idrett/mosjon (se bort fra gym i skolen)? (sett kryss)

Ja	
Nei	

2. Hvis du svarte **nei** på spørsmål 1, gå videre til *DEL B*

   Hvis du svarte **ja**, kan du oppgi hva slags form for fysisk aktivitet du driver med? (sett kryss for de alternativene som passer).

Organisert trening i idrettslag	
Treningssenter (Sats eller lignende)	
Trener/mosjonerer for meg selv	
Sykle/gå til skolen	
Annet (spesifiser)	

3. Hvilke idretter/mosjonsaktiviteter driver du med, hvor mange økter i uka har du i hver aktivitet, og varierer det med årstid?

Type idrett/mosjonsaktivitet	**Vinter**	**Vår**	**Sommer**	**Høst**

Eksempel på hvordan det kan fylles ut

Type idrett/aktivitet/mosjon	**Vinter**	**Vår**	**Sommer**	**Høst**
Langrenn	5	4	3	5
Orientering		2	2	2
Sykler til skolen			5	5

4. Anser du en av dem som din hovedidrett/-aktivitet?

Ja	
Nei	

Hvis **ja**, kan du oppgi hvilken idrett/aktivitet?_____

5. Hvor lenge (år) har du vært aktiv i de forskjellige idrettene/mosjonsaktivitetene? Ta med både nåværende og tidligere aktiviteter som du driver/har drevet med (sett kryss for det alternativet som passer best for hver idrett/aktivitet)

Type idrett/mosjonsaktivitet	**> 5 år**	**3-4 år**	**1-2 år**	**< 1 år**

6. Hvor mange ganger trener/mosjonerer du vanligvis per uke (**inkludert** konkurranser/kamper)?

1 gang per uke	2-3 ganger per uke	4-6 ganger per uke	>6 ganger per uke

7. Omtrent hvor mange timer trener/mosjonerer du vanligvis per uke (**inkludert** konkurranser/kamper)?

Type trening	**Timer**
Utholdenhetstrening	
Styrketrening	
Ballidrett	
Annet	
Totalt antall timer per uke	

8. I en **gjennomsnittlig uke**, hvor mange av de oppførte timene består av teknikk-/taktikktrening, langkjøring og intervalltrening?

Type trening	**Teknikk/taktikk**	**Langkjøring**	**Intervaller**
Timer			

DEL B

Eventuelle endringer i fysisk aktivitet siden sist du var inne til testing på NIH

1. Sett kryss i de rutene som er aktuelle

Jeg deltok i prosjektet i 2015	
Jeg deltok i prosjektet i 2016	

2. Har du **sluttet** med noen idretter siden sist du var her og testet?

Ja	
Nei	

Eventuelt hvilke?_____

Når sluttet du?_____

3. Har du **begynt** med noen nye idretter siden sist du var her og testet?

Ja	
Nei	

Eventuelt hvilke? _____

Når begynte du?_____

4. Har det skjedd noen endringer i hvor mye du trener, eller måten du trener på nå i forhold til det du gjorde ved forrige test? Sett kryss for det som passer.

Har sluttet å trene	
Trener nokså likt som ved forrige test	
Trener færre timer per uke nå	
Trener flere timer per uke nå	
Trener mindre systematisk nå	
Trener mer systematisk nå	

Eventuelle kommentarer: _____

_____

_____

5. Har du hatt skade- eller sykdomsavbrudd i treningen som har vart lengre enn 1 måned i løpet av det siste året?

Ja	
Nei	

Hvis **ja**, kan du oppgi **hva** og **når**?

_____

_____

_____

_____

Takk for at du tok deg tid til å fylle ut skjemaet!