ASSOCIATIONS OF MAXIMUM OXYGEN UPTAKE AND BODY COMPOSITION WITH ARTERIAL STIFFNESS AND BLOOD LIPIDS

A cross-sectional study among physically active females in two different training periods

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ABSTRACT

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In general, physical activity and especially endurance sports are known to reduce the risk of cardiovascular diseases and all-cause mortality. It is also established that increased arterial stiffness and un-optimal levels of blood lipids increase the risk of cardiovascular diseases. Previous studies have shown that endurance training decreases arterial stiffness, particularly in people who have greater arterial stiffness at baseline. In addition, endurance training can lead to increased HDL-cholesterol and decreased LDL-cholesterol and triglyceride levels. The main purpose of this thesis was to determine if the arterial stiffness, blood lipid levels, maximum oxygen uptake or body composition of adult elite Finnish female endurance athletes vary between the training periods of preparation period and competition period. The aim was also to determine if better physical performance and body composition have any associations to arterial stiffness or blood lipid levels. Developmental females and female rugby players were used as controls. Data of this study was based on an on-going three-year follow-up study NoREDS conducted at the University of Jyväskylä.

The results of this study showed that there were no significant differences between two timepoints but there were significant differences between endurance athletes and other groups in maximum oxygen uptake, body composition and blood lipids. Group differences in arterial stiffness and associations with maximum oxygen uptake or body composition with arterial stiffness were not found. Maximum oxygen uptake and body composition had associations with blood lipids. Maximum oxygen uptake had a positive relation to TC ($r = 0.33$, $p = 0.035$) and to HDL ($r = 0.326$, $p = 0.037$) in the preparation period. There was a negative relation in total body weight to non-HDL in preparation period ($r = -0.35$, $p = 0.025$) and in competition period ($r = -0.34$, $p = 0.028$); BMI to TC ($r = -0.32$, $p = 0.040$) and HDL ($r = -0.33$, $p = 0.034$) in preparation period and to TC ($r = -0.33$, $p = 0.038$) in competition period; TLM to non-HDL in preparation period ($r = -0.38$, $p= 0.014$) and in competition period ($r = -0.52$, $p = <0.001$), to LDL in preparation period ($r = -0.40$, $p = 0.009$) and to TC ($r = -0.46$, $p = 0.003$), TG ($r = -0.39$, $p = 0.013$) and LDL ($r = -0.52$, $p = <0.001$) in competition period.

The findings in this study suggests that body composition might have a bigger role than physical performance in maintaining favourable blood lipid profile in preventing cardiovascular health but the periodization of training in endurance sports does not seem to effect these levels significantly. Association of body composition might be stronger with blood lipids than physical performance’s, but the impact of nutritional and dietical changes towards competition period must be noted. For general population it could be more important to have enough muscle mass despite the absolute amount of fat mass. Because of the higher risk of amenorrhea and RED-S among endurance athletes leading to hormonal and endothelial changes, future studies should be more comprehensive and include the assessment of athletes’ nutritional and hormonal status, psychological stress and the amount of training to be able to evaluate their potential risks for athletes’ cardiovascular health.

Key words: arterial stiffness, body composition, endurance sports, pulse wave velocity, blood lipids, maximum oxygen uptake, athlete’s health

TIIVISTELMÄ

Haaja, E. 2024. Maksimaalisen hapenottokyvyn ja kehonkoostumuksen yhteydet valtimojäykkyyteen ja veren rasva-arvoihin: Fyysisesti aktiivisia naisia vertaileva poikkileikkaustutkimus. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, Liikuntalääketieteen pro gradu tutkielma 76 s., 3 liitettä.

Fyysisellä aktiivisuudella ja kestävyysurheilulla tiedetään olevan sydän- ja verenkiertoelimistön sairauksia ennaltaehkäisevä ja kuolleisuutta vähentävä vaikutus. Valtimojäykkyys ja veren rasva-arvot kuvaavat sydän- ja verenkiertoelimistön terveyttä. Aiempien tutkimusten perusteella kestävyysharjoittelu laskee valtimojäykkyyttä etenkin henkilöillä, joilla valtimojäykkyys on jo hieman koholla. Veren rasva-arvoissa kestävyysharjoittelu voi näkyä HDL-kolesterolin nousuna ja LDL-kolesterolin ja triglyseridien laskuna. Tämän tutkielman tavoitteena oli selvittää, onko suomalaisten aikuisten naiskestävyysurheilijoiden valtimojäykkyydessä, veren rasva-arvoissa, maksimaalisessa hapenottokyvyssä ja kehonkoostumuksessa vaihtelua peruskestävyyskauden ja kilpailukauden välillä. Tavoitteena oli myös selvittää, onko parempi fyysinen suorituskyky tai kehonkoostumus yhteydessä alhaisempaan valtimojäykkyyteen tai parempiin veren rasva-arvoihin. Tavoitteellisia kuntoilijoita ja rugbyn pelaajia käytettiin vertailuryhminä. Data saatiin Jyväskylän yliopiston käynnissä olevasta kolmivuotisesta NoREDS -pitkittäistutkimuksesta.

Tuloksista ilmeni, että missään muuttujissa ei ollut harjoittelukausien välillä tilastollisesti merkitseviä eroja, mutta kestävyysurheilijoiden ja muiden ryhmien välillä oli tilastollisesti merkitseviä eroja hapenottokyvyssä, kehonkoostumuksessa ja veren rasva-arvoissa. Valtimojäykkyydessä ei ollut eroa ryhmien välillä eikä valtimojäykkyyden ja hapenottokyvyn tai kehonkoostumuksen välillä ollut yhteyksiä. Hapenottokyvyllä oli positiivinen yhteys kokonaiskolesteroliin (r = 0,33, p = 0,035) ja HDL-kolesteroliin (r = 0,326, p = 0,037) peruskestävyyskaudella. Negatiivinen yhteys oli kehon painolla non-HDL-kolesteroliin peruskestävyyskaudella (r = -0,35, p = 0,025) ja kilpailukaudella (r = -0,34, p = 0,028); BMI:llä kokonaiskolesteroliin (r = -0,32, p = 0,040) ja HDL-kolesteroliin peruskestävyyskaudella (r = -0,33, p = 0,034) ja kokonaiskolesteroliin kilpailukaudella (r = -0,33, p = 0,038); TLM non-HDL-kolesteroliin peruskestävyyskaudella (r = -0,38, p= 0,014) ja kilpailukaudella (r = -0,52, p = <0,001), LDL-kolesteroliin peruskestävyyskaudella (r = -0,40, p = 0,009) ja kokonaiskolesteroliin (r = -0,46 , p = 0,003), triglysereihin (r = -0,39, p = 0,013, d) ja LDL-kolesteroliin (r = -0,52, p = <0,001) kilpailukaudella.

Tulokset osoittivat, että kehonkoostumuksen rooli ihanteellisten veren rasva-arvojen ylläpidossa sydän- ja verisuoniterveyden edistämiseksi voi olla suurempi kuin hapenottokyvyn. Harjoittelukausien jaksotuksella ei ollut merkitsevää vaikutusta, mutta mahdolliset muutokset ruokavaliossa kilpailukaudelle siirryttäessä tulisi ottaa huomioon. Kestävyysurheilijoilla on suurempi riski endoteeli- ja veren rasva-arvojen muutoksiin amenorrhean ja suhteellisen energiavajauksen aiheuttamien hormonaalisten muutosten vuoksi, joten tulevaisuudessa tulisi kestävyysurheilijoihin kohdentaa enemmän tutkimuksia, jotka huomioivat kokonaisvaltaisemmin harjoittelumääriä, ravitsemustilannetta, psyykkistä kuormitusta ja hormonitoiminnan statusta, sekä näiden tekijöiden negatiivisia vaikutuksia sydän- ja verisuoniterveyteen.

Asiasanat: valtimojäykkyys, kestävyysurheilu, pulssiaallon nopeus, maksimaalinen hapenottokyky, kehonkoostumus, veren lipidit, urheilijan terveys

ABBREVIATIONS

AS	arterial stiffness
ATP	adenosine triphosphate
BIA	bioelectrical impedance analyses
BP	blood pressure
BMI	body mass index
CVD	cardiovascular disease
CRF	cardiorespiratory fitness
DXA	dual energy x-ray absorptiometry
ECG	electrocardiography
FFM	fat free mass
FFMI	fat free mass index
HDL	high density lipoprotein
LDL	low density lipoprotein
PWV	pulse wave velocity
RED-S	relative energy deficiency in sports
RPE	rating of perceived exertion
SBP	systolic blood pressure
TC	total cholesterol
TD2	type 2 diabetes
TG	triglycerides
TTE	time to exhaustion
Vo_{2max}	maximal oxygen uptake
WHO	World Health Organization

CONTENTS

ABSTRACT

1 INTRODUCTION

Physical activity and especially endurance type physical activity are known to reduce risk for cardiovascular diseases (CVD) and all-cause mortality (Ashor et al. 2014, Cornelissen & Smart 2013, Paluch et al. 2024 & Valenzuela et al. 2023). CVDs, especially ischemic heart disease and stroke are the main causes of death globally (Smith 2019, 123 & WHO 2020). The traditional risk factors associated with CVDs, like lipid profile, increased insulin sensitivity and blood pressure (BP), are reduced by endurance training and increased aerobic capacity (Cornelissen & Smart 2013, Mann et al. 2014, Paluch et al. 2024 & Valenzuela et al. 2023). However, previous studies have indicated that in healthy individuals, it is not entirely clear how large amounts of endurance training is effective.

Arterial stiffness (AS) describes the rigidity of arterial walls and is an independent indicator of cardiovascular health (Vlachopoulos et al. 2010). Age and atherosclerosis are known to increase AS but previous studies have shown that endurance training decreases it, particularly in people who have greater AS at baseline or high BP (Ashor et al. 2014, Lopes et al. 2021 & Vlachopoulos et al. 2010). Low physical activity and poor cardiorespiratory fitness (CRF) have been identified as determinants of early arterial stiffening (Boreham et al. 2004 & Fernberg et al. 2017). Enhancements in blood lipid levels through physical activity and endurance training can be seen mainly as increasement in high density lipoprotein (HDL) and decreasements in low density lipoprotein (LDL) and triglyseride (TG) levels (Mann et al. 2014).

Still, the associations of CRF and physical activity with AS and blood lipids are not well known and only few have investigated whether CRF and body composition are associated with them in healthy adults, adolescents or endurance athletes. Athletes in general are apparently healthy, physically highly active, and engage less often to unhealthy behaviors, such as smoking and alcohol abuse, than general population. Even though they have less CVD risk factors, they are not immune to them. Sport related demands like mental stress from competing and high training load can predispose athletes also to other health risks. From traditional risk factors high BP is the most common risk factor in athletes and when it becomes chronic it could lead to the stiffening of arteries and other pathological cardiovascular conditions. (Berge et al. 2015, Caselli et al. 2017 & Pelliccia et al. 2017)

Elite endurance athletes train perhaps the most of all athletes. International level long distance runners are known to train 11-14 times per week on average with the total training hours rising to up to 20-30 hours per week (Haugen et al. 2022). Within their annual training periodization, they usually try to achieve their best physical performance and adequate body composition before the competing season (Heydenreich et al. 2017).

With large training amounts combined in favoring of light and lean body figure nutritional and health issues become challenging. This can lead to relative energy deficiency in sports (RED-S) syndrome both in women and in men. Especially in women, changes in endothelial function and blood lipids are possible because of the effects of low estrogen levels in RED-S. (Mountjoy et al. 2018, Rickenlund et al. 2005 & Salerni et al. 2015) While healthy young normal weight individuals with higher CRF may have lower AS, being lean or even underweight may have negative effects (Fernberg et al. 2017 & Kyte et al. 2022). Hence, there is a need for an increased understanding of vascular health in endurance trained athletes and if these seasonal changes might be harmful for athletes' overall health.

2 CARDIOVASCULAR HEALTH

Traditional risk factors for CVDs are high BP, AS, high low-density lipoprotein (LDL) cholesterol, diabetes, smoking, secondhand smoke exposure, obesity, unhealthy diet, and physical inactivity (Duodecim 2020b, Grundy et al. 2018). This section describes the main concepts of AS, blood lipids and body composition and defines their recommended values for preventing CVDs.

2.1 Arterial stiffness

AS describes the rigidity of arterial walls and is an individual indicator of cardiovascular health (Ashor et al. 2014, Avolio 2013, Safar et al. 2018 & Vlachopoulos et al. 2010). Recently in health care the assessment of it in clinical use has increased (Laurent et al. 2006 & Safar et al. 2018) because increased AS can individually increase the risk for atheroscleroris and eventually CVDs (Ashor et al 2014). Large arteries can accommodate almost half of the heart's stroke volume by distending their walls. This ability is the result of a high elastin content in the arterial walls. (Covic & Siriopol 2015) Proximal arteries are more elastic than the peripheral ones because of a dominant collagen content in the peripheral arteries making AS increase from heart towards periphery (Covic & Siriopol 2015 & Safar et al. 2018; Figure 1).

Pulse wave velocity (PWV) has been recognized as a valid non-invasive measure in assessing AS (Covid & Siriopol 2015, Laurent et al. 2006, Salvi et al. 2019 & Townsend et al. 2015). The pulse wave is transmitted through the arterial system, and its speed is inversely related to the distensibility of the arterial wall itself: the higher the velocity, the lower the arterial distensibility. PWV is expressed in m/s meaning the distance between measurement locations (m) divided by transit time (s). When arteries become stiffer, the rise of pulse wave's speed is shown as an increase in PWV. (Avolio 2013, Laurent et al. 2006 & Salvi et al. 2019) Usually PWV increases from 4–5 m/s in the ascending aorta to 5–6 m/s in the abdominal aorta and further 8–9 m/s in the iliac and femoral arteries. (Covic & Siriopol 2015, Laurent et al. 2006 & Safar et al. 2018; Figure 1)

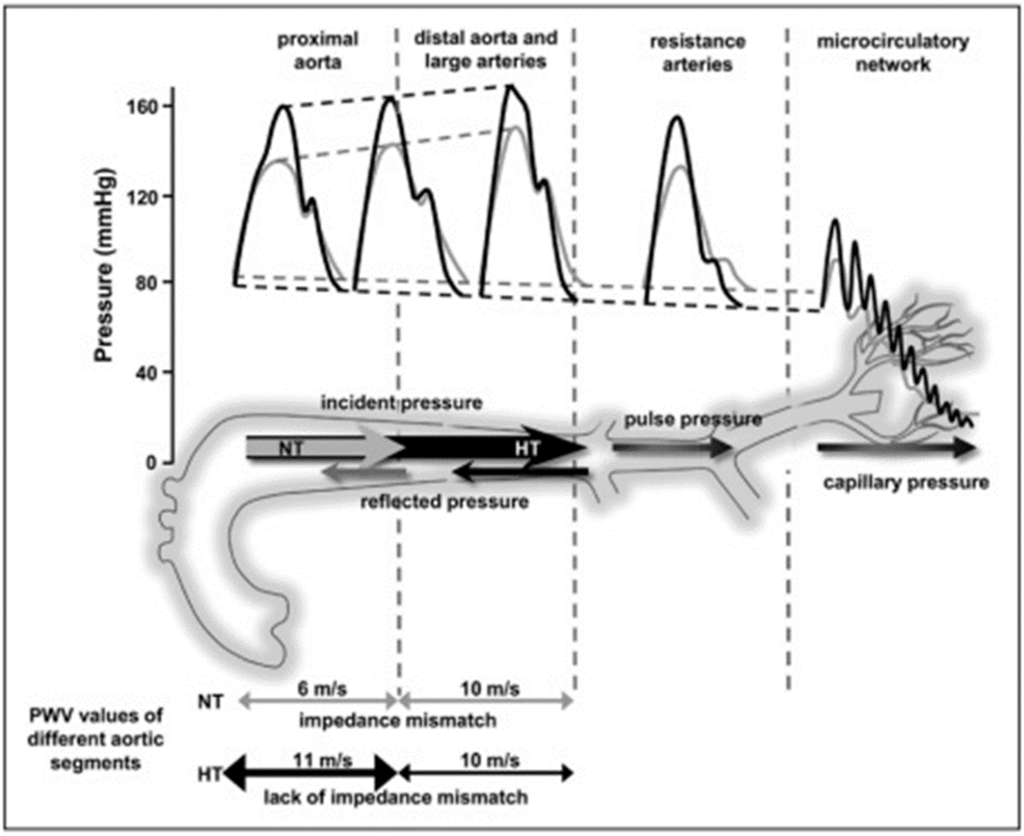

FIGURE 1. Pressure wave reflection from different aortic segmentations showing the increasement in PWV towards peripheral arteries (Safar et al. 2018).

Age is known to increase AS and first the elastin and collagen contents in arterial walls change leading to fragments in elastin making the arteries stiffer. In general, arterial stiffening leads to hypertrophic cardiomyopathy and eventually to heart failure. Atherosclerosis is congestion of the arteries and leads to decreased oxygen uptake and strokes. (Ashor et al. 2014, Lopes et al. 2021, Safar et al. 2018 & Vlachopoulos et al. 2010) Normal mean value of PWV for under 30 years old healthy population with no CVD risk factors is 6.2 m/s ranging between 4.7–7.6 m/s. For clinical practice to indicate the risk for CVD there is a threshold of 10 m/s for PWV. (Boutouyrie et al. 2010)

AS can be measured directly and non-invasively at various sites along the arterial tree. At the moment carotid-femoral PWV (cf-PWV) is world widely mostly recommended and has the most evidence linking it to cardiovascular outcomes. It also requires little technical expertise which provides benefits to measurement reliability. (Covid & Siriopol 2015, Laurent et al. 2006, Salvi et al. 2019, Safar et al. 2018 & Townsend et al. 2015) PWV measured from other vascular segments like ankle-brachial, cardiac-ankle, carotid-radial or single point estimates are not as suitable because they might lack the evidence of cardiovascular outcome prediction (Townsend et al. 2015).

A number of devices based on a probe or a tonometry technology have been used in published research. Tonometry-based techniques (e.g, the SphygmoCor device, AtCor Medical, West Ryde, NSW, Australia) use tonometer that is placed at any 2 sites where a pulse is detectable. Only 1 tonometer is attached to the unit, so PWV measurements require 2 sequential 10-20 second readings, gated to the electrocardiography (ECG). Some devices use cuffs placed around the limbs or the neck and they record the arrival of pulse-wave oscillometrically like e.g Arteriograph device (Arteriograph; TensioMed Ltd., Budapest, Hungary, Figure 2).

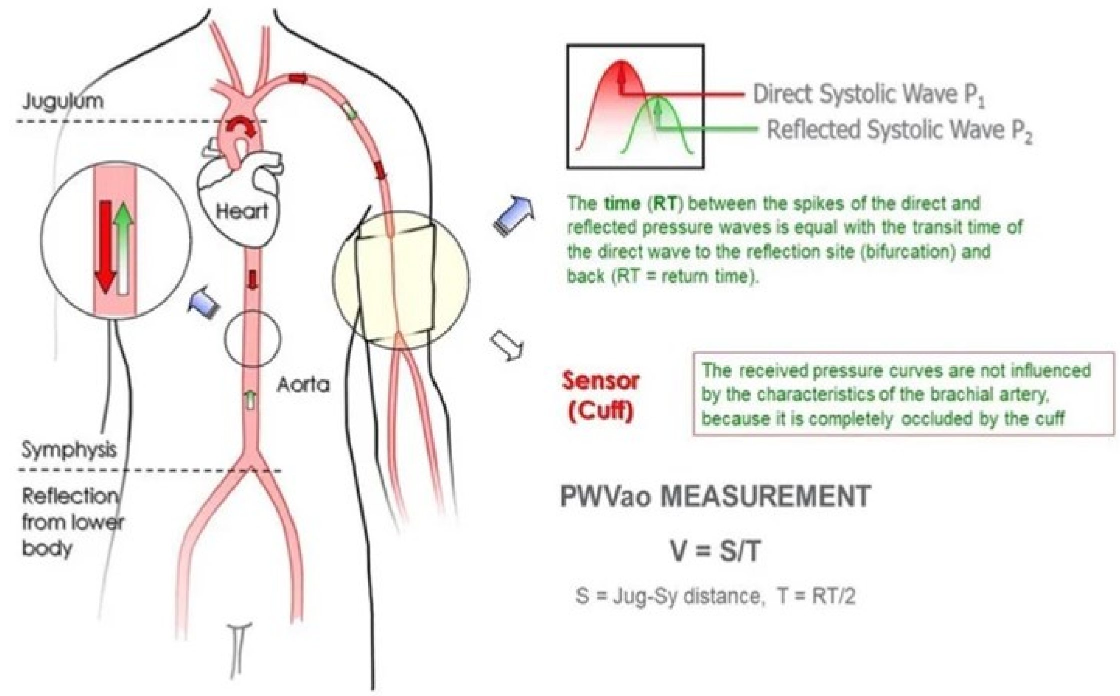

FIGURE 2. PWVao measurement by Arteriograph device (Tensiomed 2023).

Some cuff-based measurements (e.g, Mobil-O-Graph, IEM, Stolberg, Germany) capture brachial BP and brachial waveforms (casual and at 24 hours) to estimate central aortic pressures and to estimate cfPWV. Most cuff-based measurements like Mobile-O-Graph and Arteriograph devices define PWV from a single point and the measurement is based on algorithms and estimates for which they are often criticized. (Townsend et al. 2015)

2.2 Blood lipids

In general, maintaining ideal blood lipid profile and preventing dyslipidemias is important in preventing atherosclerosis and CVDs (Grundy et al. 2018 & McArdle et al. 2016, 867). The blood lipid profile is commonly defined by assessing individual's serum's total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyserides (TG) from venous blood. In recent years also the assessment of non-HDL cholesterol has increased and seen as an early sign among healthy young adults to an increased risk to have CVDs in the next 25 years (Duodecim 2022 & Pencina et al. 2019; Table 1).

TABLE 1. Recommendations for adults' serum lipids in preventing cardiovascular outcomes (Adapted and modified from Duodecim 2022 & Pencina et al. 2019).

TC	Non-HDL	LDL	HDL	TG
< 5 mmol/l	< 3.8 mmol/l	< 3 mmol/l	> 1.0 mmol/l for men > 1.2 mmol/l for women	< 1.7 mmol/l

For normal population it is recommended to define blood lipid levels in every five years internationally (Duodecim 2022 & McArdle et al. 2016, 867), and measurements of blood lipid levels should be made already in early adulthood, but at least before 40 years of age, for future CVD risk assessment (Pencina et al. 2019). The role of TC is smaller than distributed lipoproteins LDL and HDL. Especially increased HDL levels have been shown to have a causal relation to a lower risk for CVDs. (McArdle et al- 2016, 868) In population treated with statins there is evidence that non-HDL and LDL are associated to cardiovascular events, and the association of non-HDL is stronger than the association of LDL (Boekholdt et al. 2012).

2.3 Body composition

For normal population in preventing health care, defining body composition is widely used as a tool to assess the risk factors of being overweight and gaining diseases like metabolic syndrome or CVDs, but no significant reference values have been shown to strictly cause those risks (Duodecim 2023 & McArdle et al. 2016, 731-732). Athletes in general can be hypothesized to have a more favorable body composition than normal population, dependent of the sports type. Body mass index (BMI) is a traditional weight and height-based assessment of body mass calculated by dividing the body mass in kilograms with the square meter of height in meters. Internationally BMI over 25 kg/m2 means overweight and under 18.5 kg/m2 underweight. BMI is widely used in clinical health care but also criticized not be accurate enough if individual has sufficient or eminent swelling or muscle mass. A larger amount of muscle mass counts subgroups like wrestlers, bodybuilders and weightlifters. Measurement of waist circumference has become more evident in defining visceral fat and health risks in normal population when BMI is over 30 kg/m2. Especially for athletes the assessments of body composition by body's fat mass percentage (BF%) and lean muscle mass percentages are preferred. (Duodecim 2023 & McArdle et al 2016, 733-735)

Some sports are seen as weight-based sports and defining body composition is justified at competing level, even though no generally accepted optimum values for body weight or percentage of fat mass in different sports exist (Sundgot-Borgen et al 2013). Endurance sports are seen as sports that favor lighter and leaner body figure because athletes must be able to move their own body weight for prolonged times. Lighter and leaner body uses less energy and improves the economy of performance. (Heydenreich et al. 2017 & Vuorimaa 2016) For male long-distance runners the lowest fat percent can range from 1 to 8 % whilst for female the lower limit is approximately 12% (McArdle et al. 2016, 741). For normal population recommendations for normal fat % are not as standardized as BMI values are.

Body composition can be measured by low-cost and easily performed skinfold techniques and different bioelectrical impedance analyses (BIA) that can be conducted almost everywhere. Laboratory measurements like magnetic resonance imaging (MRI), hydrodensitometry, air displacement plethysmography (BodPod) or dual energy X-ray absorptiometry (DXA) are seen more reliable techniques, but they require also more time, investments and experience (Duodecim 2023 & Wagner et al. 1999). Skinfold techniques are usually not recommended to athletes hence they underestimate the amount of fat tissue on individuals with higher muscle mass and do not provide information about the amount of muscle mass. This measurement is based on the fact that body fat distributes equally throughout the body while fat is stored mainly in the subcutaneous fat tissue. BIA techniques utilize the transit time of a low-voltage electric current through the body and the estimation of body composition is based on body fluids. This measurement has errors for athletes too, hence it doesn’t take acute food intake, fluid ingestion, individual’s positioning, previous physical activity or hydration status into account. Measurements accuracy and reliability can be affected by previous strenuous physical activity or exercise reducing muscles glycogen reserves and dehydration from sweat-loss. As a consequence fat free mass (FFM) can be overestimated or body percentage underestimated and the phase of menstrual cycle affects the results on measurements made to females. (Marra et al. 2019, McArdle et al. 2016, 754-755 & Wagner et al. 1999)

Laboratory measurements like hydrodensitometry and air displacement plethysmography are based on the body weight displacing air or water through measurement of body’s density. Both of these methods are prone to bias due to temperature, pressure and humidity changes. From body composition measurements that require laboratory environments DXA has become the most reliable tool in assessing not only fat and muscle mass of the whole body but also regional and in addition mineral bone content and density. During the test participant is lying in supine position while DXA detector probes pass over the body for a 12-minute period. Measurement is based on how low-emission X-ray beams are attenuating through different body tissues. Bone tissue is attenuated more than fat tissue. Despite the radiation, DXA has been used widely, because the effective radiation doses involved are quite small (1–7 μSv). But for health and participant’s security reasons DXA measurements are recommended to be made maximum of two times annually. MRI measurements have the highest costs, and the measurements take more time. (McArdle et al. 2016, 761, Marra et al. 2019, Nana et al. 2014 & Wagner et al. 1999)

3 ENDURANCE TRAINING AND CARDIOVASCULAR HEALTH

Regular and continuous exercise training, long exercise training sessions and competitions and good CRF are characteristics of endurance sports. Running, cycling, swimming, cross-country skiing and biathlon are the most typical endurance sports. When it comes to running, distances from 800 metes to marathon are considered endurance running. Succeeding in competitions needs different physical characteristics from athletes hence the competition can last from two minutes to several hours. (Vuorimaa 2016) Endurance athletes' training is periodized and their performance and body composition is hypothesized to fluctuate depending on the on-going training season. Main goal and purpose of this periodization is to make sure athlete's performance is at its highest in the competing season. (Bompa & Puzzichelli 2018, 165 & Heydenreich et al. 2017) Among all athletes, endurance athletes' training amounts are probably the highest. This section outlines the main physiological adaptations followed through endurance training, the periodization of endurance training, the assessment of physical performance by maximum oxygen uptake and main health benefits and risks of endurance training.

3.1 Main physiological adaptations through endurance training

Endurance training has multiple acute and chronic effects on human body's cardiovascular system, energy metabolism and muscles that are behind improved physical performance through enhanced oxygen transportation and energy production. Endurance training has also positive adaptations in other organs or organ systems such as nervous system, adipose tissue and bone tissue. (Kenney et al. 2022, McArdle et al. 2015 & Valenzuela et al. 2023; Figure 3)

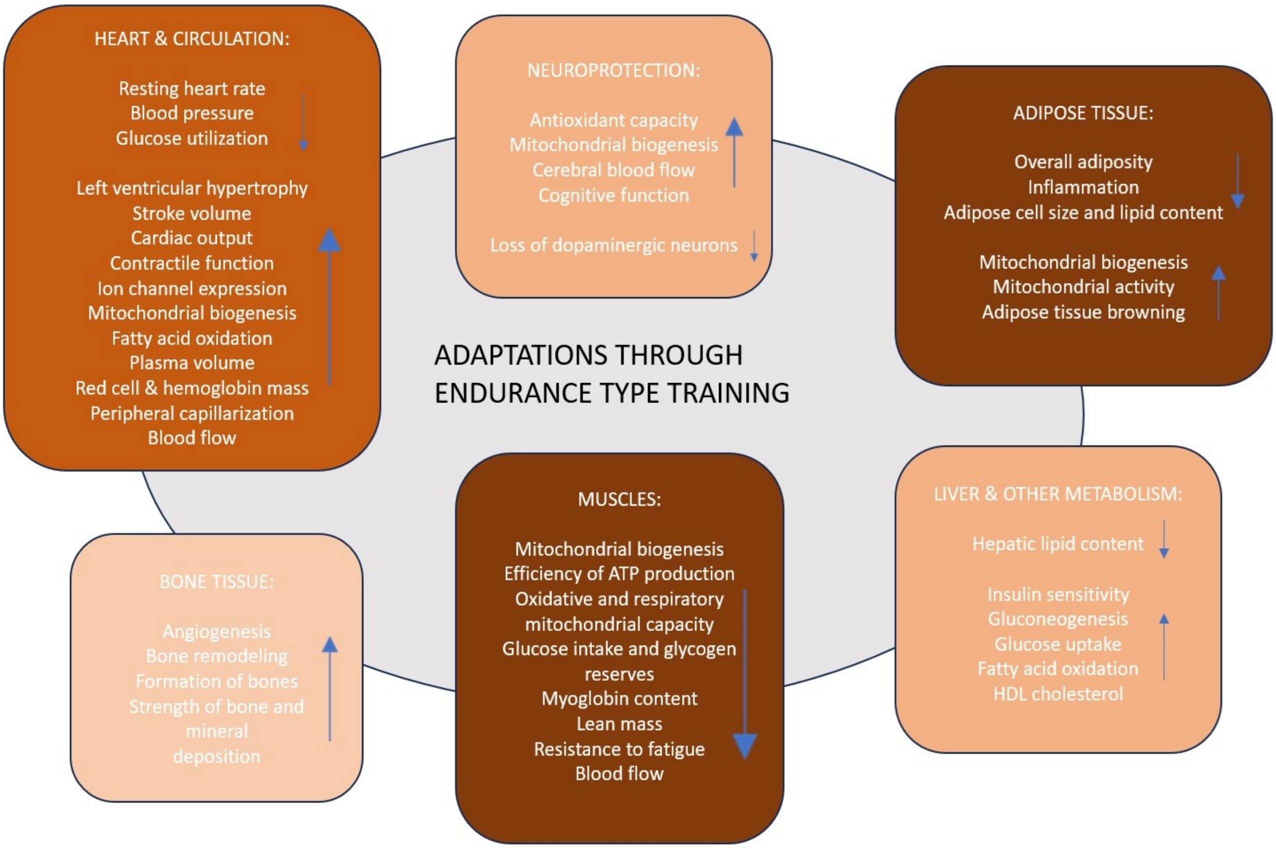

FIGURE 3. Main adaptations through endurance training (Kenney et al. 2022, McArdle et al. 2015 & Valenzuela et al. 2023; modified).

3.1.1 Adaptations in the cardiovascular system

Adaptations in the cardiovascular system consists adaptations in blood volume, heart and blood flow. Endurance training increases plasma volume fast; approximately 10-20% enhancement is possible already after three to six aerobic training sessions. Red blood cell mass changes and the total amount of red blood cells and hemoglobin are typically elevated in highly trained athletes. Because of this there is more oxygen-carrying capacity. (Kenney et al. 2022, 286-295 & McArdle et al. 2015, 467-471; Figure 3). However, even if the absolute hemoglobin and red cell masses are increased, the increasement of plasma volume causes dilution and laboratory values like hematocrit and hemoglobin are often decreased leading to “athlete’s anemia” (Kenney et al. 2022, 286-295 & McArdle et al. 2015, 467-471), and it has been estimated that 3-10% of male athletes and 15-35% of female athletes have iron deficiency (Peeling & McKay 2023). Hemoglobin levels can be manipulated by using iron supplements (Peeling & McKay 2023 & Solberg & Reikvam 2023) or training in high altitudes where the red cell volume adapts to lower oxygen levels (Turner et al. 2022). Higher hemoglobin levels, including hemoglobin concentration, hemoglobin mass and hematocrit, are associated with higher physical performance (Webb et al. 2023).

The increased work demand from training and increased plasma volume induces cardiac muscle mass and ventricular volume to increase. This leads to mainly left ventricle's enlargement and enhanced heart's stroke volume. The heart's contraction vitality becomes more efficient and the stiffness of arteries and the heart decrease. These changes lead to enhanced cardiac output (heart rate x stroke volume) which is one of the key factors behind physical performance. Heart rate becomes lower at rest and during physical activity. Endurance athletes can have heart rates lower than 30-40 beats per minute and approximately 25% greater stroke volume than inactive individuals. (Hedman et al. 2015, Kenney et al. 2022, 286-295 & McArdle et al. 2015, 467-471; Figure 3)

In addition to adaptations in the plasma volume and the heart, the oxygen transportation enhances through adaptations in blood flow in the working muscles. Blood and oxygen flow and distribution to working muscles enhances through local changes in tissues and improvements in gas exchange. The peripheral capillarization increases through increased blood flow that forces the body to develop new capillaries. The recruitment of these capillaries enhances, and this combined to increased blood volume leads to more effective blood flow distribution to the working muscles. (Kenney et al. 2022, 292-293 & McArdle et al. 2015, 472-474; Figure 3)

3.1.2 Adaptations in muscles and energy metabolism

Main adaptations in the muscles consists of changes in mitochondria, enzyme activity, myoglobin content and muscle fiber characteristics. After every exercise initial signaling responses in muscles and muscle tissues occur leading to chronic adaptations through cumulative effect (Hawley 2002). Mitochondria are the cell's energy producers and through endurance training new mitochondria are formed. Also the size of mitochondria grows and their ability to produce energy as adenosine triphosphate (ATP) is improved. This adaptation is necessary for better ability to use oxygen and produce ATP hence that is straightly dependent of the number and size of mitochondrions. (Hawley 2002 & Kenney et al. 2022, 297-300; Figure 3)

Endurance training also increases the activity of certain mitochondrial oxidative enzymes that are specialized proteins connected to the ATP production. These enzymes and their activity are necessary to form ATP from catalyzed nutrients. Myoglobin is a molecule very similar to hemoglobin that transports the oxygen molecules from the cell membrane to the mitochondria. The increased myoglobin content is needed as an oxygen reserve when oxygen comes limited through muscle action, especially in the gap between the beginning of an exercise before the cardiovascular delivery of oxygen (increased heart rate and ventilation) starts. (Hawley 2002 & Kenney et al. 2022, 297-300; Figure 3)

Training responses in muscle cell types and energy production are specific to the type of training. Through low to moderate intensity aerobic exercises the enlargement of type 1 (slow-twitch) fibers occurs while high-intensity interval training leads to enlargement of type II (fast-twitch) muscle cells. (Hawley 2002 & Kohn et al. 2011) The overall energy metabolism in muscles, including carbohydrate and lipid metabolism, changes. Because of aerobic training muscles are able to use glucose and fat for energy more efficiently. After 2 weeks of aerobic training oxidation of fat acids in rest and in submaximal performance enhances. Especially low to moderate intensity training improves the oxygen related energy production and muscles' ability to use body's fat reserves and carbohydrate metabolism enhances. Muscles can use their glycogen reservoirs more efficiently, leading to improved blood glucose stability. More intensive training improves the use of immediate energy production (ATP). Improvement in energy production also develops benefits for the body's ability to buffer lactate produced in exercises with higher intensity. (Hawley 2002 & McArdle et al. 2015, 465-474)

3.2 Cardiorespiratory fitness and maximum oxygen uptake (Vo_{2max})

Maximum oxygen uptake (Vo_{2max}) describes the body's ability to transport oxygen to muscles during activity and the ability of skeletal muscle to use oxygen. It has become the most preferred laboratory measure for physical performance (Albouaini et al. 2007 & McArdle et al 2016, 166). On clinical use in health care defining Vo_{2max} enables the prediction of risk factors of physical activity or exercising when diagnosed with CVDs or other unhealthy conditions or diseases. (Albouaini et al. 2007 & Ross et al. 2016) Among athletes it is generally used to define especially endurance athletes' physical performance and fitness and is found to be the key predictor of endurance performance. An athlete with high

Vo_{2max} can perform faster and do more muscle work more economically. (Kenney et al. 2022, 286-287 & McArdle et al. 2015, 165 – 166) The Vo_{2max} and oxygen transport system are also dependent on the function and combination of pulmonary ventilation, hemoglobin concentration, blood volume, cardiac output, peripheral blood flow and aerobic metabolism, all being part of the main adaptations through endurance training. (McArdle et al. 2015; 166, 349 & Vuorimaa 2016; Figure 1) Athletes' performance's economy depends also from sufficient sports related technique, biomechanics and individual differences (Vuorimaa 2016).

Vo_{2max} is the maximum amount of oxygen delivered to muscles in a minute. It is often expressed in milliliters of oxygen per kilogram of body mass per minute (ml/kg/min) (Albouaini et al. 2007 & McArdle et al 2016, 167). Compared to sedentary population, endurance athletes Vo_{2max} values are usually two times higher. The highest Vo_{2max} values have been measured in cross-country skiers for over 84+/-5 ml/kg/min on male and over 73+/-5 on female and the lowest on cardiac patients with only 10ml/kg/min. (McArdle et al 2015, 167 & 236, Ohtonen & Mikkola 2016 & Ross et al. 2016) The usual way of dividing to total body weight Vo_{2max} may underestimate cardiopulmonary fitness in obese individuals. Therefore, it should be defined to lean body mass or muscle mass. (Krachler et al. 2015) Males usually have higher Vo_{2max} than females when adjusting it to whole body weight, but when adjusting Vo_{2max} to lean body mass the difference in male and female elite athletes may be less significant (Higgor et al. 2023).

Vo_{2max} is traditionally tested by a treadmill or cycle ergometer intervals. On each interval the elevation or speed is gradually and progressively increased until the test participant cannot tolerate or maintain the workload to continue the test. This test until volitional exhaustion can also be defined as time to exhaustion (TTE). Especially for athletes it is recommended that the test is made similar to the main sport; runners on treadmill, cyclists on cycle ergometer, swimmers on swim flume and skiers on ski mat. (McArdle et al. 2015, 165 & 236-238) On clinical use in health care to assess patients' cardiopulmonary function, cycle ergometer is more preferred (Beltz et al. 2016).

Heart rate and ECG are monitored during the test. Ventilation and respiratory gas parameters for oxygen and carbon dioxide are monitored via breathing mask during the exercise test. A breath-by-breath analysis and respiratory rate are used to calculate average minute volumes.

A non-rebreathing valve in mouthpiece is used to prevent mixing of inspired and expired air and respiratory volumes are computed by integrating the air flow signals over the time of inspiration and expiration. (Albouaini et al. 2007 & Beltz et al. 2016)

During the test assessments of rating of perceived exertion (RPE) and blood lactate concentration should also be made (Beltz et al. 2016) RPE is used to indicate the intensity of exercise by a numerical scale of participant's perceived feelings about the exertion level. A change in blood lactate concentration linear to the increase in RPE verifies the validity of the Vo_{2max} measurement. To accept an oxygen uptake value to be as near maximum, blood lactate levels should attain 8-10 mmol/l or above. For reference, at rest blood lactate values are 1-2 mmol/l and on elite athletes can rise up to 20 mmol/l and above in intense exortion. For athlete they provide also useful information about their aerobic and anaerobic thresholds to exploit in their training. (Beltz et al. 2016 & McArdle et al 2016, 238 & 482-483)

3.3 Periodization in endurance training

Traditionally, athlete's year is divided to periods of basic endurance, preparatory season for competitions, competing season and transition phase. On some international literature the transition phase includes the basic endurance phase or the preparatory season for competitions described in Finnish literature. (Bompa & Puzzichelli 2018; 165, Casado et al. 2022 & Vuorimaa 2016 & Ohtonen & Mikkola 2016; Figure 4) Within these traditional annual phases athletes have also smaller mesocycles and individually targeted development goals. Training is typically also periodized within single training weeks by following harder days with easier days. (Casado et al. 2022, Haugen et al. 2022 & Vuorimaa 2016)

Different characteristics cannot be trained simultaneously if the goal is to achieve the best development and to perform the best in competitions. An annual plan is mandatory also to stimulate the physiological and psychological adaptations and at the same time manage fatigue and avoid overtraining (Bompa & Puzzichelli 2018, 165). In basic endurance period low intensity endurance training is the main exercise with the goal of achieving changes in mitochondria and peripheral vascular system. Aerobic endurance is developed by exercises done in steady state or low and moderate intensity lasting from 45 minutes to several hours. Endurance athletes should have approximately 70-80% of low or moderate aerobic endurance training of the total training amounts (Bompa & Puzzichelli 2018, 167) but elite international level runners are known to train over 80% in low intensity throughout the whole training year. Especially on the basic endurance training season these aerobic endurance's training amounts are high, scaling up to the end of this period until training amounts are at their highest. (Haugen et al. 2022, McArdle et al. 2015, 465-474 & Ohtonen & Mikkola 2016; Figure 4)

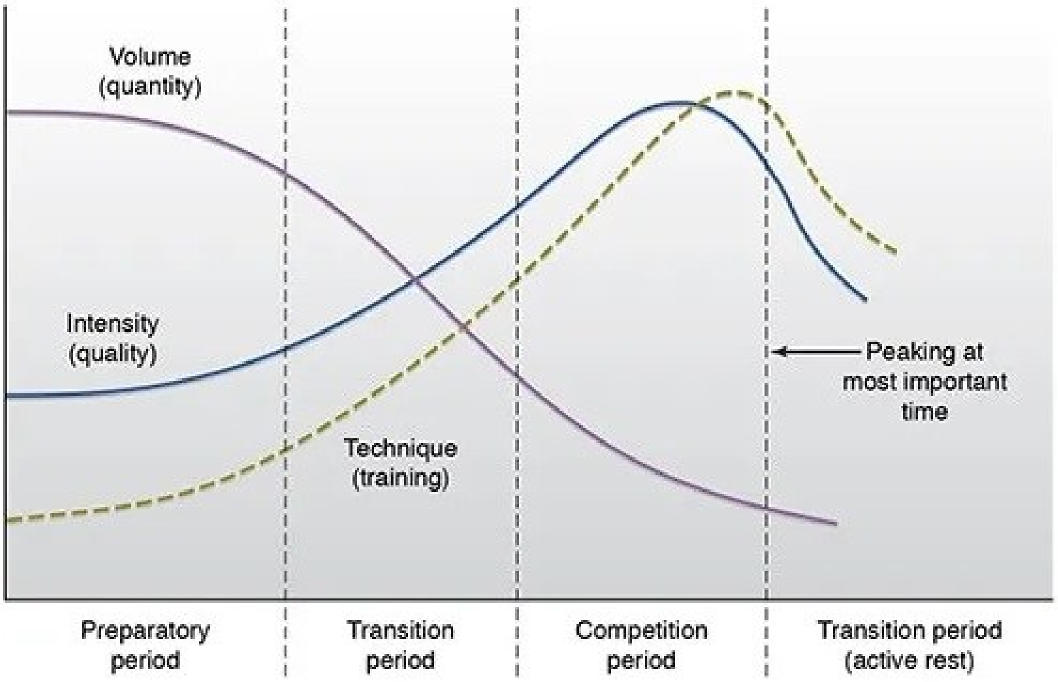

FIGURE 4. Annual periodization in endurance training; the total amount of training decreases and the intensity of training increases towards competition period (Phillips et al. 2016).

Usually, aerobic speed training and strength training are integrated to basic endurance period. At the end of this period anaerobic training is also done but kept moderate. (Haugen et al. 2022, McArdle et al. 2015, 465-474 & Ohtonen & Mikkola 2016) Speed and anaerobic training improve the body's ability to perform fast and provide anaerobic energy to the working muscles. Usual training methods are different interval exercises. Strength training is needed to provide enough strength to build up speed and improve the economy of performance. (Vuorimaa 2016 & Ohtonen & Mikkola 2016)

The preparatory season before competing season prepares the athlete for more extensive rise in the intensity of training and competing in the competitive season. On this phase athletes do more intense exercises like interval training and anaerobic training to achieve improvement in their anaerobic and maximum performance. These exercises are targeted to enlarge the left chamber of heart and Vo_{2max} and make the anaerobic energy system more efficient. Sports related skills and technique exercises are also important. On endurance athletes the preparatory season is usually twice as long as the competing season, depending on the sport and climate. For advanced athletes the preparation phase can be reduced whilst novice and young athletes need longer preparation. (Bomba & Puzzichelli 2018, 166 & 168 & McArdle et al. 2015, 472-474; Figure 4)

On competition season training and competing is more intensive but total amounts are smaller in order to manage the total load from training and competitions and at the same time recover and maintain the best physical performance. Training amounts can be bigger or more intensive than this if the timing of athlete's main competitions or championships makes it possible. (Bompa & Puzzichelli 2018; 169, Casado et al. 2022 & Ohtonen & Mikkola 2016; Figure 4)

After competitive season before the next annual training cycle is transition phase when training is lighter and non-scheduled. The purpose of this phase is to make sure the athlete is able to rest both physically and psychologically, recover, and regenerate biologically. This phase is necessary in preparing the athlete to following seasons and skipping it may lower athlete's capacity in developing and performing their best in the future competitions. Without a proper recovery athlete's risk for injuries increase. On competitive elite athletes the transition phase is approximately 1-2 weeks but for younger athletes it can last up to 4-6 weeks. (Bompa & Puzzichelli 2018, 174 & Ohtonen & Mikkola 2016; Figure 4)

3.4 Main benefits for cardiovascular health from endurance training

Previously described main adaptations through endurance training lead to health benefits and decreased overall risk for diseases (Figure 5) and endurance training like any other regularly practiced physical activity should be involved in preventing and managing not only CVDs but also other common conditions like obesity, dyslipidemias, metabolic syndrome, type 2 diabetes (TD2), nonalcoholic fatty liver disease (NAFLD), cognitive diseases like dementia, depression and even cancer (AHA 2024 & Paluch et al. 2014). For preventing CVDs it is known that physical activity can explicate 50-70% of the positive changes in preventing

CVDs. Especially the beneficial effects on human body's cardiovascular system, body composition and obesity, blood lipid levels and energy metabolism through endurance training makes it an important way to maintain cardiovascular health. (AHA 2024, Edwards et al. 2023, Grundy et al. 2018, Mann et al. 2014 & Paluch et al. 2014).

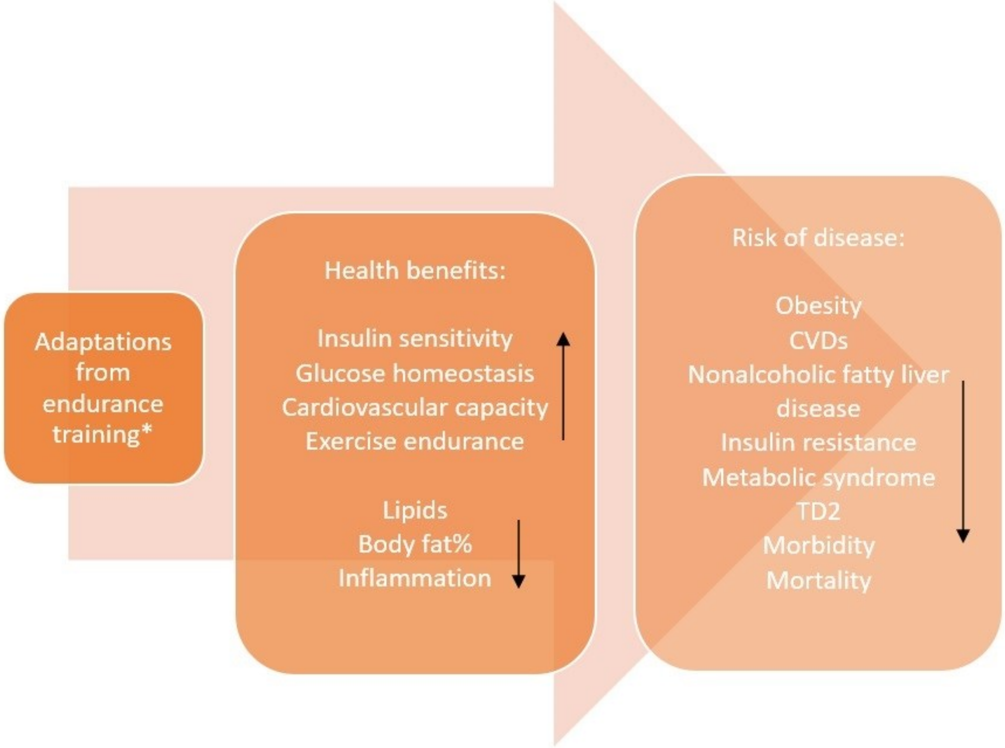

FIGURE 5. Health benefits through endurance training. *Described in Figure 3.

Endurance training has been shown to decrease not only BP but also AS in people who have greater AS at baseline or high BP. (Ashor et al. 2014, Edwards et a. 2023, Lopes et al. 2021, Safar et al. 2018 & Vlachopoulos et al. 2010) Individuals who exercise regularly have lower BP than inactive individuals. Moderate endurance training decreases BP approximately 8/5 mmHg in individuals with increased BP. (Cornelissen & Smart 2013, Duodecim 2022 & Edwards et al. 2023) The impact on BP can be seen as early as two months after starting endurance type training but BP also decreases after each endurance exercise for the next 72 hours. (Hedman et al. 2015, Kenney et al. 2022, 286-295 & McArdle et al. 2015, 467-471) The evidence of endurance training's benefits to AS is less clear than to BP. Few studies have previously resulted in reduced AS through aerobic exercise or endurance training and these findings could suggest that interventions for improving aerobic capacity may reduce the stiffening of the arterial tree that normally comes in conventional aging. (Ashor et al. 2014 & Li et al. 2015)

For obesity the adaptations of decreased body fat%, increased lean body mass and metabolical adaptations (Figure 5) are beneficial (Paluch et al. 2014). Endurance training without nutritional changes can reduce especially visceral fat tissue for few kilogrammes. Though, losing weight is more efficient when physical activity is combined to nutritional changes. (Duodecim 2023 & Paluch et al. 2014) The adaptations in insulin resistance and insulin sensitivity and improved glucose homeostasis lead to lower risk for metabolic syndrome and TD2. This is also beneficial for managing eating behaviors and again preventing obesity. (Duodecim 2023)

Because of the beneficial changes in lipid metabolism from endurance type training, endurance training is also part of the general lifestyle recommendations when manipulating blood lipid levels. HDL usually increases after 3-6 months of regular endurance training. Better HDL content can also improve the heart’s contractility. For overweight individuals the increase is smaller if they don’t lose weight simultaneously. (Fikenzer et al. 2014, Mann et al. 2014 & McArdle et al. 2016, 869) Increase in HDL can be seen when training regularly with moderate intensity but the decreased LDL and TG levels usually need exercises with higher intensity (Mann et al. 2014). LDL decreases are more likely to happen if training is combined with nutritional improvements. In case individual has not been physically active before, approximately 5% enhancements can occur. Decreased TG levels can be seen immediately after a single exercise but this effect is usually only momentary. (Duodecim 2022 & Fikenzer et al. 2018)

3.5 Main risks for cardiovascular health from endurance training

On the opposite, endurance training could also have negative outcomes for cardiovascular health. In endurance sports nutritional issues become challenging when athletes must maintain appropriate energy intake to support normal body function and to gain optimal response to training while manipulating optimal body composition. In weight-sensitive sports, athletes may struggle with unrealistic expectations of body composition in hopes of better physical performance. If there is need for manipulating body composition at all, a proper nutritional plan should be made with special nutritionists and coaches to avoid any negative impacts. (Sundgot-Borgen et al. 2013)

Favoring light and lean body can thrive athletes to restrict energy intake leading to negative energy balance. When prolonged, it is possible to gain RED-S, previously known as female athlete triad. However, nowadays it is known that this counts to both sexes, women and men. (Mountjoy et al. 2018, Kyte et al. 2022 & Sundgot-Borgen et al. 2013) RED-S in female athletes can be easier to recognize because it often leads to irregular menstruation and amenorrhea. In some sports groups amenorrhea can reach over 40% of athletes when in normal population it occurs in 2-5% of women. It must be noted though, that a female athlete can still have RED-S even if her menstrual cycle is regular and menstrual disturbance can also be hidden by the usage of hormonal contraception. (Ackerman et al. 2019, Cheng et al. 2021 & Mountjoy et al. 2018) Clinicians can also be blind to the fact that athlete's BMI can be relatively normal but very low body fat% especially combined to decreased physical performance and occured stress injuries can be a sign of RED-S (Ackerman et al. 2019).

Low energy availability where athlete's energy intake does not match with energy expended in exercise leaves athlete's body inadequate to support the functions to maintain optimal overall health and high performance. RED-S has multiple negative results in metabolic rate, menstrual function, bone health, immunity, protein synthesis, and cardiovascular health. (Mountjoy et al. 2018) Similar symptoms as in early menopause before the age of 40 years through low estrogen levels have been shown to increase the CVD risk especially when combined with inappropriate consuming of energy (Grundy et al. 2018 & O'Donnell et al. 2011) and athletes with low energy availability are 2.5 times more likely to engage unhealthy cardiovascular outcomes (Ackerman et al. 2019). Hypoestrogenism and amenorrhea can be associated to early atherosclerosis already in young athletes and premenopausal physically active women, despite the level of physical activity. (Mountjoy et al. 2018, O'Donnell et al. 2009 & 2011, Rickenlund et al. 2005)

In healthy cardiovascular system, estrogen plays a role in regulating BP, vasodilation of the blood vessels in corporation of nitric oxide production, decreasing vascular inflammation and atherosclerosis and improving vascular reactivity. The unfavorable changes in endothelial function like increasement of the vascular tone and the disturbance in estrogen action in vascular cells and estrogen receptors lead to elevated BP. (O'Donnell et al. 2009 & 2011, Pradhushankar et al. 2014, Rickenlund et al. 2005 & Shufelt et al. 2017) Estrogen has an impact to blood lipid metabolism as well and hypoestrogenism can lead to increased lipid levels mainly in LDL and TC cholesterols, but also on TG (Grundy et al. 2018 & O'Donnell et al. 2011, Rickenlund et al. 2005 & Vishakha 2019).

4 LITERATURE REVIEW OF PREVIOUS STUDIES

A systematic advanced search was made to PubMed (Medline) and SPORTDiscus (EBSCO) databases with six different search phrases (Figure 6). The purpose was to find previous studies that either 1) compared vascular stiffness and blood lipid profile of endurance athletes to less active, 2) searched for associations of physical performance or body composition to AS or lipids and 3) searched for fluctuations in any of these outcomes among endurance athletes in the different training periods. In addition to this main search for previous studies, multiple other search phrases were used to find definitions for the main concepts introduced in the beginning of this thesis.

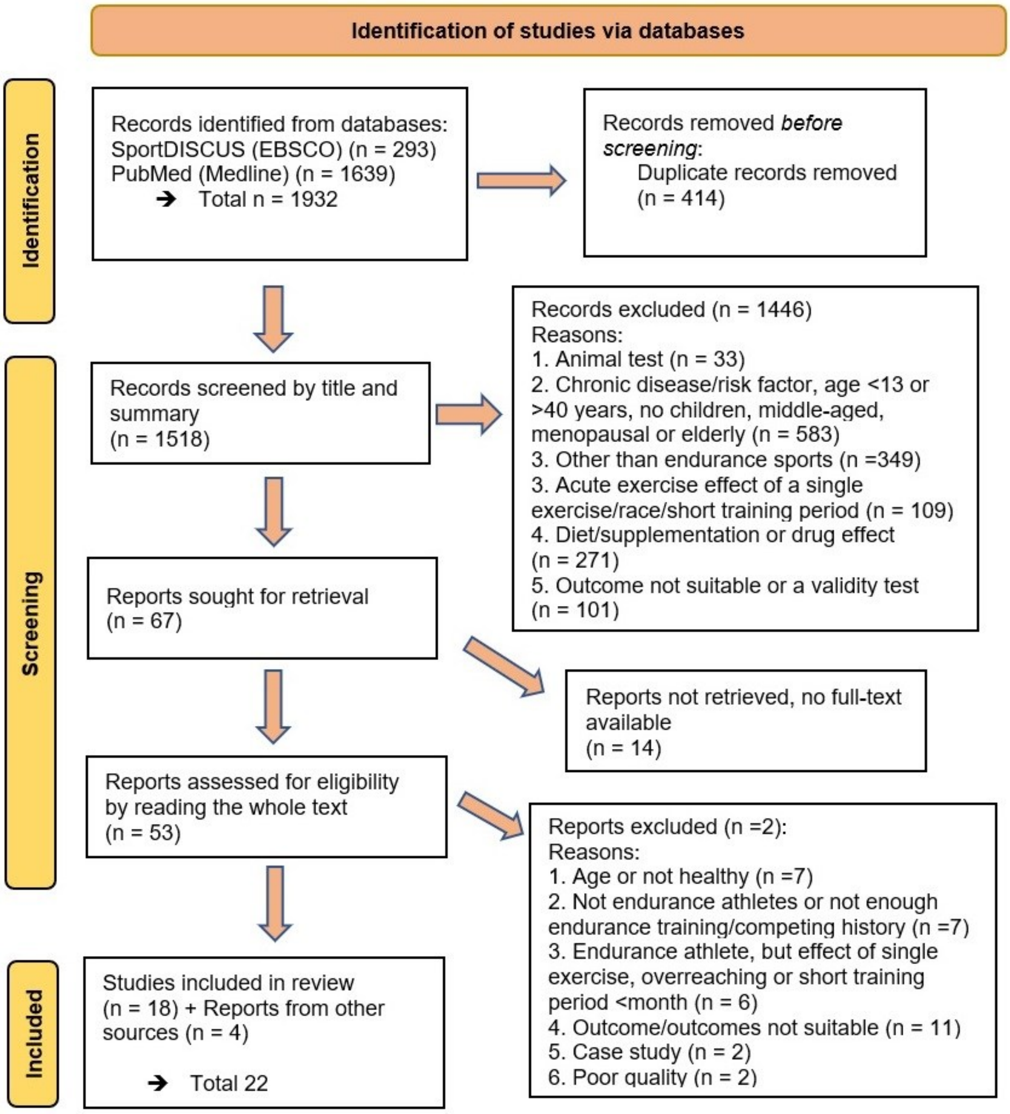

FIGURE 6. Flow chart of the search process of previous studies.

Articles were included by following criteria; 1) English peer-reviewed article published 2000-2023, full-text available 2) study participants healthy adolescents or adults, aged 13-40 years, 3) studies assessing endurance athletes must have a suitable amount of training or competing history 4) outcomes AS (PWV), blood lipids (TC, HDL, LDL and TG), body composition and physical performance (Vo_{2max}). Articles assessing animals, children, middle-aged or postmenopausal women, elderly population or individuals with chronic diseases or their risk factors were excluded. Articles were also excluded if the research was only about the acute effects of a single endurance exercise or race or a short period of endurance training or a case study of a single athlete. If research had multiple subgroups defined by age or different sport groups, the eligible subgroups were taken to account. (Figure 6)

The references of relevant articles found were also exploited if eligible and 4 articles were found outside the initial search. Total of 22 eligible articles published between 2001-2023 were selected (Figure 6; Appendices 1-3). The quality and potential biases were evaluated by Joanna Briggs Institute's (JBI 2023) critical tools for analytical cross-sectional studies, cohort studies and systematic reviews.

4.1 Arterial stiffness and blood lipid profile of endurance athletes compared to less active population

Defining an athlete from normal population as an individual or cohort by training status is not standardized. Relative Vo_{2max} is the most cited parameter in literature to define participant groups and it can be used to define athletes from well-trained or recreational athletes or totally inactive population. Elite level athletes also have other qualities that separate them from other populations. Unfortunately, there is no common perspective or terminology to characterize the training status of an individual or cohort. (De Pauw et al. 2013 & McKay et al. 2022) The body composition and physical performance of athletes can be hypothesized to be better than less active population. This chapter discusses previous studies defining differences in AS and blood lipid profiles of endurance athletes compared to less active population. Studies included in this search had to have competitive athletes with an adequate training history. All participants were healthy and non-smoking adults or adolescents, if not later mentioned otherwise. At baseline there were no differences between athletes and controls in BP or age, which can in general be seen as risk factors for CVDs. In all of these study reports it is shown that athletes' body fat percentage and BMI was lower than controls' and their Vo_{2max} or Vo_{2peak} was higher than the less active controls'.

In the literature search six studies assessing AS with PWV were found. The way of measuring PWV varied (Appendix 1 & 2). Augustine et al. (2015), Bjärnegård et al. (2018), Kyte et al. (2022), Tomsci et al. (2021), Hashimoto & Okamoto (2023) & Otsuki et al. (2007) assessed PWV and compared adult endurance athletes to less inactive population of the same age. In these studies, only Hashimoto & Okamoto (2023) and Otsuki et al. (2007) implicated lower PWV in athletes. Similar to Tomschi et al. (2021), in addition to less active population, Otsuki et al. (2007) compared endurance athletes also to strength athletes. PWV of endurance athletes was lower than other athletes and less active control groups in both studies.

Different from other studies made to endurance athletes, Augustine et al. (2015) compared amenorrheic athletes as a subgroup to eumenorrheic athletes and less active controls. They found out that carotid-femoral PWV was significantly lower in the eumenorrheic athletes than the controls, but not in amenorrheic athletes' subgroup. Carotid-radial and femoral-posterior tibial PWV showed no significant difference. Overall, their study suggests that systemic AS in amenorrheic athletes is similar to eumenorrheics' despite training status or vessels observed in young healthy women. Kyte et al. (2022) reported three amenorrheic participants and 40% of participants in risk of RED-S based on a questionnaire but they did not make any subgroup analyses of them. Other studies did not report the absence of amenorrhea or RED-S and any of these studies did not report the phase of menstrual cycle of female participants.

In three of the six studies assessing PWV of endurance athletes had also measurements of lipid profile as a baseline measurement. In two of these studies were no differences in blood lipid levels between groups, but in Kyte et al. (2022) study among runners showed significantly higher HDL-cholesterol compared to controls. Only two studies focusing on assessing blood lipid levels and comparing endurance athletes to less active population were found (Appendix 1) and both of them were focused on adolescent distance runners. In Mitsusono & Ube (2006) study assessing only females HDL levels were more favourable in runners than non-athletes and TG was higher in non-athletes than runners. Eisenmann et al. (2001) assessed blood lipids in both sexes among young distance runners. Their study indicated that HDL was the only blood lipid that appeared to be enhanced compared with the general population.

For AS it could be concluded that in previous studies endurance trainings' effect to AS has been considered to have a lowering effect to PWV but these studies have mostly been targeted to the research of acute trainings' effects or to non-professional athletes or population who already have traditional CVD risk factors. (Ashor et al. 2014 & Lopes et al. 2021) In studies comparing endurance athletes to less active were no significant difference in PWV between these groups. It may differ between male and female athletes and amenorrheic women could have increased AS. From the results of this search it is shown that endurance athletes may have lower PWV than other athletes like strength sport athletes. Naturally there is no definite conclusion considering the fact that studies comparing different athletes were not the main target of this search and only studies who had less active control group were included.

From previous literature it is well known that endurance training affects fat metabolism and blood lipid profile but mainly the difference is in HDL cholesterol levels (Duodecim 2022, Fikenzer et al. 2018 & McArdle et al. 2015, 465-474). This search strengthens the fact that endurance athletes do not have superior blood lipid profile compared to normal population despite HDL cholesterol. It could be possible that comparative studies of endurance athletes' lipid profiles are not widely committed in recent years. From the references of chosen studies it could be noticed that lipid profile studies have been made among endurance athletes but they are committed earlier than year 2000 which was the criteria to exclude studies from this search. Also, studies about the effects of different diets of athletes were excluded and this limits the search results.

4.2 Associations of cardiorepiratory fitness and body composition with arterial stiffness and lipids

Eight studies in healthy adolescents and adults and three in endurance athletes were included in the literature review (Appendix 1 & 2). Associations of CRF with AS was evaluated in six studies and four of them showed clear relation of higher CRF with lower AS. In Boreham et al. (2004) longitudinal study from adolescence to young adulthood CRF was inversely associated with PWV independent of body fatness and lifestyle variables and adjustments for blood lipid levels did not decrease the association. Gando et al. (2016) committed also a longitudinal study to healthy adults in which they showed that changes in PWV were significantly higher during the two-year study period in participants with lower CRF than they were in participants with higher CRF group. Their study suggests that higher CRF is associated with slower progression of age-related arterial stiffening, but the lack to conclude this might be the length of their study. Also, they reported that CRF was measured only once at the baseline. This makes it impossible to know if there could have been changes in participants CRF during the follow up period which would have had modifications to participants' AS. In both of these studies no differences in male or female participants were found.

Fernberg et al. (2017) and Haapala et al. (2022) made cross-sectional studies combining associations of CRF and body composition with AS. Fernberg et al. (2017) study showed that young adult females with lower Vo_{2max} had higher PWV, but no differences in male participants were found. Haapala et al. (2022) study had adolescent and young adult female participants, and in their study CRF was also inversely associated to PWV in all age groups. Their subgroup of young adults had higher fat free mass index (FFMI) and CRF than adolescents. Haapala et al. (2022) also made adjustments with BF% and FFMI. They found out that BF% was directly associated and FFMI inversely associated with PWV. In Fernberg et al (2017) study results indicated that females with lower CRF had higher PWV, but no differences in males were found. In addition, their study included also obese participants and they had higher PWV than normalweight or underweight participants.

Even though Fernberg et al (2017) and Haapala et al. (2022) had similar results about the associations of CRF with AS like Boreham et al. (2004) and Gando et al. (2016), the relationship between Vo_{2max} and AS was not seen on two studies made to adolescents and young adults. Conversely to others, Namgoong et al. (2018) found no associations between Vo_{2max} and AS on healthy young adults. Similar to this in Meyer et al. (2017) study to adolescents, opposite to average hypotheses, PWV increased with higher CRF. Both studies had male and female participants and when sex groups were assessed separately and adjustments with age and other confounding factors were made the associations remained similar.

Budimir et al. (2012) and Wildman et al. (2003) searched for associations of body composition with AS in both sexes. In Budimir et al. (2012) participants were healthy and non-obese but smoking occured. They used multiple body composition assessments and found no significant associations of body composition to PWV when confounding factors like age, BP, lipids and smoking were not adjusted. Wildman et al. (2003) had normalweight and obese participants. They found higher body weight, body mass index, waist and hip circumferences, and waist-hip ratio were strongly correlated with higher PWV, independent of age, systolic , race, and sex. In their study PWV values were 0.4-0.9 m/s higher for obese individuals compared with normal-weight individuals. Their study demonstrates that excess body weight affects on vascular stiffness in adults as young as 20 years. This could mean that excess weight begins to affect the vascular system at a very early stage of vascular aging. The same way Haapala et al. (2022) suggested that for women maintaining sufficient muscle mass and favorable body composition could be beneficial across the lifespan.

On the three studies about associations made to endurance athletes the results varied, similar to normal population. Bjarnegård et al. (2015) & Augustine et al. (2015) found no associations between body composition and AS. This might be explained by the fact that athletes are usually normalweight. Hashimoto’s and Okamoto’s (2023) study results suggest that higher V̇O2max is associated with lower peripheral AS in addition to central AS among endurance-trained athletes. Bjärnegård et al. (2015) & Augustine et al. (2015) studied only female athletes, whilst Hashimoto & Okamoto (2023) only men. It must be notable and discussed that female and male physiology are not the same. In Augustine et al. (2015) study amenorrheic athletes were assessed a subgroup and correlations with participants’ Vo$_{2\text{max}}$, percent body fat and salivary estradiol were calculated; higher Vo$_{2\text{max}}$ was associated with lower carotid-femoral PWV, but no associations of participants’ percent body fat or salivary estradiol with any vascular parameters were found. In Haapala et al. (2022) study was reported that part of the participants were classified “physically highly active (training 6 times a week)” or ”young female athletes, competing”, though athletes as a subgroup were not discussed separately.

Fernberg et al. (2017) and Haapala et al. (2022) studies could conclude that higher CRF and favorable body composition are associated with lower PWV. In Fernberg et al. (2017) the highest mean PWV was in participants with lower Vo$_{2\text{max}}$ & overweight and the lowest in participants with higher Vo$_{2\text{max}}$ & normalweight. Also Wildman et al. (2003) study showed body composition’s relation to AS. These studies strengthens the hypothesis that body composition has an evident relationship with AS. But the overall results and methods of previous studies vary. From this search it could be concluded that associations between better CRF and favorable body composition with AS or blood lipids have been made but not widely to endurance athletes.

Blood lipid levels and body composition were measured in most of the studies but mainly as a baseline measurement. Associations with blood lipids were evaluated in only one study. In Namgoong et al. (2018) study Vo_{2max} had a significant association with TGs. Participants with better Vo_{2max} had lower TG levels. This literature search focused on the parameters related to the study questions of this thesis and all possible confounding factors are not discussed in this part. For instance, in some studies, the lacking information about study participants’ precise physical activity, sedentary behaviour, diet quality, nutritional status, total energy expenditure, time of menstrual cycle or contraceptive use or any traditional cardiovascular risk factors and their role in the presented results is impossible to know. In all of these studies the menstrual cycle phase of female participants was not recorded or reported, or reported to be not available, because of a prospective approach. In some studies participants were reported to be healthy but still included participants who smoked or consumed alcohol. In these studies, adjusted statistical analyses were made.

Even though the associations vary, it could be assumed that for normal population, better CRF, physical activity and favorable body composition have associations with lower AS and in some studies sports related physical activity is seen better than only leisure time physical activity. This supports the fact that for the public health the improvement of CRF and participating in sports activities and maintaining favorable body composition are important tools for the primary prevention of CVDs. The effects of these possible associations via prolonged endurance training and competitive sports are not yet discovered. Associations with blood lipid levels among normal healthy population and endurance athletes needs to be studied more.

4.3 Fluctuations between different training periods of endurance athletes

Endurance athletes are known to periodize their training like described previously. Third phase of literature search of previous studies aimed to find longitudinal studies of endurance athletes' possible changes in physical performance, body composition, AS and blood lipid levels in different training periods of their annual training (Appendix 3). Especially to competing athletes it is important to achieve their best physical performance before main competitions (Bompa & Puzzichelli 2018, 165 & Heydenreich et al. 2017). Durkalec-Michalski et al. (2022) studied Vo_{2max} fluctuations and changes in body composition in triathletes. Their performance was improved during the competition period when they gained longer TTE in incremental cycle test. They also had lower Vo_2 at the ventilatory threshold.

Trinshek et al (2020) found no differences in Vo_{2max} between general preparation period, the beginning of the specific preparation period, the beginning of the pre-competition period and the beginning of the competition period in endurance athletes. However, Vo_{2max} increased in the control group. Endurance athletes had competitive sport history of 8.0 ± 2.4 years and control group had no previous or current professional sports experience, but their training was endurance-orientated. It could be possible that changes in high-level are only minimal, but when training background is smaller changes are bigger and faster. In addition, the control groups Vo_{2max} levels were lower at the baseline than endurance athletes'.

Body composition of endurance athletes is generally hypothesized to be leaner in competition period. In Heydenreich et al. (2017) systematic review of 82 reports was shown that endurance athletes had significantly higher body mass and FFM in the competing season compared to the preparation and transition phases. For the percentage of fat mass, no differences were detected between the seasonal training phases. The more recent results of Durkalec-Michalski et al. (2022) and Trinshek et al. (2020) are similar. In Trinshek et al. (2020) study endurance runners had significantly lower absolute and percentage fat mass than controls in almost all examinations, except for the general phase. In general phase the difference between these groups was non-significant. Endurance runners' fat mass% was significantly higher in the general training phase than in the subsequent training phases. The controls had non-significant change, even though there were slightly lower fat mass% values in the competition phase. Endurance athletes had higher percentage lean body mass than controls in all training phases, except for the general phase. Both absolute lean body mass and lean body mass% increased significantly between the general and the subsequent training phases in endurance athletes' group but in control group they had no significant change but lean body mass slightly increased at the two last timepoints measured. For skeletal muscle mass there was no significant changes in neither of these groups.

Durkalec-Michaski et al (2022) found no significant differences between training and competition period, but triathletes' body mass slightly decreased in competition period mostly because body fat was reduced. Different to Trinshek at al. (2020) DXA measurements, they used bioimpedance method and described their study participants to have more fat mass than triathletes usually do but no reference to this was given. Naturally, Heydenreich et al. (2017) review had multiple body composition assessment methods which may lower the reliability of their results.

After competition period athletes usually have a transition phase (Bompa & Puzzichelli 2018, 174 & Ohtonen & Mikkola 2016). Different to other studies, Ormsbree & Arciero (2012) studied detraining's effects to Vo_{2peak}, body composition and blood lipids after competition period in swimmers. In their study Vo_{2peak} decreased significantly (-7,7%). In maximum heart rate, respiratory quotient and RPE were no differences, but TEE was significantly lower after detraining period. Lean mass remained unchanged but body fat mass% increased significantly(+12%). Also, waist circumference increased. This study was the only one recognizing changes in blood lipids. TC, HDL, and LDL remained unchanged whilst TGs showed a trend to increase. The lack in Ormsbree's & Arciero's (2012) study is the very small sample size and the fact that detraining period is longer than described in literature earlier. Detraining phase in their study was 5 weeks (35–42 days) but usually endurance athletes' transition phase is 1-2 weeks (Bompa & Puzzichelli 2018, 174 & Ohtonen & Mikkola 2016). For this reason, it could be assumed that Vo_{2max} in this study decreased not because of the detraining but because of the increasement of body fat and total weight.

Longitudinal studies of fluctuations of AS among endurance athletes have not been conducted almost at all. Koshiba & Maeshima (2015) examined the effects of detraining on temporal changes in AS in endurance athletes. They found out that detraining may result in increased AS from 3 months onward. This could be useful to know if an athlete is forced to detrain because of a prolonged injury or sickness. In detraining the increase in PWV was observed earlier than an increase in DBP. This could suggest that PWV is a useful vascular index for understanding the effects of detraining at an early stage, also in normal population. (Koshiba & Maeshima 2015)

From this literature research it can be conducted that there is a lack of longitudinal researches targeted to evaluate changes in endurance athletes' physical performance, body composition and especially blood lipids and AS in different phases of their annual training cycle. Only few longitudinal studies have been made and most of them have studied only Vo_{2max} and body consumption. From these studies it seems that among endurance athletes there is increasement in Vo_{2max} towards competitions like usually hypothesized but Vo_{2max} is dependent of body weight. In body composition the total body mass may stay unchanged with increasement of lean body mass and slightly decrease in body fat. Studies evaluating the effects of detraining could be useful to understand or estimate the changes in athletes' health if training has ended because of an injury or some other reason.

5 PURPOSE OF THE STUDY AND STUDY QUESTIONS

The associations between CRF and physical activity and body composition towards AS are seems to be studied but among endurance athletes only marginally. Because of prolonged endurance training, regular stress from competing and sport related physical demands endurance athletes have a risk for hormonal and endothelial changes that could affect their AS or blood lipids. In elite level of endurance sports it is common to try to achieve the peak performance and adequate body composition before the competing season. Previous studies show the lack of longitudinal studies on this research field.

The purpose of this thesis was to determine how much these features change between two different training periods and does physical performance and body composition associate to more favorable AS or blood lipids on adult elite endurance athletes. To more explanatory approach, purpose was also to use small groups of developmental endurance type trained females and female rugby players as controls. The study questions were:

1. Does AS, blood lipids levels, or body composition of adult female endurance athletes, developmental endurance type trained females or female rugby players differ between the preparation period and the competition period?
2. Is there any differences between these groups at these two timepoints?
3. Does better maximum oxygen uptake or favorable body composition associate to favorable AS or blood lipid levels?

6 METHODS

In this section the study design and study participants are described together with the methods used for the collection of NoREDS study data, the measurements of study's outcomes and the statistical methods used in this thesis.

6.1 Study design and study participants

All data in this study is based on the data from Athletic Performance and Nutrition (NoREDS) conducted at the University of Jyväskylä, Finland. NoREDS is an on-going longitudinal three-year follow-up study where athletes' body composition and energy and nutrient intake are examined in different phases of the athletic season. NoREDS consists total of 388 Finnish national to international-level male and female athletes, aged 16–35 years at baseline. Study participants are followed up from 2021 to 2024 and they represent ball sports, gymnastics, endurance sports and track and field sports. Study follows the Declaration of Helsinki and was approved by the Ethics Committee of the University of Jyväskylä (NoRED-S 514/13.00.04.00/2021). Participants gave written informed consent before the enrollment in the study.

For this study the repeated measures of female endurance athletes, developmental females and female rugby players from NoREDS were used for a cross-sectional design. Endurance athletes' group consisted of athletes from duathlon, cross-country skiing, running, orienteering and speed walking. Participants with missing data on key variables were excluded. The final study sample in this thesis consisted of 23 female endurance athletes, 6 developmental females and 12 rugby players. According to McKAY's et al. (2022) Participant classification framework the endurance athletes group represents level 4-5 “Elite/international level” and “World class” participants. The two control groups' participants represent level 2 “Trained/Developmental”. Measurements were made in two timepoints; one in preparation period and the other in competition period. Time between these periods was approximately 3-4 months in endurance athletes, 6-8 months in developmentals and 6-7 months in rugby players. Table 2)

TABLE 2. Descriptive statistics of the study groups.

Group	n	Age* ± SD	Height (m)* ± SD	Weight (kg)* ± SD	BMI* ± SD
Endurance athletes**	23	24.7 ± 4.5	1.71 ± 0.06	61.6 ± 4.6	21.1 ± 1.5
Developmentals	6	26.7 ± 3.4	1.70 ± 0.04	68.6 ± 3.6	23.7 ± 1.7
Rugby players	12	29.5 ± 3.6	1.67 ± 0.04	69.6 ± 9.7	24.8 ± 2.8
Total	41	26.4 ± 4.5	1.70 ± 0.05	64.9 ± 7.4	22.6 ± 2.6

*first measurement; mean in preparation period, SD = strandard deviation

**duathlonists (1), runners (3), cross-country skiers (9), orienteerers (8), race walkers (2)

6.2 Measurements and data collection

In this chapter the methods and protocols used in NoREDS and this study are described.

6.2.1 Arterial stiffness

In this study participants rested in a supine position for ten minutes before the measurements. Oscillometric pulse wave analysis was then performed following the manufacturer's instructions from the right upper arm using the Arteriograph device (Arteriograph; TensioMed Ltd., Budapest, Hungary) in the supine position. In the measurement Arteriograph device's cuff is inflated 45 mmHg above the participant's SBP. A pressure sensor detects the pressure variations and transfers them to computer. First systolic peak wave comes from the systolic volume ejection in the aorta and the second lower peak wave comes from the pressure reflection from peripheral arteries. PWV is then calculated from the return time which is the difference between these two peak waves (Tensiomed 2023; Figure 2). All measurements were made in the morning.

6.2.2 Blood lipids

In this study venous blood samples were collected in the morning in a fasted state. Blood samples were drawn from antecubital vein at a supine position between 7:00 and 10:00 AM. For serum separation, whole blood was left to clot for 30 minutes at room temperature and centrifuged at 2,200 ×g before aliquoting and storing the sera at −80°C until analysis. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using Indico analyzer (Thermo Fischer Scientific, Finland) according to manufacturer's instructions. Non-HDL levels were calculated manually by substracting HDL level from TC level.

6.2.3 Vo_{2max}

In this study Vo_{2max} was tested by different but suitable ways for endurance. Duathlonists were tested with running treadmill, 5/9 cross-country skiers with skiing mat, 3/9 by nordic walking and 1/9 with running treadmill, orienteerers with running treadmill, speed walkers with walking treadmill and runners with running treadmill. For developmental females and female rugby players the running treadmill was used. Before the test, participants were advised only to train light on previous day, but no restrictions to food or drink were given. RPE and lactate levels were measured during the test.

6.2.4 Body composition

In this study body composition was estimated by DXA (LUNAR Prodigy, GE Healthcare). The participants were measured in a fasting state and were positioned supine in the center of the table. They were scanned using the default scan mode for total body scanning automatically selected by the Prodigy software (enCORE 2005, version 9.30 and Advance 12.30). The system software provides the mass of lean soft tissue (LM), fat (FM), and bone minerals. Quality assurance was performed every measurement day with multipoint phantom. Precision of the repeated measurements expressed as the percent coefficient of variation was 2.2% for percentage of fat and 1.0% for LM in our laboratory. BMI values of each participant in this study were calculated manually by dividing the body mass in kilogrammes with the square meter of height in meters using the data of participants weight and height from NoREDS data.

6.3 Statistical methods

All statistical analyses were performed using the IBM SPSS Statistics version 28.0 software (Chicago, IL, USA). First the normality of distribution was evaluated for each variable in each group. Due to the small sample size the Shapiro–Wilk test was used. In addition, the normal Q-Q plots were evaluated and skewness and kurtosis were evaluated by dividing them by standard errors. It appeared that some of the variables were slightly not normally distributed. Due to this and the fact that sample sizes were small, non-parametrical tests were used for these variables. This consists of triglyceride and TC of endurance athletes, total LDL and non-HDL levels of all participants and BMI, total fat mass and Vo_{2max} of rugby players.

The Paired Samples T-test was used for the normally distributed variables to compare the differences in means between two timepoints. Data were presented as means and standard deviations (SD), mean differences and standard deviations, and confidence intervals of the means (95% CI). For abnormally distributed variables the Wilcoxon Signed-Rank test was used to compare the differences in medians between two timepoints. Data was presented in means and standard deviations and medians. Exact p-values and Monta Carlo's p-values were used to confirm the test results to reject or retain the null hypothesis. Null hypothesis was that there would not be differences within groups at two timepoints.

Because of the fact that sample sizes were quite small in all groups and included also abnormally distributed variables, the non-parametrical Kruskall-Wallis test was used to define if there was significant differences between the study groups variances of means of each variables at preparation period or at competition period. Null hypothesis was that the mean values of each variable do not differ between groups. Pairwise comparisons between groups were made if the test showed significant difference and in that case significance levels were adjusted by the Bonferroni correction for multiple tests. Eta squared ($ŋ^2$) was calculated separately based on the information from statistical analyses.

The associations of Vo_{2max} to AS and blood lipids and body composition to AS and lipids were evaluated using Spearman's correlation and was targeted to all participants. The significance value was set to <0.050 in all tests.

7 RESULTS

7.1 Changes in means and medians between preparation and competition period

7.1.1 Arterial stiffness

No significant changes in PWV were found in any of the study groups between the two timepoints. There were only small differences and the confidence intervals show that there were both positive and negative outcomes. (Table 3)

TABLE 3. Changes in PWV (m/s); Paired Samples T-Test.

Group	MeanP* ± SD***	MeanC** ± SD	Median*	Median**	Mean difference ± SD	95% CI****
Endurance	6.0 ± 0.6	6.0 ± 0.5	6.1	5.9	0.02 ± 0.35	-0.13 – 0.17
Developmental	6.6 ± 0.9	6.5 ± 0.6	6.5	6.6	0.12 ± 0.64	-0.56 – 0.80
Rugby	6.2 ± 0.4	6.2 ± 0.4	6.3	6.2	0.08 ± 0.46	-0.15 – 0.30

*preparation period, **competition period, ***SD=standard deviation, ****CI=confidence interval, of mean difference

7.1.2 Blood lipids

No significant changes in blood lipid levels were found in any of the study groups between the two timepoints. There were only small changes between two timepoints and the confidence intervals and ranks show both positive and negative outcomes, but no clear change to positive or negative. (Table 4 & 5)

TABLE 4. Changes in blood lipid levels; Paired Samples T-Test.

Group	MeanP* ± SD***	MeanC* ± SD	Median*	Median**	Mean difference ± SD	95% CI****
			TC (mmol/l)			
Developmental	3.7 ± 0.9	3.9 ± 0.9	3.6	3.8	-0.23 ± 0.35	-0.59 – 0.14
Rugby	4.0 ± 0.7	4.1 ± 0.5	4.1	3.6	-0.07 ± 0.88	-0.63 – 0.49
			HDL (mmol/l)			
Endurance	1.9 ± 0.4	1.9 ± 0.4	2.0	1.9	0.03 ± 0.27	-0.09 – 0.15
Developmental	1.6 ± 0.5	1.6 ± 0.5	1.5	1.6	-0.08 ± 0.14	-0.22 – 0.06
Rugby	1.7 ± 0.2	1.7 ± 0.3	0.6	0.8	-0.06 ± 0.24	-0.21 – 0.09
			TG (mmol/l)			
Developmental	0.8 ± 0.4	0.6 ± 0.2	0.8	0.6	0.16 ± 0.30	-0.15 – 0.47
Rugby	0.7 ± 0.2	0.8 ± 0.3	2.6	2.1	-0.09 ± 0.27	-0.26 – 0.08

*preparation period, **competition period, ***SD=standard deviation, ****CI=confidence interval, of mean difference

TABLE 5. Changes in blood lipid levels; Wilcoxon Signed Rank Test.

Group	MeanP* ± SD***	MeanC* ± SD	Median*	Median**	Ranks (n) Positive/Negative/No change
			TC (mmol/l)		
Endurance	4.8 ± 0.8	4.7 ± 1.1	4.9	4.5	9/13/1
			Non-HDL		
Endurance	2.9 ± 0,8	2.8 ± 1.0	2.9	2.8	9/14/0
Developmental	2.2 ± 0.6	2.3 ± 0.6	2.2	2.1	4/2/0
Rugby	2.4 ± 0.6	2.4 ± 0.6	2.3	2.2	6/6/0
			LDL (mmol/l)		
Endurance	2.6 ± 0.6	2.6 ± 0.9	2.8	2.5	12/11/0
Developmental	1.8 ± 0.4	2.1 ± 0.7	2.0	2.0	3/3/0
Rugby	2.3 ± 0.6	2.3 ± 0.6	1.6	1.7	7/5/0
			TG (mmol/l)		
Endurance	0.8 ± 0.2	0.9 ± 0.6	0.79	0.74	10/13/0

*preparation period, **competition period, ***SD=standard deviation

7.1.3 Vo_{2max}

No significant changes were found in Vo_{2max} in any of the study groups between the two timepoints. The confidence intervals and ranks show that the changes between two timepoints had both positive and negative outcomes. (Table 6)

TABLE 6. Changes in Vo_{2max} (ml/kg/min).

Paired Samples T-test						
Group	MeanP* ± SD***	MeanC** ± SD	Median*	Median**	Mean difference ± SD	95% CI****
Endurance	58.3 ± 6.5	59.3 ± 6.5	57.0	59.5	-1.08 ± 3.74	-2.70 – 0.54
Developmental	41.7 ± 2.8	42.7 ± 3.2	41.9	41.9	-1,05 ± 2,35	-3.52 – 1.42
Wilxocon Signed Rank Test						
	MeanP* ± SD***	MeanC** ± SD	Median*	Median**	Ranks (n) Positive/Negative/No change	
Rugby	43.2 ± 3.1	43.6 ± 2.9	44.1	43.9	7/5/0	

*preparation period, **competition period, ***SD=standard deviation, ****CI=confidence interval, of mean difference

7.1.4 Body composition

Tests showed no significant changes in total body weight, BMI, TFM, TLM or TTF, in any of the study groups between the two timepoints. There were only small changes between two timepoints and the confidence intervals and ranks show both positive and negative outcomes. (Table 7)

TABLE 7. Changes in body composition.

Paired Samples T-Test						
Group	MeanP* ± SD	MeanC* ± SD	Median*	Median**	Mean difference ± SD	95% CI****
Total body weight (kg)						
Endurance	61.6 ± 4.8	61.3 ± 4.7	62.0	61.1	0.29 ± 1.45	-0.34 – 0.92
Developmental	68.6 ± 3.6	68.5 ± 3.9	68.3	67.6	0.07 ± 1.50	-1.51 – 1.64
Rugby	69.5 ± 9.7	69.4 ± 9.0	69.0	68.4	0.07 ± 1.77	-1.05 – 1.19
BMI						
Endurance	21.1 ± 1.5	21.0 ± 4.7	21.2	21.2	0.12 ± 0.50	-0.10 – 0.33
Developmental	23.7 ± 1.7	23.7 ± 1.4	23.4	23.8	-0.01 ± 0.54	-0.57 – 0.56
TFM (kg)						
Endurance	10.5 ± 3.5	10.0 ± 3.9	10.3	9.9	0.44 ± 1.48	-0.20 – 1.09
Developmental	16.9 ± 4.4	17.5 ± 7.3	16.8	15.0	-0.66 ± 5.40	-6.33 – 5.01
TLM (kg)						
Endurance	48.4 ± 4.7	48.5 ± 4.7	48.4	48.2	-0.17 ± 1.54	-0.84 – 0.50
Developmental	48.8 ± 4.8	49.5 ± 3.9	49.4	50.1	-0.72 ± 1.61	-2.41 – 0.96
Rugby	48.9 ± 5.0	49.2 ± 5.0	48.7	49.3	-1.13 ± 1.34	-1.98 to -0.27
TTF%						
Endurance	17.8 ± 5.6	17.1 ± 6.0	16.6	15.5	0.71 ± 2.28	-0.28 – 1.70
Developmental	25.6 ± 6.5	24.3 ± 6.6	25.2	23.9	1.31 ± 2.54	-1.35 – 3.97
Rugby	27.0 ± 5.8	25.3 ± 5.7	25.8	24.3	1.61 ± 2.96	-0.27 – 3.49
Wilxocon Signed Rank Test						
	MeanP* ± SD	MeanC* ± SD	Median*	Median**	Ranks (n) Positive/Negative/No change	
BMI						
Rugby	24.8 ± 2.8	24.8 ± 2.5	24.0	24.0	7/5/0	
TFM (kg)						
Rugby	18.2 ± 6.1	17.0 ± 5.7	16.8	15.8	3/9/0	

*preparation period, **competition period, ***SD=standard deviation, ****CI=confidence interval, of mean difference

7.2 Differences between groups at two timepoints

7.2.1 Arterial stiffness

Endurance athletes' PWV was only slightly lower than the other groups and developmental females had the PWV highest levels. (Table 4; Figure 5) Only group that had small decrease in mean PWV was developmentals, but there were no significant differences between groups in both preparation period or competition period. Though, the means did not differ, the groups mean PWV levels come a little more similar in competition period, and in endurance athletes the individual differences were little bit higher than in other groups (Figure 7 & 8).

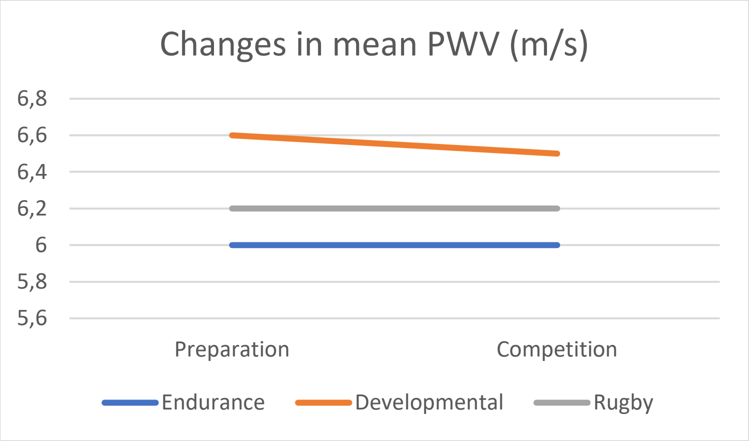

FIGURE 7. Changes in mean PWV between groups.

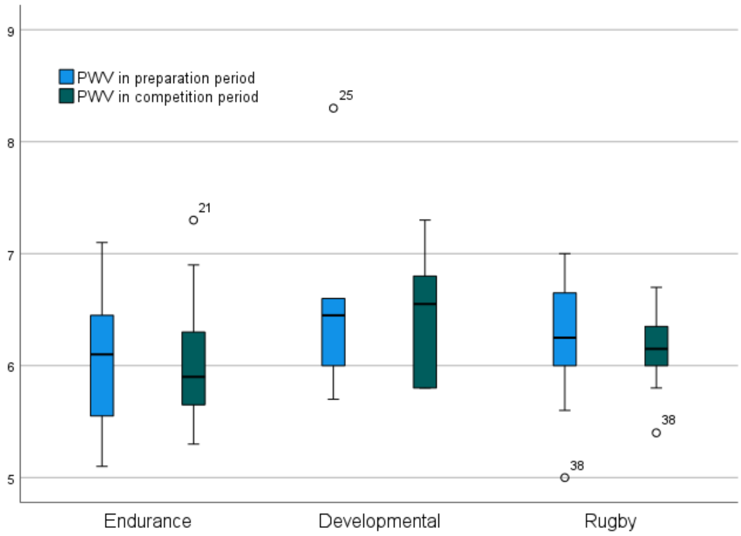

FIGURE 8. Variance of PWV between groups.

7.2.2 Blood lipids

Significant differences between groups in blood lipids were found in preparation period in TC (Kruskall-Wallis H = 11.327; degree of freedom 2, n = 41, Monte Carlo's p = 0.002, 99% confidence interval 0.001-0.003; ŋ^2 = 0.245), HDL (Kruskall-Wallis H = 6.967; degree of freedom 2, n = 41, Monte Carlo's p = 0.026, 99% confidence interval 0.022-0.031; ŋ^2 = 0.131) and LDL (Kruskall-Wallis H = 6.448; degree of freedom 2, n = 41, Monte Carlo's p = 0.034, 99% confidence interval 0.030-0.039; ŋ^2 = 0.117). The difference was between endurance athletes and developmentals and endurance athletes and rugby players. Developmentals' and rugby players' variance was similar. (Table 8) No significant differences were found in TG and non-HDL.

TABLE 8. Pairwise comparisons of mean TC, HDL and LDL between groups in preparation period.

Pair	Std. Test Statistics	p/adjusted p, 2-sided
	TC	
END-DEV	2.713	**<0.007*/0.020***
END-RUG	2.642	**<0.008*/0.025***
	HDL	
END-DEV	2.188	**0.029***/0.086
END-RUG	2.006	**0.045***/0.135
	LDL	
END-DEV	2.351	**0.019***/0.056

Abbreviations: END = endurance athletes; DEV = developmentals; RUG=rugby players. *Significally different folded.

Endurance athletes had the highest TC, HDL and LDL levels and just slightly higher TG levels than the other groups. (Table 6; Figure 7) Even though the differences in means within groups were very small, there is shown that the mean values of TC and LDL come slightly more similar in competition period and mean TG becomes slightly less similar in competition period (Figure 9).

FIGURE 9. Changes in mean blood lipid levels between groups.

Endurance athletes had more individual variance in TC than other groups. In HDL endurance athletes and developmentals had more individual variance than rugby players and in LDL endurance athletes and rugby players more than developmentals. In TG the groups’ variances were quite similar. (Figure 10)

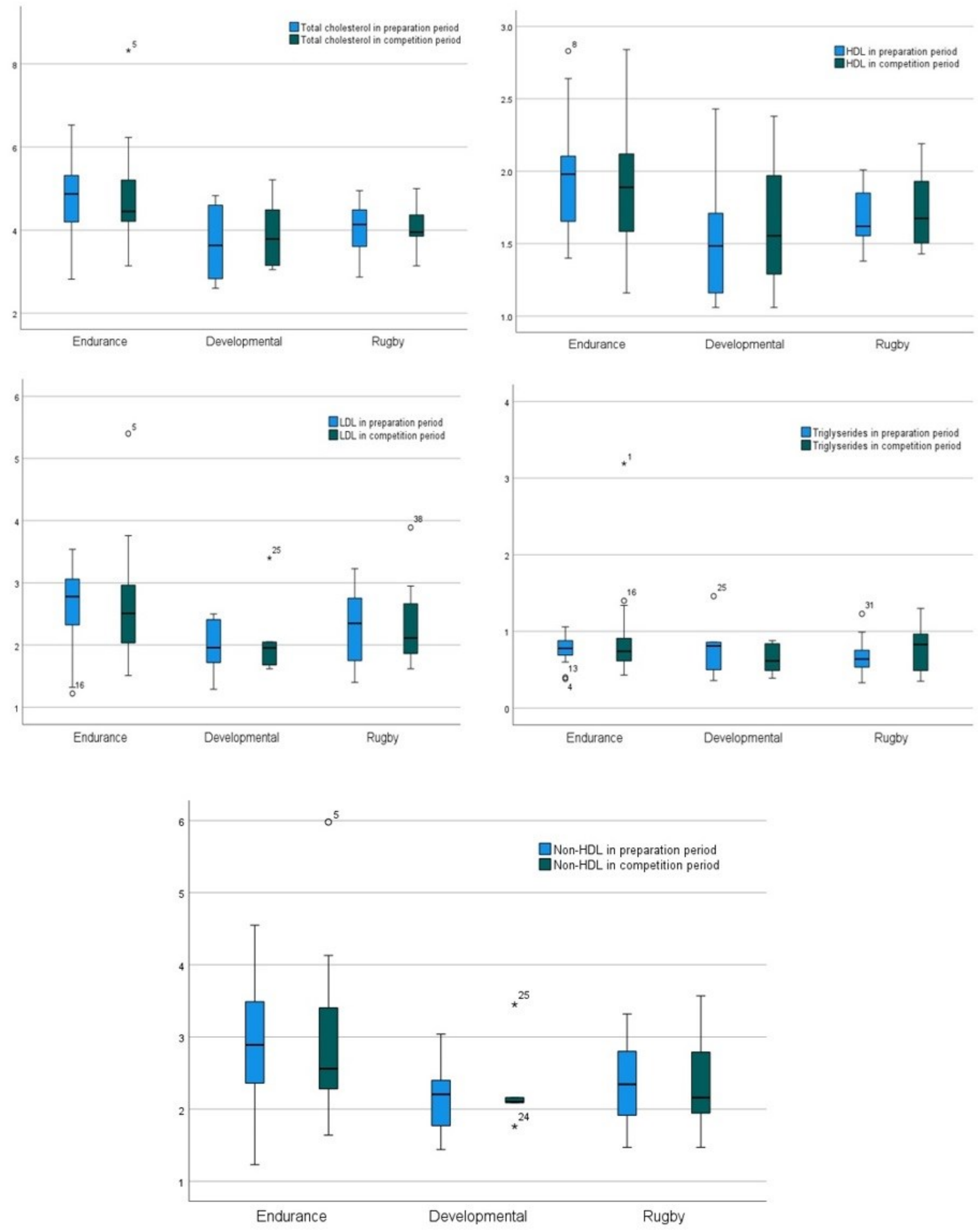

FIGURE 10. Variances of blood lipid levels between groups.

7.2.3 Vo_{2max}

Significant differences in variance of mean Vo_{2max} between endurance athletes and developmentals and endurance athletes and rugby players were found in both preparation period (Kruskall-Wallis H = 29.979; degree of freedom 2, n = 41, Monte Carlo's p = 0.000, 99% confidence interval 0.000-0.000; $\eta^2 = 0.736$) and competition period (Kruskall-Wallis H = 26.819; degree of freedom 2, n = 41, Monte Carlo's p = 0.000, 99% confidence interval 0.000-0.000; $\eta^2 = 0.653$). (Table 9)

TABLE 9. Pairwise comparisons of mean Vo_{2max} between groups.

Pair	Preparation period		Competition period	
	Std. Test Statistics	p/adjusted p, 2sided	Std. Test Statistics	p/adjusted p, 2-sided
END-DEV	4.189	**<0.001*/0.000***	3.833	**<0.001*/0.000***
END-RUG	4.514	**<0.001*/0.000***	4.377	**<0.001*/0.000***

Abbreviations: END = endurance athletes; DEV = developmentals; RUG=rugby players. *Significally different folded.

In all groups there was only small increasement in mean Vo_{2max}. Endurance athletes had the highest Vo_{2max} values and developmental females the lowest. Endurance athletes' group had also more individual variance, but their group had also more participants. (Table 9; Figure 11 & 12)

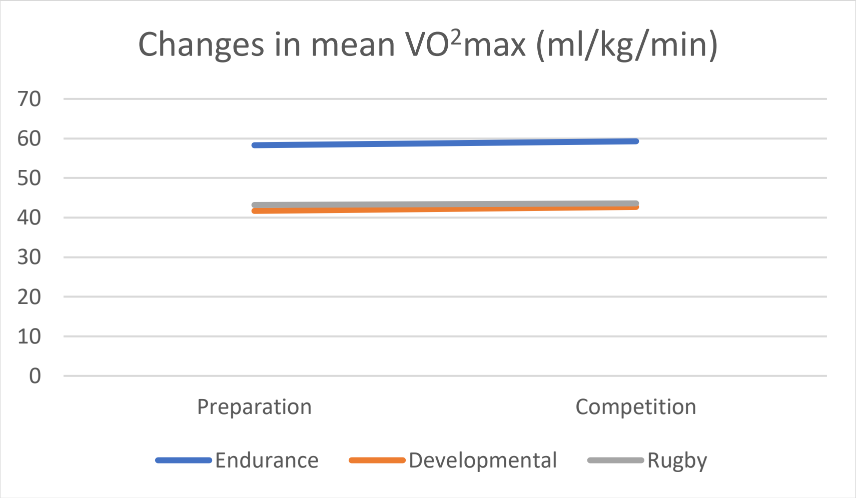

FIGURE 11. Changes in mean Vo_{2max} between groups.

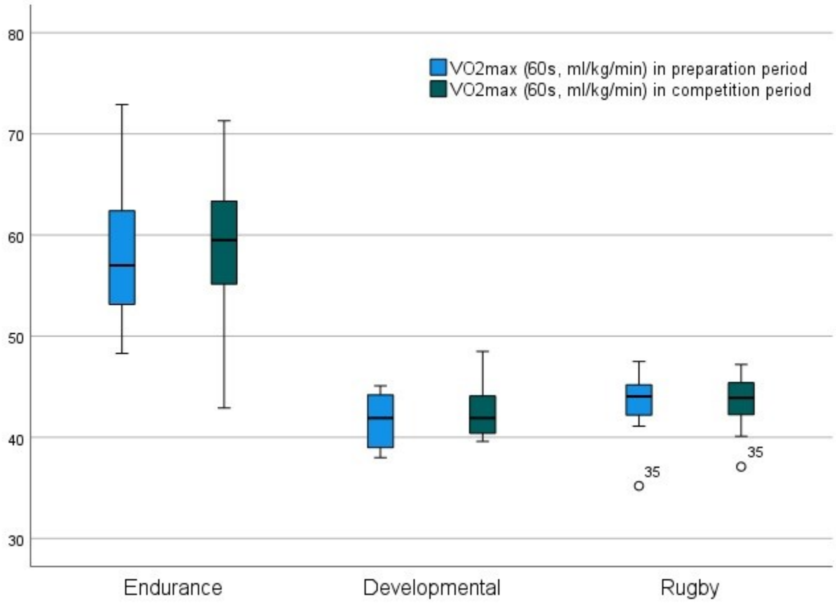

FIGURE 12. Variance of Vo$_{2max}$ between groups.

7.2.4 Body composition

Significant differences between groups were found in mean total body weight in preparation period (Kruskall-Wallis H = 10.431; degree of freedom 2, n = 41, Monte Carlo's p = 0.002, 99% confidence interval 0.001-0.004; $\eta^2 = 0.222$) and competition period (Kruskall-Wallis H = 12.273; degree of freedom 2, n = 41, Monte Carlo's p = <0.001, 99% confidence interval 0.000-0.001; $\eta^2 = 0.27$); BMI in preparation period (Kruskall-Wallis H = 18.963; degree of freedom 2, n = 41, Monte Carlo's p = 0.000, 99% confidence interval 0.000-0.000; $\eta^2 = 0.446$) and competition period (Kruskall-Wallis H = 23.099; degree of freedom 2, n = 41, Monte Carlo's p = 0.000, 99% confidence interval 0.000-0.000; $\eta^2 = 0.555$); TFM in preparation period (Kruskall-Wallis H = 17.486; degree of freedom 2, n = 41, Monte Carlo's p = 0.000, 99% confidence interval 0.000-0.000; $\eta^2 = 0.408$) and competition period (Kruskall-Wallis H = 15.737; degree of freedom 2, n = 41, Monte Carlo's p = <0.001, 99% confidence interval 0.000-0.001; $\eta^2 = 0.362$), and in TTF% in preparation period (Kruskall-Wallis H = 14.521; degree of freedom 2, n = 41, Monte Carlo's p = <0.001, 99% confidence interval 0.000-0.000; $\eta^2 = 0.33$) and in competition period (Kruskall-Wallis H = 11.921; degree of freedom 2, n = 41, Monte Carlo's p = <0.001, 99% confidence interval 0.000-0.002; $\eta^2 = 0.261$). The biggest differences in all these were between endurance athletes and rugby players. (Table 10; Figure 13) No significant difference between groups were found in TLM in both preparation and competition period.

TABLE 10. Pairwise comparisons of mean total body weight, BMI, TFM and TTF% between groups.

Pair	Preparation period		Competition period	
	Std. Test Statistics	p/adjusted p, 2-sided	Std. Test Statistics	p/adjusted p, 2-sided
Total body weight				
END-DEV	-2.597	**0.009*/0.028***	-2.673	**0.008*/0.023***
END-RUG	-2.542	**0.011*/0.033***	-2.894	**0.004*/0.011***
BMI				
END-DEV	-2.672	**0.008*/0.023***	-2.928	**0.003*/0.010***
END-RUG	-4.026	**<0.001*/0.000***	-4.453	**<0.001*/0.000**
TFM				
END-DEV	-2.731	**0.006*/0.019***	-2.713	**0.007*/0.020***
END-RUG	-3.780	**<0.001*/0.000**	-3.512	**<0.001*/0.001**
TTF%				
END-DEV	-2.263	**0.024***/0.071	-2.160	**0.031***/0.092
END-RUG	-3.558	**<0.001*/0.001***	-3.171	**0.002*/0.005***

Abbreviations: END = endurance athletes; DEV = developmentals; RUG=rugby players. *Significantly different folded.

Endurance athletes mean total body weight & BMI was lower than the other groups. Rugby players had the highest total body weight and BMI. Though, the changes within groups were not significant, there can be seen small changes in TFM, TLM and TTF%. There was small improvement in TLM in all of these groups and in developmentals the change is the biggest. In competition period the groups' TLM values come a little bit more similar than in preparation period. TTF% of all groups decreased slightly, while TFM slightly reduced in endurance athletes and rugby players, and total body weight and BMI remained almost the same. (Table 10; Figure 13)

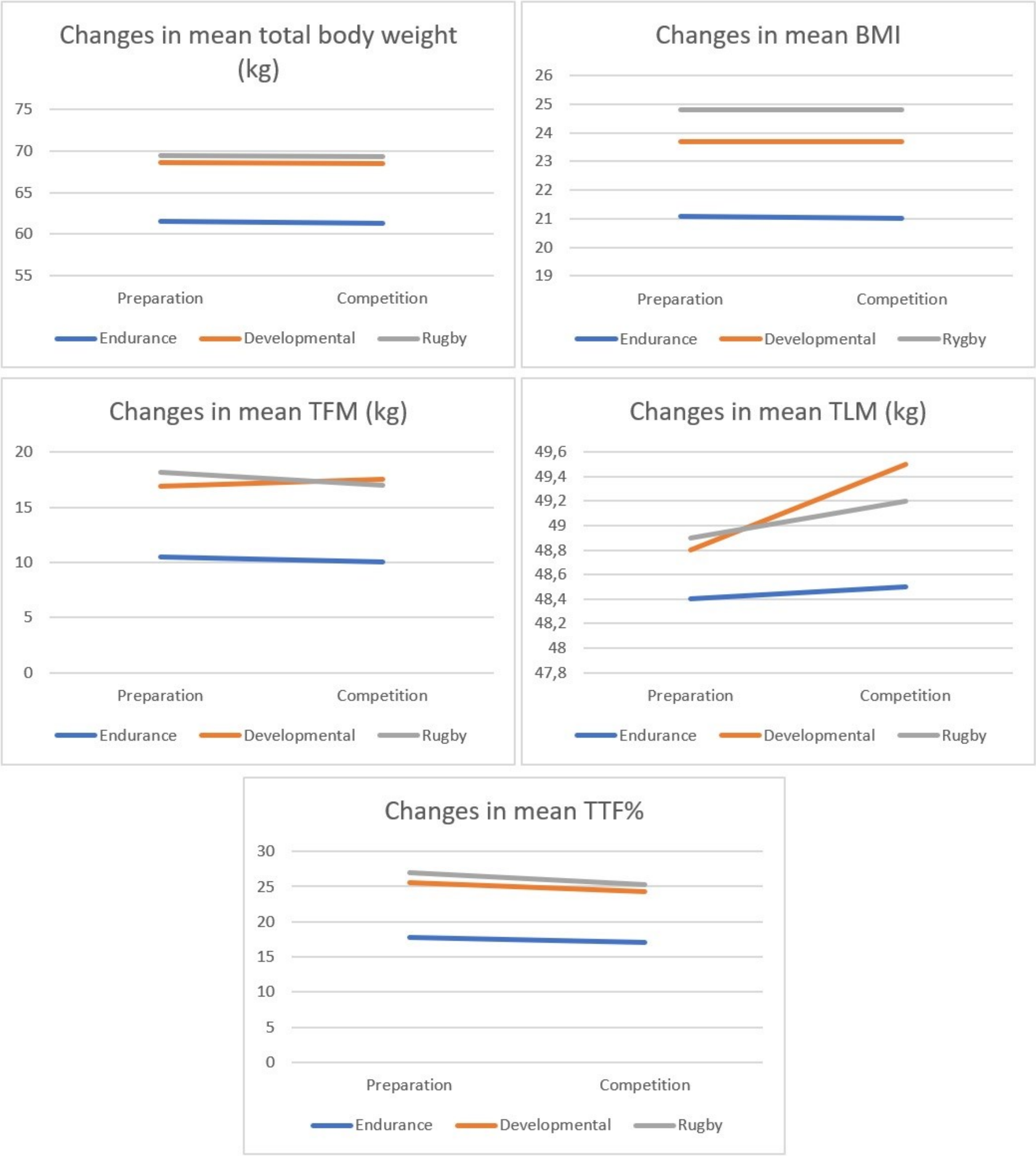

FIGURE 13. Changes in body composition.

Individual variance is seen also in body composition. Rugby players have more individual variance especially in total body weight and BMI. The variance in BMI comes smaller in competition period. (Figure 14)

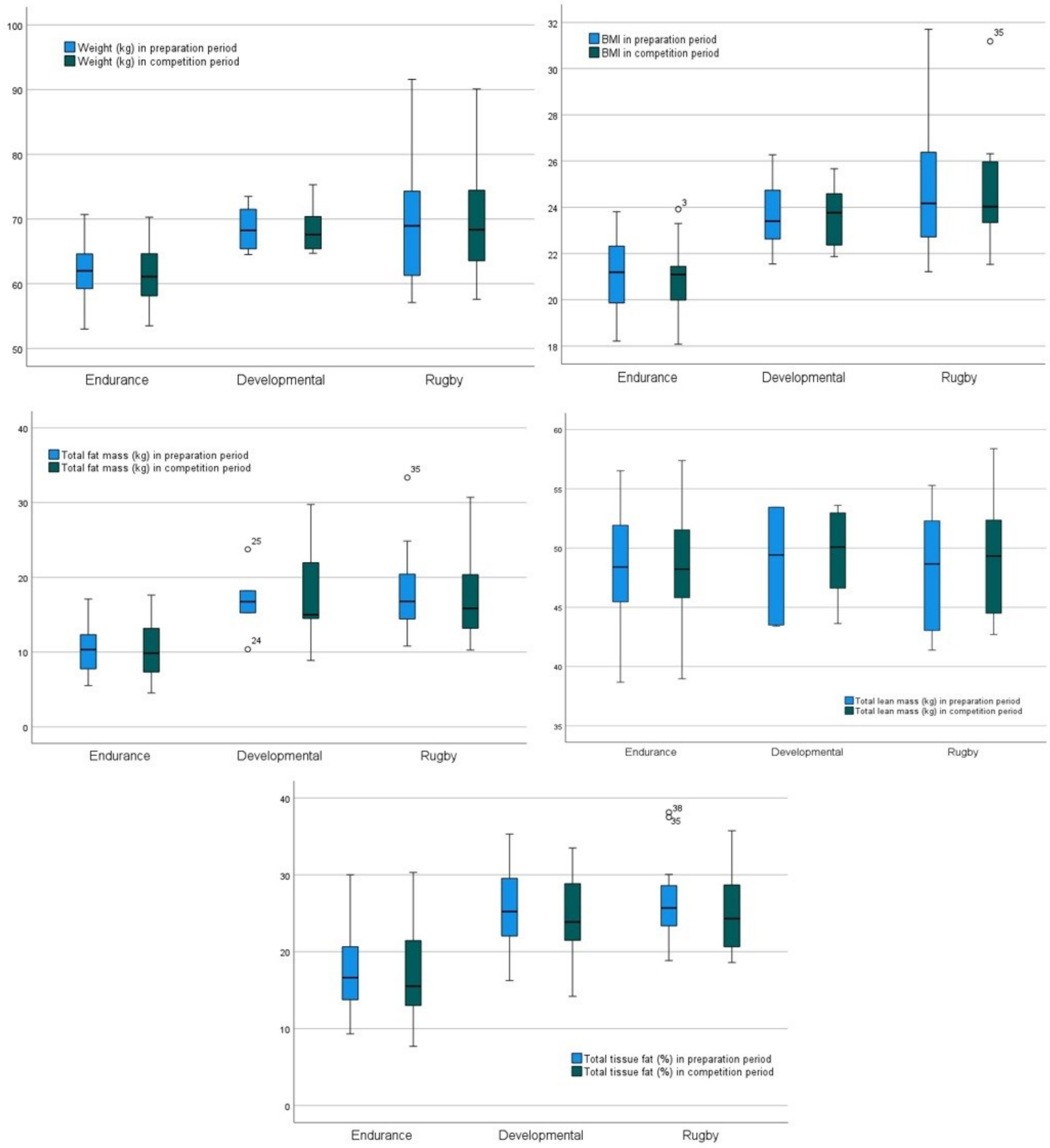

FIGURE 14. Variances of changes in body composition.

7.3 Associations of physical performance and body consumption to arterial stiffness and blood lipid levels

Possible associations of physical performance and AS or blood lipid levels and associations of body composition and AS or blood lipid levels were evaluated in the total study participants (n=41) since they were healthy, normal-weighted and their PWV and blood lipid levels were within the recommendations.

7.3.1 Vo$_{2max}$ and associations to PWV and blood lipid levels

Significant relations between Vo$_{2max}$ and PWV were not found on preparation period or competition period when data of all study participants together was evaluated. Vo$_{2max}$ had significant positive, but only low to moderate relation to some blood lipid levels in preparation period but not in competition period. In the preparation period there was a significant low to moderate positive relations to TC (r = 0,33, p = 0.035, two-tailed) and to HDL (r = 0.326, p = 0.037, two-tailed). Study participants with higher Vo$_{2max}$ had higher TC and HDL levels in preparation period. (Figure 15)

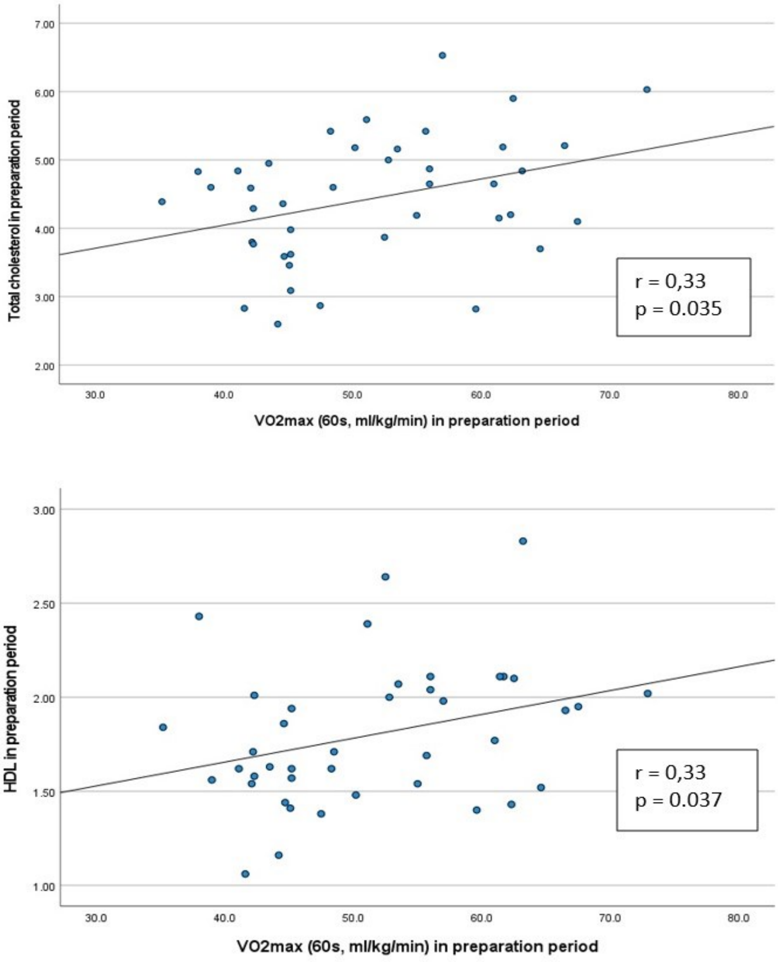

FIGURE 15. Positive relation of Vo$_{2max}$ and TC and HDL levels in preparation period (n=41).

7.3.2 Body composition and associations to PWV and blood lipid levels

Body composition's variables showed no significant relations to PWV on preparation period or competition period. Significant negative, but only low to moderate relations to blood lipid levels were found only with total body weight, BMI and TLM in both preparation and competition period. Total body weight was significantly negatively related to non-HDL in preparation period (r = -0.35, p = 0.025, two-tailed) and in competition period (r = -0,34, p = 0.028, two-tailed). BMI was significantly negatively related to TC (r = -0.32, p = 0.040, two-tailed) and HDL (r = -0.33, p = 0.034, two-tailed) in preparation period and in to TC (r = -0,33, p = 0.038, two-tailed) in competition period. Study participants who had higher total body weight had lower non-HDL in both timepoints and participants who had higher BMI had lower TC levels in both timepoints but also lower HDL levels in preparation period. (Figure 16)

TLM was significantly negatively related to non-HDL in preparation period (r = -0,38, p= 0.014, two-tailed) and in competition period (r = -0,52, p = <0.001). TLM was also significantly negatively moderately related to LDL in preparation period (r = -0.40, p = 0.009, two-tailed) and to TC (r = -0.46 , p = 0.003, two-tailed), TG (r = -0.39, p = 0.013, two-tailed) and LDL (r = -0.52, p = <0.001, two-tailed) in competition period. Study participants who had higher TLM had lower non-HDL and LDL in both timepoints and lower TC and TG levels in competition period. (Figure 17)

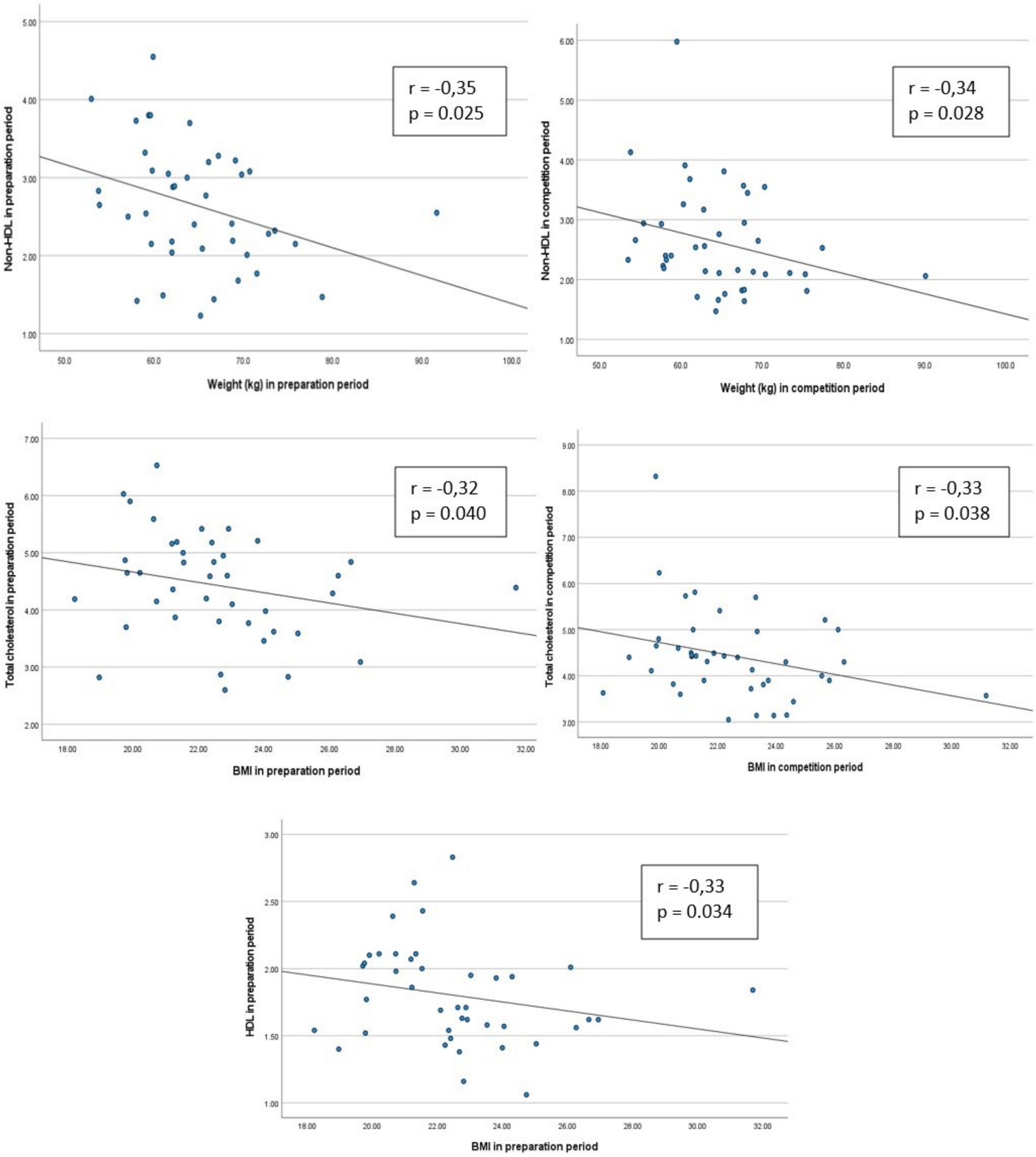

FIGURE 16. Negative relations of total body weight and BMI with blood lipids (n=41).

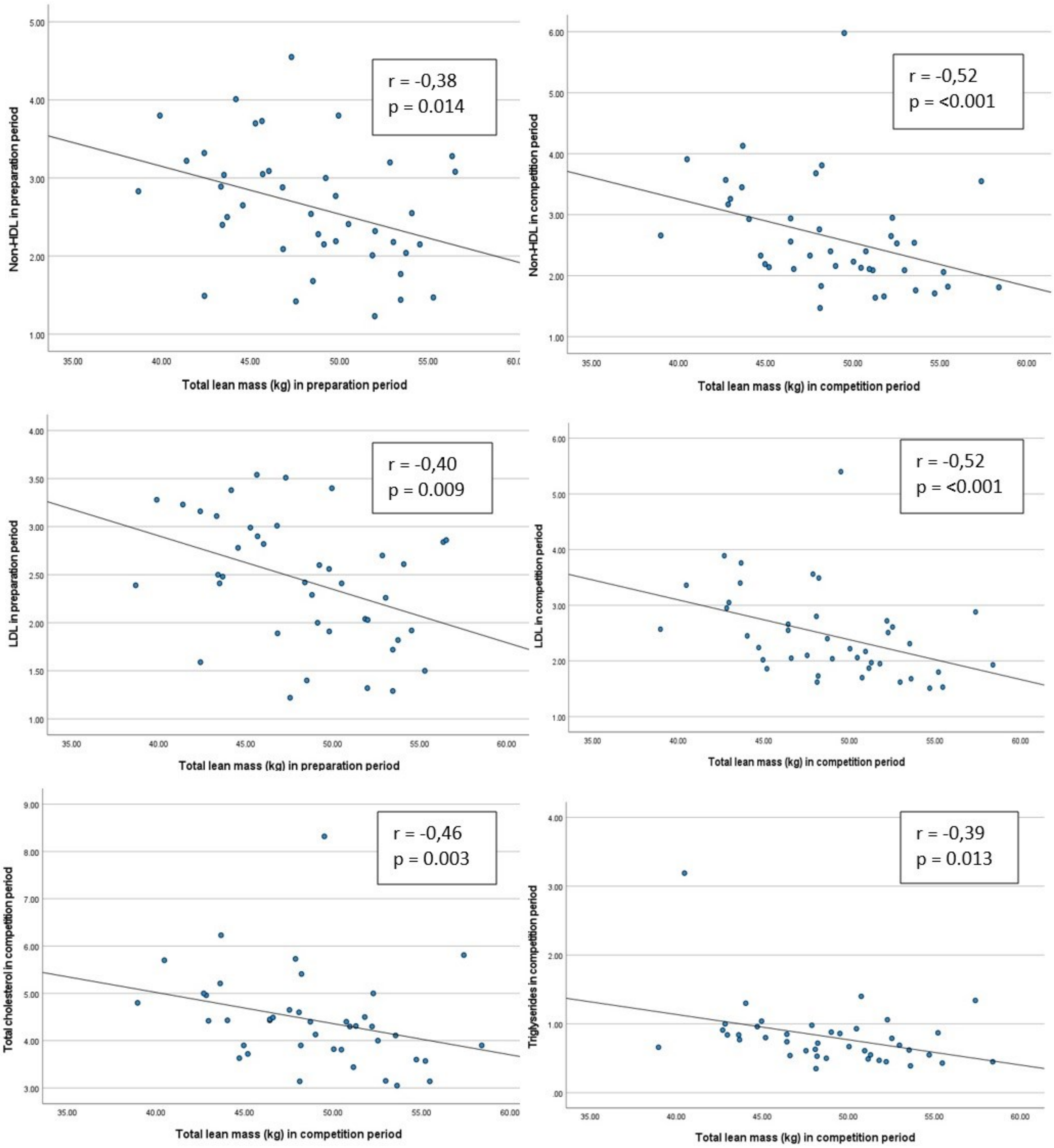

FIGURE 17. Negative relations of TLM and blood lipids.

8 DISCUSSION

In this section the ethical considerations in this thesis and the limitations and reliability of this thesis' study methods and results are discussed. The main results of this study and the possible conclusions for future research are discussed with the research found in the literature review.

8.1 Main results

In this study the participants were healthy without any cardiovascular outcomes. This study showed no significant changes between the two timepoints in means and medians in any of the outcomes, but there was a lot of variance between individuals. Different to this study, previous studies have shown that between different training periods and towards competition period Vo_{2max} increases but this change could be stronger in non-elite athlete level (Trinshek et al. 2020). In competition period endurance athletes have higher body mass and FFM (Durcalek-Michalski et al. 2022, Heydenreich et al. 2017 & Trinshek et al. 2020) but there is lack of longitudinal studies about the fluctuations between different training periods among endurance athletes and this study found no evidence to add to previous knowledge. This chapter discusses the main cardiovascular outcomes in this thesis; PWV and blood lipids, but focuses more on the significant findings in this study; group differences in blood lipids, Vo_{2max} and body composition and the associations found in blood lipids with body composition.

8.1.1 PWV and associations with Vo_{2max} and body composition

The mean PWV levels of each group in this study were in the normal range of 4,7-7,6 recommendation for under 30 years old healthy population (Boutouyrie et al. 2010) In the previous studies participants were also healthy, but some studies had little bit younger participants than this study. Similar to most of the earlier studies comparing endurance athletes to less active control groups there was no significant differences in PWV values between groups in this study. Previously only Tomschi et al. (2021) and Otsuki et al. (2007) studies showed lower PWV values for endurance athletes than the less active control groups. In two studies endurance athletes had lower PWV values than strength training athletes (Otsuki et al. 2007 & Tomschi et al. 2021).

Significant associations of neither higher Vo_{2max} or favorable body composition with PWV was not found on this study. Endurance athletes had the highest mean Vo_{2max} values but their mean PWV was only slightly lower than the other groups'. Developmental females had the highest PWV values and there was a reduction towards competition period. Because of the fact that that their Vo_{2max} was similar to rugby players and no significant improvement was seen in neither of these groups it is impossible to say if this change in their PWV occurred because of improvement in physical performance. All participants were healthy young adults within the recommended PWV values, and this could be the reason why significant differences or associations with physical performance were not found. Participants were also healthy in all previous studies, but the results varied. For associations of PWV with higher physical performance the results of this study are similar only with Namgoong et al. (2018) study. Significant relations of lower AS with higher CRF were found in most of the studies made to healthy adults (Boreham et al. 2004, Fernberg et al. 2017, Gando et al. 2016 & Haapala et al. 2022) and in one study made to endurance athletes (Hashimoto & Okamoto 2023). It is possible that this study had more homogenic groups than these previous studies that showed stronger associations.

In body composition the group differences suggest that endurance athletes had more favourable body composition than other groups, but developmental females and rugby players also were in the range of normalweight according to BMI. Previously Budimir et al. (2012), Fernberg et al. (2017), Haapala et al. (2022) & Wildman et al. (2003) showed significant relations of PWV with body composition. Opposite to these and similar to this study, among endurance athletes these associations were not found in Bjärnegård et al. (2015) and Augustine et al. (2015) studies which also had only female participants similar to this study. Even though the participants were healthy in all of the studies, the results varied within the studies made not only to endurance athletes but also within the studies made to healthy adults with no competing or high physical activity level reported. Only Haapala et al. (2022) reported the physical activity levels of participants like in the studies committed among endurance athletes.

Koshiba & Maeshima (2015) had committed the only longitudinal study to endurance athletes and showed that detraining increased PWV but this was not seen until three months of detraining had occured. This study compared PWV levels between preparatory and competition period and it could be possible that there is more change if measurements were made after competition period in the end of transition period (detraining) and compared to multiple timepoints. In this study the participants were physically active in all groups, and it must be noted that there could be bigger changes and differences if endurance athletes were compared to less active populations than in this study. There might be also differences and stronger associations depending on the sports type if strength training would have been compared.

This study did not provide new evidence to strengthen or weaken the evidence of previous studies about the changes of PWV in different training periods or associations with better physical performance or body composition and the role of endurance training as a beneficial training type for maintaining favorable PWV values for preventing CVDs remains unclear. In some of the previous studies their results show that better physical performance or body composition can associate beneficially with PWV in healthy adults.

Among endurance athletes there is need for future research and when studying females' hormonal issues and the possibility of amenorrhea and RED-S should be considered. In the previous studies made to female endurance athletes only Aubergine et al. (2015) had amenorrheic athletes as a subgroup, but their study found no differences in PWV between eumenorrheic and amenorrheic participants. All previous studies did not take the phase of menstrual cycle, hormonal disturbance, or use of contraceptives into account when measuring PWV. Because of the facts that despite the level of physical activity, unfavorable changes in endothelial function and increased vascular tone through hypoestrogenism and amenorrhea can be associated to early atherosclerosis already in young athletes and premenopausal physically active women (Mountjoy et al. 2018, O'Donnell et al. 2009 & 2011, Rickenlund et al. 2005, Pradhushankar et al. 2014, Rickenlund et al. 2005 & Shufelt et al. 2017) future research should include information of participants' hormonal and nutritional issues to gain evidence of their possible effects on cardiovascular health.

8.1.2 Blood lipids and associations with Vo$_{2\text{max}}$ and body composition

In this study, the mean blood lipid levels of each group were also in the range of normal values of Finnish recommendations (Duodecim 2022) and no significant changes between the two timepoints were found. In most of all the studies found in the review, blood lipid measurements were made as a baseline measurement to participants and the groups were similar at baseline. Like described previously, moderate endurance training adaptations lead to HDL increases while decreased levels of LDL and TG may need training adaptations from more higher intensity exercises like interval training (Duodecim 2022, Fikenzer et al. 2014 & Mann et al. 2014). The training intensity changes in the preparatory period for competitions (Bomba & Puzzichelli 2018, 166 & 168 & McArdle et al. 2015, 472-474) and this could suggest that the LDL levels could be more favorable towards the competition period.

Only three studies comparing endurance athletes to less active groups showed similar significancy for better HDL (Eisenmann et al. 2001, Kyte et al. 2022 & Mitsusono & Ube 2006) and TG (Mitsusono & Ube 2006) in the endurance athletes' group. Ormsbree's & Arciero's (2012) longitudinal study showed that after 5 weeks detraining phase after competition period TC, HDL, and LDL had remained unchanged and TGs increased. These results strengthen the hypothesis that endurance athletes do not have a superior blood lipid profile compared to normal population despite HDL cholesterol, but detraining might lead to unfavorable changes also among healthy athletes. Unfortunately, Omsbree's & Arciero's (2012) study was made among swimmers and the transition phase after competition was longer than usually described for endurance athletes (Bompa & Puzzichelli 2018, 174 & Ohtonen & Mikkola 2016) so it might not be eligible enough to use their results as comparison.

Similar to previous studies, in this study HDL of endurance athletes was also higher than in the other groups, but different to earlier studies, TC and LDL were higher among endurance athletes. These differences were significant only in preparation period and there was seen that towards competition period the groups became more similar. In this study there was small increase in Vo_{2max} towards competition period in all groups. When it comes to periodization and more favourable lipid profile through enhancement of physical performance, there is very little evidence from previous studies. Previously only Namgoong et al. (2018) study showed associations of higher Vo_{2max} with blood lipids. Different to this study, their study showed that participants with better Vo_{2max} had lower TG levels. In this study participants with higher Vo_{2max} had higher HDL and TC, but these associations were seen only in preparation period and they were only low to moderate. It is possible that there is some dietary changes towards competition period that have a bigger impact on blood lipids than the modifications in training.

Even though there were no significant changes in body composition towards competition period, and total body weight and BMI remained similar in all groups, small changes in TFM, TTF% and TLM was seen. Towards competition period TFM decreased in endurance athletes and rugby players and TTF% decreased in all groups. There was small increase in TLM in all groups and the largest increase was observed in developmental females. Previous studies made to endurance athletes about the associations of body composition and blood lipids were not found in the literature review. In this study there was significant associations found in non-HDL with total body weight and TLM, TC and HDL with BMI and LDL, TC and TG with TLM. Study participants who had higher total body weight had lower non-HDL in both training periods and participants who had higher BMI had lower TC levels in both timepoints but also lower HDL levels in preparation period.

TLM had more associations with blood lipids and the correlations were slightly stronger than the ones with total body weight and BMI (Figures 16 & 17). From these results it could be suggested that body composition might have a bigger role than physical performance in maintaining favourable blood lipid profile in preventing cardiovascular health but the periodization of training in endurance sports does not seem to effect these levels significantly. Yet, like in PWV, there might be larger differences in blood lipid levels if the measurements would have made in multiple timepoints and not only in preparation period and competition period.

In this study smaller TFM or TTF% did not associate to better blood lipid levels but TLM did. This might suggest that if gaining and maintaining muscle mass is important in preventing cardiovascular outcomes even in the normalweight and physically active population, despite the body's fat status, it must be even more important for those with extra fat tissue and perhaps also overweight population could maintain satisfying blood lipid levels more easily with sufficient muscle mass. Although, it must be noted that for overweight population dietary and nutritional issues have more impact on blood lipid levels than endurance type training (Duodecim 2022, Fikenzer et al. 2018 & Mann et al. 2014). In addition, similar to studies of PWV, especially among females, hormonal and nutritional issues should be included into studies about blood lipids also hence hypoestrogenism can lead to increased levels of LDL, TC and TG (Grundy et al. 2018 & O'Donnell et al. 2011, Rickenlund et al. 2005 & Vishakha 2019). In this study endurance athletes had the smallest

TTF% and they had the highest TC, HDL and LDL levels and little higher TG levels than the other groups. Unfortunately, there was no information available about the menstrual and hormonal status of all participants. Future research is needed to gain more knowledge and evidence of possible harmful effects to blood lipids among athletes with low estrogen levels, amenorrhea or RED-S.

8.2 Ethical considerations

In this thesis the search process, study methods and results from the literature review and this thesis are described as accurate as possible without distortion, and the ethical considerations and rules of research integrity (Tutkimuseettinen lautakunta 2023) were utilized during the process. When using study data prospectively, considerations about the study methods and their effects to study participants used in the original study can be made even though the prospective design does not cause any new disturbance to participants physical integrity. In No-Reds the physical integrity of participants was disturbed by health measurements like taking blood samples, causing physical stress by testing their physical performance and predisposing them to radiation by using DXA as measurement of their body composition. The participants in No-RED-S study had given informed consent before the enrollment of the study and were able to discontinue the participation if they wanted. No-RED-S was approved by the Ethics Committee of the University of Jyväskylä. Participants had also given the information that they have a right to know who and how their personal data is used and the information how to get access to this information.

Before receiving the study data for this thesis a written agreement about the data protection, data management and reporting and delivering the results of this thesis was made with the author of this thesis and Jyväskylän yliopisto and the lead researcher of No-RED-S study. After the agreement the study data was minimized and pseudonymized before it was received for this thesis. No personal information and only the data relevant for this thesis' study questions was delivered with encrypted methods (secured email). During the process data was not given to anyone else and after the process the saved data and results was delivered back to the head researcher of No-RED-S study to be exploited in the future if needed. Considerations about reporting the information and results in this thesis were made during the process hence part of the participants were endurance athletes from a rather small country where there are not so many international and national level athletes. Even with no personal data received, this thesis includes measurements of participants' anthropometry and information about the sports type. When combined to age, this information could have risks of participants being recognized even without the information of the athlete's home city or sports. For this reason, no specific information of single individuals' measurements with age and the type of sport are not included in the report of results in this thesis.

Ethical considerations were made also in the studies found in the literature review. Participants consent to study participation was reported in all articles and all selected studies were approved by ethical committee. It was reported that there were no funding sources or conflicts of interest that would have affected the original authors' interpretations of the results.

8.3 Limitations and reliability

When using study data prospectively it is not possible to manipulate the way of measurements made in the original study and it is inpossible to know if any clinical or technician based errors could have occurred. All measurements for the outcomes of interest in this thesis in No-RED-S were traditional standardized measurements. No limitations were seen in the standardization protocols in measurements of blood lipids and body composition, but some small limitations were seen in measurements of PWV and Vo_{2max}.

Blood samples and DXA measurements were reported to be made in the morning in a fasted state and according to the manufacturers' instructions. For body composition measurements the positioning and details of the DXA machine and software and analysing protocols were reported as recommended (Nana et al. 2014). For initial blood lipid testing it is accepted also to take blood samples in a non-fasted state if there are not additional laboratory tests that require fasting. Consideration of taking a fasted test should be made if TG concentration is > 5 mmol/l (Nordesgaardt et al. 2016) but TG levels this high was not found on participants of this study.

Laurent et al. (2006) & Townsend et al. (2015) have given recommendations for minimizing confounding factors when measuring PWV (Table 11). In No-RED-S all measurements were reported to be made in the morning and according to these recommendations except for the recommendation of menstrual cycle phase. This was also the situation in the previous studies assessing PWV where the measurements from female participants were not made in the similar phase of menstrual cycle. The arteriograph device used in No-RED-S defines PWV from a single point and the measurement is based on algorithms and estimates for which they are often criticized compared to measurements from multiple points (Townsend et al. 2015). In this thesis this could be seen only as a small limit since all participants were healthy and the PWV of all participants was in the range of Boutouyrie et al. (2010) general recommendations of the age group.

TABLE 11. Recommendations for standardization of participant's conditions when measuring PWV (Adapted and modified from Laurent et al. 2006 & Townsend et al. 2015)

Confounding factor	**In practice**
Room temperature	Controlled environment kept at 22±1°C
Rest	At least 10 min in recumbent position
Vigorous physical activity	Refrain at least 12 hours before measurements
Time of the day	Similar time of the day for repeated measurements, for menstruating women measurements should be made in a similar menstrual cycle phase
Smoking, eating	Participants have to refrain, for at least 2-4 h before measurements, particularly from drinking beverages containing caffeine and large meals
Alcohol	Refrain from drinking alcohol 10 h before measurements
Position	Supine position is preferred, position (supine, sitting) should be mentioned
White coat effect	Influence on blood pressure and pressure-dependent stiffness
Cardiac arrythmia	Be aware of possible disturbance

The measurement of Vo_{2max} was made with a reasonable method for endurance sports and mostly according to the athletes main sport. For cross-country skiers it is recommended to test Vo_{2max} in a ski mat (McArdle et al. 2015, 165 & 236-238) but only 5/9 was tested with skiing mat. This was because the measurements were made in two different test labs and the ski mat was not always available. For the validity of the test also assessments of perceived exortion (RPE) and blood lactate concentration were made during the test as recommended (Beltz et al. 2016). It is possible that the food and fluid intake and previous exercises effect the physical performance of the test day (Beltz et al. 2016 & McArdle et al 2016, 238 & 482-483). For some reason there was no information given for the participants of No-RED-S about rest or the intensity of previous exercises and fluid and food intake. It is possible that because of this there could be some results that define the participants' physical performance in a skewed way.

Another limitation in this thesis in defining Vo_{2max} is that it was evaluated according to the individual's total body weight even though it should be evaluated according to muscle mass or lean mass (Krachler et al. 2015). Endurance athletes in No-RED-S were reported to represent McKayS's et al. (2022) level 4-5 "Elite/international level" and "World class" participants but the results showed some overlapping with the Vo_{2max} levels of endurance athletes and the other groups. This might occur because of the limitation of not adjusting Vo_{2max} to lean body mass or just some unexpected incidents in the study participants and their preparation to the test, or changes in their usual training cycle. In addition to the measurements, the time between measurements was shorter among endurance athletes (3-4 months) and longer in the other groups (6-7 months). For example, it is always possible that the measurements in competition season, meant to be taken when the participants should have their best performance were not timed properly. This can either make the possible differences smaller or larger both for the individuals but also for the differences between groups. Also, there was no information about the absolute amount of training hours, volume and intensity of training in different groups which can lead to suspicion of a more homogenic sample than would have been preferred for conducting an eligible comparison between the groups.

Naturally, the small amount of study participants is also a limit in this thesis. During the thesis process and because of it's time limit, some participants had to be excluded due to the lack of measurement data. No-RED-S is an on-going study and with enough time you could hypothesize to have more eligible study participants included. The small number of participants also weakens the power of the statistical tests made hence the nonparametric methods can be seen less powerful. In this thesis the use of non-parametric tests is justified because of the number of participants and the fact that some variables were not normally distributed. Naturally, statistical calculation errors can also occur even if multiple checkups have been made.

In addition, also considerations of the quality and reliability of the previous studies found in literature search and their comparability to this thesis can be discussed. The quality of previous studies found in the literature review were assessed separately with suitable critical appraisal tools for each study design. Within discussion of limitations and reliability were discussed in all of them. If there were aspects that might decrease the quality of the study, they are listed in the appendices at the end of this thesis (Appendix 1-3). It appeared that studies among endurance athletes assessing PWV, blood lipids, body composition and physical performance and the associations between them are not widely made, especially longitudinally. Naturally, it is possible that the exclusion and inclusion criteria was too strict or even more search phrases could have been used. Some articles were eligible according to title and abstract, but they could not be taken into account because there was no full-text available in English.

Finally, this thesis does not take account any other possible confounding factors like dietary issues or nutritional status, psychological stress, hormonal contraceptives or menstrual status of participants. It is possible that some of the participants could have RED-S and there were few participants that could be assumed to be underweighted and having a very low BF%. In No-RED-s, information about participants menstrual status and usage of hormonal contraceptives was gathered but the lack of information from all participants made it impossible to assess the possible associations of amenorrhea with the outcomes in this thesis. This thesis had only female participants and the previous studies selected to the review had different layouts by gender having either only female participants, male participants or participants from both genders. This makes the comparison between these studies a bit more challenging when noted that the physiology and bases to enhance e.g. physical performance between males and females are different. Also there was no information about the total amount of training hours of participants that could have made it possible to evaluate if the groups were heterogenic in this part.

8.4 Conclusions

There is a lack of longitudinal studies focusing on the changes in endurance athletes' physical performance, body composition and especially blood lipids and AS in different phases of their annual training cycle. In this study there was only small differences between preparation period and competition period, but individual variance was large in all groups. Group differences were found in Vo_{2max}, body composition and blood lipids. The results of this study strengthens the fact that endurance athletes do not have superior PWV levels or blood lipid profile but their physical performance and body composition is better than the average population.

In conclusion, this study suggests that body composition might have a bigger role than physical performance in maintaining favourable blood lipid profile in preventing cardiovascular health but the periodization of training in endurance sports does not seem to affect these levels significantly. Association of body composition might be stronger with blood lipids than physical performance's, but the impact of nutritional and dietical changes towards competition period must be noted. Even among healthy and physically active population, the amount of lean body mass had the strongest negative association to blood lipids, and this could suggest that for general population it could be more important to have enough muscle mass despite the absolute amount of fat mass.

Because of the facts that athletes can have risk factors for CVDs too and the sport-related demands of body composition in endurance sports can drive towards RED-S leading to hormonal and endothelial changes, it could be useful to target future studies to endurance athletes. These studies should be more comprehensive and take into account other parameters like BP, psychological stress, nutritional and hormonal status and the amount of training. Especially in women, hormonal and menstrual status varies highly and associations to AS and overall cardiovascular health are possible. Further studies with subgroup analyses to amenorrheic participants or participants with RED-S could provide more information about the harmful effects of hormonal and endothelial changes in amenorrheic women or RED-S. It must be taken to account that identifying RED-S is not easy and men should also be included in the studies. Lately, knowledge and discussion about RED-S has taken interest and recognition and management of it has been taken into consideration. It could be assumed that also research on this phenomenon will be increased in the future to provide a better understanding of its role and overall athletes' health. In addition, some of the earlier studies showed increasements after detraining in PWV and blood lipids. Studies evaluating the effects of detraining could be useful to understand or estimate the changes in athletes' health despite the previous training background in case training has ended because of an injury or the active competing phase is over.

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APPENDIX 1 Literature review – Arterial stiffness and blood lipid profiles of endurance athletes compared to less active population

*Quality assessments made using Joanna Briggs Institute's (JBI) critical appraisal tools for analytical cross-sectional studies, cohort studies and systematic reviews

Study	Design/Characteristics	Outcomes/Main results	Quality//Limitations/Comments*
Augustine et al. 2015 Subclinical atherosclerotic risk in endurance-trained premenopausal amenorrheic women	**Cross-sectional** Athletes (n=28): 10 amenorrheic athletes (age 21±3) & 18 eumenorrheic athletes (age 22±3) training >30 min of exercise 5-7 times/week, age Recreationally active controls (n=15, age 23±4) training<30 min of exercise 5-7 times/week All female	**PWV m/s** (Carotid-femoral; applanation tonometry SphygmoCor Technology, Atcor, Sydney, Australia) Carotid-femoral PWV (5.0 ± 1.0 m/s) of amenorrheic athletes were similar to those of eumenorrheic athletes (PWV, 4.6 ± 0.5 m/s) and controls (PWV, 5.4 ± 0.7 m/s, p = 0.15) ➔ Mean carotid-femoral PWV was significantly lower in the eumenorrheic athletes than the controls **Lipids** No significant differencies **Body composition** (Air displacement phlethysmography BodPod; COSMED, Concord, CA) Body fat% amenorrehic athletes 18.8 ± 3.9, eumenorheic athletes 21.3 ± 4.8, controls 29.2 ± 4.9, p=<0.001 ➔ Significantly lower in athletes **Vo_{2Peak}** (indirect calorimetry TrueOne 2400 Metabolic Measurement System, ParvoMedics, Sandy, Utah) Both amenorrheic and eumenorrheic athletes had signifucantly higher $Vo_{2\ peak}$ than controls ($p < 0.05$) ➔ Higher Vo_{2peak} was associated with lower carotid-femoral	**Good/Moderate** - Small sample size - Not reported how the groups were recruited - Athletes competing level not reported

		PWV	
Bjärnegård et al. 2018 Vascular characteristics in young women—Effect of extensive endurance training or a sedentary lifestyle	**Cross-sectional** Athletes (n=47, age 20,3±0,4): Runners (n=26, age 20,2±0,4) & Whole body endurance athletes (n=18, age 19,4±0,5, biathlete, canoe, swimming, triathlon) Swedish national level athletes; at least 6 training sessions/week over the last 5 years (the average of 8,7±0,3 training sessions/week, corresponding to 12,2±0,6 hours/week) Sedentary controls (n=31, age 19,9±0,6) Low physical activity in daily life, never involved in endurance or strength training in leisure time or competitive sports Normally active controls (n=21, age 21,1±0,6) Physical activities of low intensity in their daily life and/or occasionally endurance or strength training in the past but never on a regular basis All female	**PWV m/s** (Carotid-femoral; SphygmoCor Technology, Atcor, Sydney, Australia) ➔ Mean carotid-femoral PWV was similar in between athletes and controls but carotid-radial PWV was lower in whole body endurance athletes than in runners (P < .05), indicating higher arterial distensibility along the arm ➔ long-term endurance training is associated with potentially favourable peripheral artery adaptation, especially in sports where upper body work is added. If persisting later in life, this adaptation could contribute to lower cardiovascular risk. **Vo_{2max} mL/min/kg** (cycle ergometer test) ➔ significantly higher in athletes (30-50%, p=<0.001)	**Good**
Eisenmann et al. 2001 Blood lipids in young distance runners	**Longitudinal** - annual measurements for 21	**Lipids** ➔ development of blood lipids in young distance runners was	**Good**

	males and 18 females aged 9–18 years competing and consistently placing within the top 5 of road races 10 km or more by age and sex - participants were divided into whole-year age groups (i.e., 11.0–11.99)	similar to youth in the general population: TC and LDL remained stable, HDL declined during adolescence (especially in males), and TG increased with age ➔ runners did not possess a superior blood lipid profile except for HDL ➔ sex differencies: age-related trends were statistically significant for HDL and TG in boys only TC and LDL were slightly greater in boys at all ages except 11, 15, and 17 yr ➔ HDL was similar between the sexes until 13 yr when values became greater in girls ➔ No sex differences for TG	
Kyte at el. 2022 Vascular Function in Norwegian Female Elite Runners: A Cross-Sectional, Controlled Study	**Cross-sectional** Athletes (n=16, age 27±3) Norwegian national level athletes; 11.0(ranging 9,0-14,5) hours/week (running & trailrunning) Controls (n=17, age 26±2) Maximum training restricted to 2 hours/week, resulted as 1,0 hours/week All female	**PWV m/s** (Carotid-femoral; SphygmoCor Technology, Atcor, Sydney, Australia) ➔ No significant difference in PWV between athelets (5,4±1,5) and controls (5,2±1,6) **Lipids** HDL cholesterol (mmol/L) runners 1.9 (1.7–2.4), controls 1.5 (1.4–1.9), p=0.017 Runners showed significantly higher HDL-cholesterol and insulin sensitivity compared to controls **Vo_{2max} (mL·kg−1·min−1)** Runners 64.3 (62.5–66.7), controls 44.8 (41.8–45.4), p<0.001	**Good** - Sample size small - Amernorrheic participants reported 3/16 (19%) in athlete group - 7/16 (44%) runners reported RED-S in a questionnaire - the normality test (Shapiro-Wilk) failed for some of the variables, and data are therefore presented as median (25th–75th percentile) throughout

		Body composition (dual-energy X-ray absorptiometry (DXA); Lunar, Prodigy Densitometry, GE Medical Systems, Chicago, IL, USA) Fat mass% runners 16.9 (15.3–19.0), controls 29.7 (25.6–33.7), p=<0.001	- p-values mark for difference between groups
Mitsuzono & Ube 2006 Effects of endurance training on blood lipid profiles in adolescent female distance runners	**Cross-sectional/Longitudinal** Distance runners (n=8): Endurance training over 3 years Non-atheletes/Controls (n=7) No daily exercise All female, age 16,5-19 years Second measurement made to 6 runners after a year	**Blood Lipids** ➔ HDL higher in runners ➔ TG higher in non-athletes **Body composition** ➔ No differencies in BMI ➔ fat% of runners lower	**Good/Moderate** - No information of the recruiting of groups - Menstruation phase not reported (tarkista vielä)
Otsuki et al. 2007 Relationship between arterial stiffness and athletic training programs in young adult men	**Cross-sectional** Athletes: Endurance-trained: Short (n = 7) or long (n = 7) competitive sport careers Strength-trained: Short (n = 7) or long (n = 7) careers ➔ mean difference in sport participation between goups 3 years Sedentary healthy controls (aged, 20 years; n = 7), no regular physical activity for at least 2 years	**PWV** (Aortic, tonometry formPWV/ABI; Colin Medical Technology, Komaki, Japan) ➔ lower in endurance-trained and higher in strength-trained compared with sedentary **Vo_{2max}** (incremental cycling to exhaustion test) ➔ body weight corrected value greatest in endurance trained **Lipids**: Serum total, HDL- and LDL-cholesterol & TG ➔ only difference between groups higher HDL-cholesterol in endurance trained group **Body composition**: - weight, waist circumference and	**Good** - Very small sample sizes - Tonometry based on statistical norms of Japanese individuals, suitability for American or European populations?

	All male	BMI higher in strength trained group - BMI in endurance trained and sedentary the same but fat mass not measured	
Tomschi et al. 2021 Brachial and central blood pressure and arterial stiffness in adult elite athletes	**Cross-sectional** Adult elite athletes (n=136, 70 male, 69 female, age 23.3 ± 3.8) performing on top-national and international level Controls(n=50, 26 male, 24 female, age 23.0 ± 3.0); moderately active studentswith maximum of 4 h of physical activity per week ➔ measurements compared in terms of sex, sport category, and age of the athletes	**PWV** (Aortic; Mobil-O-Graph device, IEM, Stolberg, Germany) ➔ no difference between athletes and controls in any parameter ➔ PWV is positively correlated with age, women exhibit lower BP and PWV than men ➔ different sport categories showed that endurance athletes exhibit lower BP and PWV compared to other athletes ➔ as conclusion: brachial and central BP and PWV values of athletes, suggesting that high-performance sport does not negatively impact AS	**Good** - Endurance athletes sample size quite small - Exact training amounts of athletes not reported but competing - Multiple sports compared - Participants' coffeine intake/pre-measurement activity and female participants' time of menstrual cycle not registered

APPENDIX 2 Literature review - associations of cardiorepiratory fitness and body composition with arterial stiffness or blood lipids

*Quality assessments made using Joanna Briggs Institute's (JBI) critical appraisal tools for analytical cross-sectional studies, cohort studies and systematic reviews

Study	Design/Characteristics	Outcomes/Main results	Quality/Limitations/Comments*
Boreham et al. 2004 Cardiorespiratory Fitness, Physical Activity, and Arterial Stiffness. The Northern Ireland Young Hearts Project	**Longitudinal** Startpoint in age 12 or 15 years At the present/final assessment 202 male and 203 female, aged 20-25 years	**Vo_{2max}** (submaximal cycle ergometer test) **Lipids & Body consumption** as a baseline measurement **PWV** ➔ CRF was inversely associated to PWV independent of body fatness and lifestyle variables ➔ adjustments for blood lipid levels did not decrease the association ➔ no sex differencies	**Good** - 36-36.1% smokers and 76.4-85.6% using alcohol, otherwise healthy - analyses performed in several steps including adjustments for age, sex, mean arterial pressure, and body height and weight and potential confounders such as smoking status, alcohol consumption and intake of fat (as % of total energy intake), and/or intermediate (ie, in the pathway between the determinants and the outcome) variables, such as body fatness (as expressed by the sum of 4 skinfolds) were investigated - menstrual cycle phase not reported
Budimir et al. 2012 Sex-specific association of anthropometric measures of body composition with arterial stiffness in a healthy population	**Cross-sectional** 352 healthy participants as a part of larger genetic epidemiology research program, aged	**Body composition** (BMI, BF% by skinfold measurements, waist circumference – WC, waist-hip ratio – WHpR, and waist-height ratio – WHtR)	**Good** - blood lipids only as a baseline measurement - multiple body composition assessment methods but

	Premenopausal female (n=200) Male (n=152)	**PWV** (Arteriograph (TensioMed Ltd., Budapest, Hungary) ➔ all participants non obese and healthy but i.ge smoking accured ➔ sex differencies: BMI, WC and WHtR are predictors of AS in females and BMI in males ➔ no significant associations of body composition to PWV if confounding factors like age, BP, lipids and smoking were not adjusted	skinfold measurements not as reliable as DXA/impedance devices - menstrual phase not reported
Fernberg et al. 2017 Arterial stiffness is associated to cardiorespiratory fitness and body mass index in young Swedish adults: The Lifestyle, Biomarkers, and Atherosclerosis study	**Cross-sectional** 834 participants, aged 18–25 years Female (n=577) Male (n=257)	**Vo_{2max}** (ergometer bike test) **Body composition** (impedance device, Tanita) **PWV** (SphygmoCor; AtCor Medical Pty Ltd, SphygmoCor, Sydney, Australia) ➔ females with low Vo_{2max} had higher PWV, no differencies in male ➔ study included obese participants and they had higher PWV than normalweight or underweight participants ➔ highest mean PWV was in group low Vo_{2max} & overweight and the lowest in high Vo_{2max} & normalweight	**Good** - blood lipids only as a baseline measurement - menstrual phase not reported
Gando et al. 2016 Cardiorespiratory Fitness Suppresses	**Longitudinal**	**Vo_{2peak}** (graded cycle exercise test)	**Good**

Age-Related Arterial Stiffening in Healthy Adults: A 2-Year Longitudinal Observational Study	470 healthy men and women, aged 26 to 69 years Measurements made at baseline and after 2 years follow-up After baseline measurements divided to subgroups age-specificically (20–29, 30–39, 40–49, 50–59, and 60–69 years) and based on Vo_{2peak} (low, middle, high)	**Lipids & body composition** (BMI & DXA) as a baseline measure **PWV** (brachial-ankle; Omron Colin, Kyoto, Japan) ➔ two-year changes in PWV were significantly higher during the study period in participants in the low CRF group than they were in the high CRF group ➔ higher CRF is associated with slower progression of age-related arterial stiffening ➔ no sex differencies	- brachial-ankle PWV, statistical norms of Japanese? - Vo_{2peak} was measured only once at the baseline; impossible to know if there could have been changes in participants CRF during the follow up period - Menstrual cycle phase of female participants not reported
Haapala et al. 2022 Associations of cardiorespiratory fitness, body composition, and blood pressure with arterial stiffness in adolescent, young adult, and middle-aged women	**Cross-sectional** Females (n = 146), aged 16–58 years Data from 4 different studies, divided to 3 subgroups of adolescents (n=36), young adults (n=74) and middle-aged adults (n=36)	**Vo_{2peak}** (maximal exercise test with respiratory gas analysis on a cycle ergometer/treadmill) **Aortic PWV** (Arteriograph device, PWVao**)** **Body composition** (body fat percentage & FFMI; bioelectrical impedance or DXA) ➔ young adults had higher FFMI and Vo^{2}peak than adolescents ➔ CRF inversily associated to PWV ➔ associations to PWV remained similar after adjustment of BF% ➔ BF% was directly associated and FFMI inversely with PWV	**Good** - part of participants in the young adult group defined as “physically highly active (training 6 times a week)” or ”young female athletes, competing” - menopausal participants not in the interest of this search, but to mention that menopausal status had no effect on these associations and when scaled for FFM there were no differencies in Vo_{2peak} but regardless of similar Vo_{2peak} PWV was higher in middle-aged group - menstrual cycle phase not reported (not available for

		➔ As a conclusion higher CRF, higher FFMI and lower BF% were associated with lower PWV	the researches)
Hashimoto & Okamoto 2023 Peripheral Arterial Stiffness is Associated with Maximal Oxygen Uptake in Athletes	**Cross-sectional** Athletes (n=21) Endurance-trained athletes and national-level college athletes, comprising 3 triathletes, 14 canoeists, 1 fin swimmer, 1 futsal player, 1 surf lifesaver and 1 touch rugby player. Mean endurance (e. g., running, cycling, swimming, and rowing) training time for athletes was 6.1±0.5 days/week (18.4±7.8 h/week) Controls (n=12) Not habitually performed endurance training for at least one year, mean training time 1.6±1.9 days/week (1.0±1.1 h/week) All male	**Vo_{2max}** (incremental cycle ergometer testing) **PWV** (carotid-femoral velocity/femoral-ankle) ➔ Both carotid-femoral PWV (P=0.019) and femoral-ankle PWV (P=0.028) were lower in athletes than in controls. ➔ $\dot{V}o_{2max}$ was significantly higher in athletes compared to controls (P<0.001) ➔ Significant correlations were found between carotid-femoral PWV and $\dot{V}o_{2max}$ (r=–0.510, P=0.018) and between femoral-ankle PWV and $\dot{V}o_{2max}$ (r=–0.472, P=0.031) in athletes ➔ Correlations not evident in controls ➔ As conclusion higher $\dot{V}o_{2max}$ is associated with lower peripheral AS in addition to central AS among endurance-trained athletes	**Good/Moderate** - Sample size small - Other than endurance sports included (futsal, lifesaver?) - Measurements made oscillometrically, based on statistical norms of Japanese individuals, suitability for American or European populations?
Meyer et al. 2017 Controversies in the association of cardiorespiratory fitness and arterial stiffness in children and adolescents	**Cross-sectional** 646 healthy children and adolescents (316 females, age 13.9±2.1 years)	**CRF** (6-min indoor run test; maximum heart rate had to be over 85%) **PWV** (oscillometric cuff-based Mobil–O-Graph (IEM Healthcare, Stolberg Germany) device)	**Good** - Big sample but very diffenert results, discussed in the report - Different CRF assessment

		➔ PWV increased with higher CRF controversily to other studies, also when sex groups assessed separately and adjustments with age and other confounding factors	than in the other studies
Namgoong et al. 2018 The relationship between arterial stiffness and maximal oxygen consumption in healthy young adults	**Cross-sectional** 13 men and 10 women with mean age of 22.9 ± 0.7, 23.6 ± 0.4 years	**PWV brachial-ankle** **Vo$_{2max}$ graded exercise test** ➔ No significant correlation to PWV **Lipids** ➔ Vo$_{2max}$ had a significant association with triglyceride	**Good** - Very different results than others, discussed in report
Wildman et al. 2003 Measures of Obesity Are Associated With Vascular Stiffness in Young and Older Adults	**Cross-sectional** 186 young adults (20 to 40 years, 50% African American) and 177 older adults (41 to 70 years, 33% African American)	**PWV cm/s** ➔ Higher body weight, body mass index, waist and hip circumferences, and waist-hip ratio were strongly correlated with higher pulse-wave velocity, independent of age, SBP, race, and sex overall and among both age groups ($P<0.01$ for all) ➔ Even among the 20- to 30-year-olds, obese individuals (body mass index>30) had a mean pulse-wave velocity value 47 cm/s higher than non-obese individuals ($P<0.001$).	**Good** - 50% of study participants African-American in age group 20-40 - Body fat% not assessed

APPENDIX 3 Literature review – Fluctuations between different training periods of endurance athletes

*Quality assessments made using Joanna Briggs Institute's (JBI) critical appraisal tools for analytical cross-sectional studies, cohort studies and systematic reviews

Study	Design/Characteristics	Outcomes/Main results	Quality/Limitations/Comments*
Durkalec-Michalski et al. 2022 Do Triathletes Periodize Their Diet and Do Their Mineral Content, Body Composition and Aerobic Capacity Change during Training and Competition Periods?	**Longitudinal** 20 triathletes, aged 32 ± 7 years 2 females and 18 males Moderate but competitive and professional level athletes; average training experience 8.5 ± 4 years Measurements made in the preparation period and competion period	**Body Composition** (bioelectrical impedance analysis) ➔ no significant differences were recorded between training vs. competition periods ➔ Body mass slightly decreased from 80.5 ± 14.4 kg in the training period to 78.6 ± 11.2 kg in the competition period, especially due to the insignificant reduction of body fat **Physical performance** (incremental cycling test (ICT) ➔ performance was improved during the competition period (longer TTE) ➔ lower Vo_{2max} at the ventilatory threshold	**Good** - bioelectrical impedance analyse not as accurate as DXA - no differences in energy and nutritional values of habitual diets in studied triathletes between training and competition periods - only 2 females - drop out rate% big (from 50 to 20) because of injuries and health issues
Heydenreich et al 2017 Total Energy Expenditure, Energy Intake, and Body Composition in Endurance Athletes Across the Training Season: A Systematic Review	**Systematic review** SPORTDiscus and MEDLINE from January 1990–31 January 2015 ➔ 82 original research articles that examined TEE, energy intake, and/or body composition of 18-40-year-	**Body composition** Assessed by DXA in 32.1% of studies, by bioelectrical impedance analysis (BIA) in 25.6% of studies, and by hydrostatic weighing in 25.6% of studies ➔ During the competition	**Good/Moderate** - In 71.7% of the studies, where body composition was measured, no details of standardization were provided (!) - In more than one third

	old endurance athletes engaged to competitive sports and reported the seasonal training phases of data assessment were included in the review Training periods divided to preparation phase, competition phase and transition phase	phase, both body mass and fat-free mass were significantly higher compared to other seasonal training phases ($p < 0.05$) ➔ percentage of fat mass; no differences were detected between the seasonal training phases ➔ Endurance athletes show training seasonal fluctuations in TEE, energy intake, and body composition ➔ Sex differences: in males body mass was lowest during the transition phase and absolute and relative fat mass were highest during the competition phase and FFM was lowest during the transition phase; in female absolute and relative body fat were higher during the preparation phase than during the transition phase, body mass or FFM differences between seasonal training phases were not observed	(34.5%) of the female study populations, where body composition was assessed, the menstrual status was not reported - A separate analysis between eumenorrheic and amenorrheic athletes could not be performed (cumulative number of participants during the different seasonal training phases too low) - Male athletes demonstrated the highest fat mass values during the competition phase and the lowest FFM during the transition phase might be an anomaly from the pooling of data
Koshiba & Maeshima 2015 Influence of detraining on temporal changes in arterial stiffness in endurance athletes: a prospective study	**Longitudinal** Eighteen female university athletes requiring high endurance exercise capabilities were classified into 2	**PWV cm/s** (Brachial-ankle; PWV/ABI® Omron Colin Co., Ltd., Komaki, Japan) ➔ PWV in the detraining group increased	**Good?** - Very small sample size

	groups: 10 retired players (detraining group) and 8 active players (training group) Measurements made a total of 6 times: immediately before retirement of the detraining group and at 1, 2, 3, 6, and 12 months after retirement	significantly at 3 and 12 months as compared with that at 0 months and showed a significant increase at 12 months compared with that at 1 month ➔ In the detraining group PWV was significantly higher at 3, 6, and 12 months than in the training group ➔ Detraining may result in increased AS from 3 months onward in endurance athletes	
Ormsbree & Arciero 2012 Detraining increases body fat and weight and decreases Vo_{2peak} and metabolic rate	**Longitudinal** Swimmers 4 female, aged 19.5 ± 1.2 years 4 male: age 19.5 ± 1.0 years 5 weeks (35–42 days) of swim detraining after competitive season -> possible decrements to body composition, aerobic fitness, RMR, and fasting blood lipids	**Body composition** (DXA) ➔ 12% increase in body FM and percent, lean mass was unchanged ➔ waist circumference was significantly increased **Vo^2peak** (cycle ergometer) ➔ significant 7.7% decrease in Vo_{2peak} ➔ max heart rate, respiratory quotient and RPE did not differ but TEE was significantly lower after detraining period **Lipids** ➔ TC, HDL, and LDL remained unchanged ➔ TGs showed a trend to increase	**Good/Moderate** - Very small sample size - Longer detraining/transition phase than recommended for athletes?
Trinshek et al. 2020	**Longitudinal**	**Vo_{2max}** (TEE treadmill)	**Good**

Maximal Oxygen Uptake Adjusted for Skeletal Muscle Mass in Competitive Speed-Power and Endurance Male Athletes: Changes in a One-Year Training Cycle	12 sprinters (24.7 ± 3.3 years), 10 endurance runners (25.3 ± 5.3 years), and 10 recreationally trained controls (29 ± 4.5 years) All male Measurements repeated 4 times; (1) beginning of the general preparation period, (2) beginning of the specific preparation period, (3) beginning of the pre-competition period, and (4) beginning of the competition period	➔ endurance runners had no significant changes in Vo_{2max} (in control group was) **Body composition** (DXA) ➔ endurance athletes absolute and fat mass% was significantly higher in the general phase than in the subsequent training phases ➔ LBM of endurance runners significantly increased between the general and the subsequent training phases ➔ Endurance runners had no significant change in percentage SMM during the annual training cycle	- Small sample size