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Prospective, longitudinal and cross-sectional perspectives from the HUNT Study

Doctoral thesis

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SAMMENDRAG

Kondisjon er vist å være en sterk risikofaktor for hjerte-karsykdom og død. Mesteparten av tidligere forskning på kondisjon som risikofaktor har vært gjennomført ved indirekte og mindre nøyaktige metoder sammenlignet med direkte måling av maksimalt oksygenopptak ved gassanalyse under arbeid til utmattelse, som er den mest nøyaktige måten å måle kondisjon på.

Denne avhandlingen består av tre artikler. I den første artikkelen undersøkte vi betydningen av kondisjon på risiko for kransåresykdom, som for eksempel hjerteinfarkt og angina, hos 4500 deltakere fra Helseundersøkelsen i Trøndelag 3 (HUNT3, 2006-08). Selv hos disse tilsynelatende friske deltakerne var kondisjon målt som maksimalt oksygenopptak tett forbundet med lavere forekomst av kransåresykdom i løpet av ca 10 års oppfølging. Vi så også at vi bedre kunne plukke ut individer med risiko for fremtidig kransåresykdom ved å legge informasjon om maksimalt oksygenopptak til en etablert modell for å vurdere risiko for hjerte-karsykdom.

I den andre artikkelen undersøkte vi hvordan kondisjonen endrer seg over tid ved å repetere måling av maksimalt oksygenopptak hos ca en tredjedel av deltakerne fra HUNT3 på ny i HUNT4 (2017-19), hvor vi fant at det forventede fallet i kondisjon over 10 år er høyere hos eldre enn hos yngre. Funnene skiller seg tildels fra funn i tverrsnittsstudier der man sammenligner på tvers av aldersgrupper ved en enkelt måling, og vi argumenterer for at repeterte tester gir et mer robust mål på fall i maksimalt oksygenopptak med økende alder. De som var aktive opprettholdt maksimalt oksygenopptak bedre, og bedre opprettholdelse var forbundet med en gunstigere utvikling av risikofaktorer for hjerte-karsykdom i oppfølgingsperioden.

I den tredje artikkelen undersøkte vi sammenhengen mellom maksimalt oksygenopptak og størrelse på venstre hjerteforkammer. Et forstørret venstre forkammer er en velkjent risikofaktor for hjerte-karsykdom, noe som er spesielt interessant siden utholdenhetsatleter ofte har store forkammer. Vi fant at også tilsynelatende friske HUNT-deltakere med høyt oksygenopptak hadde økt forekomst av forstørret forkammer og at denne sammenhengen ble forsterket ved økende alder. Tross denne potensielt ugunstige endringen i hjertestruktur fant vi ikke tegn til ugunstige endringer på hjertets fylningsfunksjon, noe som vanligvis er forbundet med store forkammer.

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Veiledere: Bjarne Martens Nes, Håvard Dalen

Finansieringskilde: Regionalt samarbeidsorgan for utdanning, forskning og innovasjon i Helse Midt-Norge

SUMMARY

Cardiorespiratory fitness is a well-known risk factor for cardiovascular disease and death. Most previous research has been conducted using indirect and more inaccurate methods for measurement of cardiorespiratory fitness compared to measuring maximal oxygen uptake by gas-analysis during maximal exercise.

This thesis consists of three papers. In paper I, we investigated the predictive value of cardiorespiratory fitness on coronary heart disease, e.g. myocardial infarction and angina, in 4500 participants from the third wave of the Trøndelag Health Study (HUNT3, 2006-08). Cardiorespiratory fitness measured as maximal oxygen uptake was strongly and inversely associated with coronary heart disease even in apparently healthy participants during roughly ten years of follow-up. Furthermore, by adding information on maximal oxygen uptake to an established risk prediction model, we were able to better identify individuals with higher risk of future coronary heart disease.

In paper II, we investigated how cardiorespiratory fitness changes with age by repeating measurements of maximal oxygen uptake in one third of the participants from HUNT3 in HUNT4 (2017-19). Repeated measures of maximal oxygen uptake have not been performed previously in this scale. We showed that the expected decline in maximal oxygen uptake over ten years is higher in older compared to younger individuals. These findings differ from previous cross-sectional studies, where one compares differences in maximal oxygen uptake across age groups, and we argue that repeated measures give more reliable estimates of the decline in maximal oxygen uptake with age. Those who were physically active maintained their maximal oxygen uptake better, and maintenance of a higher maximal oxygen uptake was associated with a more favorable change in cardiovascular risk factors during follow-up.

In paper III, we investigated the association between maximal oxygen uptake and the size of the left atrium. Enlargement of the left atrium is an established risk factor for cardiovascular disease, which is especially interesting as endurance athletes have large atrias. We found that even apparently healthy HUNT3 participants with a high maximal oxygen uptake were more prone to having enlarged left atria, and that this association was more pronounced with higher age. Despite this potentially unfavorable change in cardiac structure, we did not find evidence of reduced left ventricular diastolic function, which often accompanies left atrial enlargement.

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Mom and dad, thank you for always supporting me in my decisions and teaching me the value of hard work. Paul, Ida and Margrete for showing inspiring stamina by never giving up after years of Catan the board game. Thank you to all my family and friends for still inviting and including me despite this thesis having made me an antisocial hermit at times.

Lene, thank you for your support and understanding, and for always making me laugh with your quirks. It means so much to me, both during the work on this thesis and in general. Aksel, I'm so lucky to be your dad, thanks for reminding me every day how unimportant these ~100 pages really are. I love you!

Trondheim, November 2020

Jon Magne

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LIST OF PAPERS

Papers listed below are referred to by their roman numerals throughout this thesis.

I. Letnes JM, Dalen H, Vesterbekkmo EK, Wisløff U, Nes BM. Peak oxygen uptake and incident coronary heart disease in a healthy population: the HUNT Fitness Study. *Eur Heart J*. 2019;40(21):1633-1639

II. Letnes JM, Dalen H, Aspenes ST, Salvesen Ø, Wisløff U, Nes BM. Age-related change in peak oxygen uptake and change of cardiovascular risk factors. The HUNT Study. *Prog Cardiovasc Dis*. 2020;63(6):730-737

III. Letnes JM, Nes B, Vaardal-Lunde K, Slette MB, Mølmen-Hansen HE, Aspenes ST, Støylen A, Wisløff U, Dalen H. Left Atrial Volume, Cardiorespiratory Fitness, and Diastolic Function in Healthy Individuals: The HUNT Study, Norway. *J Am Heart Assoc*. 2020;9(3).

SELECTED ABBREVIATIONS

AF	Atrial fibrillation
BMI	Body mass index
BP	Blood pressure
CHD	Coronary heart disease
CI	Confidence interval
CPET	Cardiopulmonary exercise testing
CVD	Cardiovascular disease
HDL	High-density lipoprotein
HR	Hazard ratio
HUNT	Trøndelag Health Study («Helseundersøkelsen i Trøndelag»)
IDI	Integrated discrimination improvement
LA	Left atrium
LAVI	Left atrial volume indexed by body surface area
LV	Left ventricle
MET	Metabolic equivalent task
NRI	Net reclassification improvement
PA	Physical activity
RCT	Randomized controlled trial
SD	Standard deviation
VO_{2peak}	Peak oxygen uptake
$VO_{2\%pred}$	Peak oxygen uptake in percent of predicted by age and sex

DEFINITIONS

Cardiorespiratory fitness: a health-related component of physical fitness[1] that relates to the ability of the cardiovascular and respiratory systems to supply oxygen during sustained physical activity.[1,2]

Exercise: physical activity that is planned, structured and repetitive with a purpose of improving or maintaining (one or more components of) physical fitness.[1]

Maximal oxygen uptake (VO_{2max}): the highest rate at which oxygen can be taken up and utilized by the body during strenuous, dynamic exercise using large muscle groups.[3]

Metabolic equivalent task (MET): one MET equals the oxygen uptake at rest, commonly defined as an oxygen uptake of 3.5 mL/kg/min.[2]

Oxygen uptake (VO_2): defined by the modified Fick equation: VO_2 = cardiac output x arteriovenous oxygen difference. All energy-releasing processes in the body ultimately depends on oxygen consumption, and thus the oxygen uptake is an indirect but accurate measure of energy expenditure.[4]

Peak oxygen uptake (VO_{2peak}): the highest oxygen uptake achieved during a maximal oxygen uptake test to voluntary exhaustion. Although not strictly interchangeable, the term is often used as a proxy for the term "maximal oxygen uptake".

Physical activity: any bodily movement produced by skeletal muscles that results in energy expenditure.[1]

Risk factor: a factor with a causal relation with a clinical outcome which may be of a socioeconomic, environmental or behavioral characteristic, or a trait.[5] If the factor is associated with, but not causally related to the given outcome, it is known as a risk marker.[5]

1. BACKGROUND

Non-communicable diseases such as cardiovascular disease (CVD) increased considerably during the 20th century[6] with a following focus on CVD prevention. The interest in preventing CVD lead to the initiation of the Framingham Heart Study in the 1940s,[7] which also marked the start of the field of CVD epidemiology. The term "risk factor" was popularized some years later in 1961,[7] and in the decades following the birth of the Framingham Heart Study knowledge on the classical risk factors for CVD such as hypertension, diabetes and smoking e.g., was established from various epidemiological studies. In 1953, parallel to this development, Morris et al. published a seminal study which marked the initiation of the field of physical activity (PA) epidemiology.[8] They showed how London bus drivers, with their sedentary work, had a higher incidence of coronary heart disease (CHD) than the corresponding bus conductors who walked up and down stairs in the typical two-floor London buses, postulating that differences in their work-related PA explained the observed difference.[8] Although the findings challenged the understanding at the time, the findings were supported by several studies in the years to come.[9] Some years later prospective studies linking higher cardiorespiratory fitness (CRF) to lower all-cause and cardiovascular mortality emerged, perhaps best known by the 1989 study by Blair et al.[10] Since the well-known studies on PA and CRF by Morris and Blair, respectively, numerous studies have been conducted to unravel the role of CRF and PA in CVD pathophysiology. Based on the mounting epidemiological knowledge contributing to the understanding of CVD pathophysiology through the 20th century, prevention strategies have played a central role in decreasing the negative impact of CVD over the last decades.[11,12] Despite these advances, CVD is still the leading cause of death and disability-adjusted life years globally,[13] and although mortality from CVD is declining in high-income countries, the prevalence of CVD is relatively unchanged,[13,14] with accompanying burden on both societies and individuals. Furthermore, there is evidence that the decline in CVD mortality in high-income countries is slowing down, and in some countries even rising again.[15] Being ranked as the 4th leading risk factor for death in the world in the 2009 WHO report "Global Health Risks",[16] physical inactivity is an important target for preventive strategies. CRF, however, is not widely recognized and utilized as a factor conveying useful information in clinical practice, despite considerable accumulated knowledge of its significance.[17]

1.1. Cardiorespiratory fitness

Physical fitness is commonly divided in a performance-related component and a health-related component, where the latter is comprised of body composition, flexibility, muscular fitness, and CRF.[1,2] CRF, popularly coined "exercise capacity" or "aerobic fitness", is a composite measure of the capacity of several body organs and functions as it measures the body's capacity to transport oxygen from ambient air to muscle fiber mitochondria for cellular respiration during PA. The process of oxygen delivery to peripheral tissue includes pulmonary ventilation and gas-diffusion, oxygen transport, mainly by binding of oxygen to hemoglobin in red blood cells, cardiac output, as defined by the product of stroke volume and heart rate, and vascular function. Lastly, capillary and mitochondrial density and function, as well as other peripheral tissue factors, limit extraction of oxygen from the blood offered to the peripheral tissue. Maximal oxygen uptake (VO_{2max}), the gold-standard measure of CRF,[17,18] is a function of the joint capacity of these processes. The term VO_{2max} stems back to Hill et al., who concluded that each individual has an upper limit of their oxygen uptake demonstrated as a plateauing of the oxygen uptake with increasing exercise intensity.[3]

1.1.1. Physiological determinants of VO_{2max}

VO_{2max} is classically described by the modified Fick equation, originally developed as an expression of cardiac output (Figure 1):[4,19]

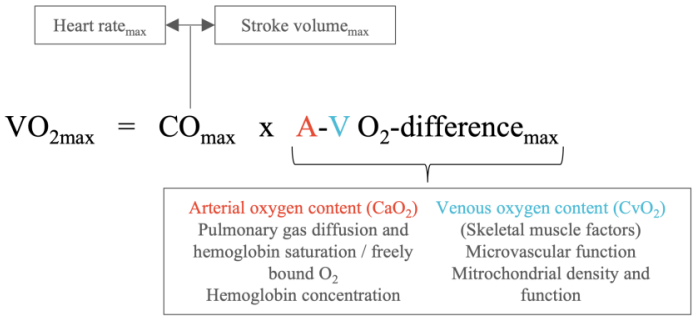

Figure 1. Modified Fick equation describing VO_{2max} and key components. CO = cardiac output.

Cardiac output, pulmonary gas diffusion capacity, and blood oxygen transport capacity are commonly referred to as central factors, describing their role in oxygen *delivery*.[3] Oxygen delivery to the working tissues is now, after some previous controversy, generally recognized as the most important factor limiting VO_{2max}.[3,20,21] Pulmonary gas diffusion capacity is not a restraining factor in healthy non-athletic adults, and the respiratory system does not adapt

considerably to exercise, but might constrain performance in elite endurance athletes through exercise-induced arterial hypoxemia narrowing the arteriovenous oxygen difference.[22–24] The oxygen transport capacity per mL blood, mainly determined by the hemoglobin concentration, is relatively unchanged in response to exercise as the blood volume increases equally to or more than the hemoglobin mass.[21,25,26] Also, whether blood volume expansion is key in improving cardiac output by exercise is still somewhat controversial.[21,27] Increasing cardiac output, defined as the product of left ventricular (LV) end-diastolic volume minus LV end-systolic volume (= stroke volume) and heart rate, is the main way of increasing VO_{2max} by exercise training.[3,20,24] Maximal heart rate does not increase and LV end-systolic volumes have not been shown to decrease as an adaptation to exercise,[20] and thus enlargement of the LV end-diastolic volume with improved diastolic filling characterizes the improved cardiac output through enlarged stroke volume in response to exercise.[20,28] Although exercise may also contribute to improvements of VO_{2max} by increase in peripheral skeletal muscle oxygen extraction by e.g. mitochondrial alterations and higher capillary density,[21,24] the central venous blood contains roughly 20-30 mL oxygen per L blood (compared to ~200mL in arterial blood) at maximal dynamic exercise involving large muscle groups, meaning that there is little oxygen left to extract, and the primary mechanism behind training-induced increases in VO_{2max} is increases in cardiac output through increased stroke volume,[3,20] although differences in training status play a role in these relationships.[24]

Identifying the true VO_{2max} can be difficult and depends on the criteria being used.[3,29] The term peak oxygen uptake (VO_{2peak}) is often used interchangeably with VO_{2max}, although the term VO_{2peak} implies that strict VO_{2max} criteria may not have been met in the given test situation. For simplicity, the term VO_{2peak} will be used throughout the thesis from here on.

1.2. Physical activity, exercise and cardiorespiratory fitness

Although often used interchangeably, PA and exercise pertain to different concepts. PA refers to any bodily movement increasing energy expenditure over resting levels,[1] meaning that e.g. walking to collect the daily mail is classified as a subtype of PA. Exercise is a subcategory of PA, often classified as leisure-time or recreational PA.[2] The term *exercise* implies that the activity is planned and has an objective of maintaining or improving one or more aspects of physical fitness,[1] e.g. endurance exercise having the aim of affecting CRF.

Although all PA, in theory, has the potential to improve CRF (depending on the individual's CRF status), it is well-established that exercise with moderate to high intensity is causally linked to a higher VO_{2peak}.[30] Short-term randomized exercise trials further indicate that high-intensity training is more efficient for increasing VO_{2peak}, compared to moderate intensity training when calorically matched,[17,31] but long-term exercise trials are scarce, with a notable exception for the Generation 100 Study showing superior effects of high-intensity exercise compared to moderate intensity and a control group on VO_{2peak} in older adults over five years of follow-up.[32] Thus, for investigation of long-term effects of exercise on VO_{2peak}, using observational designs is more feasible. High self-reported levels of PA is associated with high VO_{2peak} in cross-sectional studies,[33,34] and is also a long-term predictor of VO_{2peak} in longitudinal observational studies.[35] Inactivity in its utter form of complete bed rest, conversely, was shown to reduce VO_{2max} by 26% over the course of 20 days in Bengt Saltin's famous bed rest study.[36,37] In epidemiological studies PA is modestly correlated with VO_{2peak}, both measured by self-report[38–40] and objectively by e.g. accelerometer-measure[41] or doubly labelled water technique.[42] Correlations with VO_{2peak} are typically lower for low and moderate intensity compared to high intensity.[38,39] Two large longitudinal studies have also investigated associations between PA and maximal exercise-estimated CRF[43] and measured VO_{2peak},[44] demonstrating that higher volumes of PA in terms of absolute intensity is associated with higher CRF/VO_{2peak}, while comparisons of different relative intensities are lacking.

This touches the distinction between *relative* and *absolute* intensity of PA. Absolute intensity is often defined by energy expenditure from different activities, commonly as metabolic equivalents of task (METs, one MET reflecting an oxygen uptake of 3.5 mL/kg/min).[2] The amount of weekly or daily METs is often calculated from self-reported PA questionnaires by using standardized tables established for various activities[45] or from accelerometers.[46] Based on energy expenditure for different activities, intensity is usually classified as light (1.6 to 2.9 METs; e.g. walking), moderate (3.0 to 5.9 METs; e.g. brisk walking, tennis) or high (>6.0 METs; e.g. jogging or running), with ≤1.5 METs being defined as sedentary behavior.[45,47] However, such absolute thresholds ignores individuals' fitness levels when classifying intensity.[48] Conversely, measures of *relative intensity* of PA take into account the individual's prior fitness level and focus on some physiological measure of intensity[2] such as self-reported perceived exertion like the Borg scale[49] or objective measures of intensity as percentage of VO_{2peak}, percentage of peak heart rate, or percentage of heart rate reserve (maximal heart rate minus resting heart rate). As noted in a recent editorial,[50] much research into PA for health has

focused on the total amount of PA spent through accumulated METs, which not necessarily improves CRF notably, while high-intensity training of short duration, e.g. interval training, improves CRF without necessarily spending large amounts of METs.

Considerable evidence supports a strong association between different measures of PA and all-cause and disease-specific mortality and morbidity,[51–54] with strong trends towards an effect on mortality, also from randomized controlled trial (RCT)-level evidence recently shown in the Generation 100 Study.[32] Studies have suggested that the association between PA and all-cause mortality disappears after adjustment for CRF status,[55] suggesting that (at least some of) the effect of PA is mediated through increases in CRF or that increasing CRF is a marker of exercise response. However, the relationship between the behavior PA and the trait CRF is complex, underscored by the difficulty of measuring PA precisely in epidemiological studies, in contrast to the more precise methods for quantification of CRF.

1.2.1. Physical activity recommendations

PA guidelines for Americans,[56] as well as recommendations from Norwegian health authorities[57] and the World Health Organization,[58] all recommend a minimum of 150 minutes of moderate intensity or 75 minutes of high-intensity aerobic PA, or an equivalent combination of the two, each week for adults, as well as activities improving muscle strength two or more days weekly.

1.3. Measuring energy expenditure

The first measurements of human energy expenditure during rest and work was performed by direct calorimetry, a meticulous and time- and resource-demanding technique involving isolation of research subjects in closed chambers for direct measurement of heat production.[4] Thus, the method has obvious constraints for widespread use, due to e.g. lack of possibility to track dynamic short-term changes in energy expenditure. For measurement of energy expenditure over long periods of time in free-living individuals the Doubly labelled water technique is considered the gold-standard,[4] but this technique is not feasible for assessing fluctuations in energy expenditure over short time periods.[46] Indirect calorimetry, however, takes advantage of the fact that all processes spending energy in the body depends on oxygen consumption, founding assessments of energy expenditure on measurement of oxygen uptake. The widely used open-circuit spirometry technique lets the subject under testing breathe

ambient air with assessment of expired and inspired volumes and concentrations of carbon dioxide and oxygen instantly (breath-by-breath or mixing chamber methods) or after collection in bags (Douglas bag technique), with the latter still considered the gold-standard.[18] However, also open-circuit spirometry requires experienced personnel and some extra equipment on top of a treadmill or cycle ergometer, and cardiopulmonary exercise testing (CPET) has therefore traditionally been under-utilized in both epidemiological and clinical settings. Estimating CRF through various formulas based on submaximal or maximal treadmill- or cycle-ergometry exercise, on the other hand, is widely used in epidemiological settings, with relatively good correlation towards directly measured VO_{2max} by open-circuit spirometry.[17] However, agreement of estimated exercise capacity with corresponding directly measured VO_{2max} at the individual level is questionable.[59,60] Also, such predictions are commonly biased towards higher CRF values with higher maximal exercise levels, as they were developed for submaximal steady-state exercise.[61] Non-exercise algorithms have become increasingly popular as they are easy to use in epidemiological studies[62] and for the individual by estimating CRF from accessible information such as age, sex, self-report PA, resting heart rate, and measures of body weight and/or central obesity, despite the lower accuracy compared to directly measured VO_{2peak}.[17]

1.3.1. Cardiopulmonary exercise testing in clinical settings

Although CPET with gas analysis during maximal exercise provides direct insight into physiological alterations related to the pathophysiology of different cardiopulmonary diseases,[63] and its value in several clinical scenarios is established,[64] it remains under-utilized by clinicians.[65] CPET may differentiate between different causes of unexplained exertional dyspnea through evaluation of the various CPET parameters,[66] and assists in evaluation and management of heart failure, cardiomyopathies, surgical risk, obstructive, interstitial and vascular pulmonary disease, myocardial ischemia, and cardiovascular rehabilitation.[63,64,66] Furthermore, included in the European Association for Cardiovascular Prevention and Rehabilitation and American Heart Association 2016 focused update on CPET in specific patient populations was a call to determine the value of CPET in apparently healthy populations.[64] Similarly, in their 2016 scientific statement,[17] the American Heart Association argued that CRF should be regularly assessed in clinical practice, and for accumulating more evidence to facilitate increasing use of CRF assessment in primary care settings.

1.4. Variations in VO_{2peak} with age, sex and different populations

In a meticulous study on various measures of physical fitness in relation to age, Robinson described already back in 1938 how "the mechanism for supplying and utilizing O_2 in exhaustive work" was only roughly half that of a seventeen-year-old for men in their 8th decade.[67] Since then, a wide variety of studies have examined the influence of age and sex on VO_{2peak} using cross-sectional designs, showing declines across age groups at one time-point, and a few have used longitudinal designs and calculated declines over time using repeated measures of VO_{2peak}. The average annual decline in VO_{2peak} found in a selection of studies[34,38,41,44,68–96] examining VO_{2peak} by treadmill exercise from different populations, omitting studies on athletes, is shown in Figure 2.

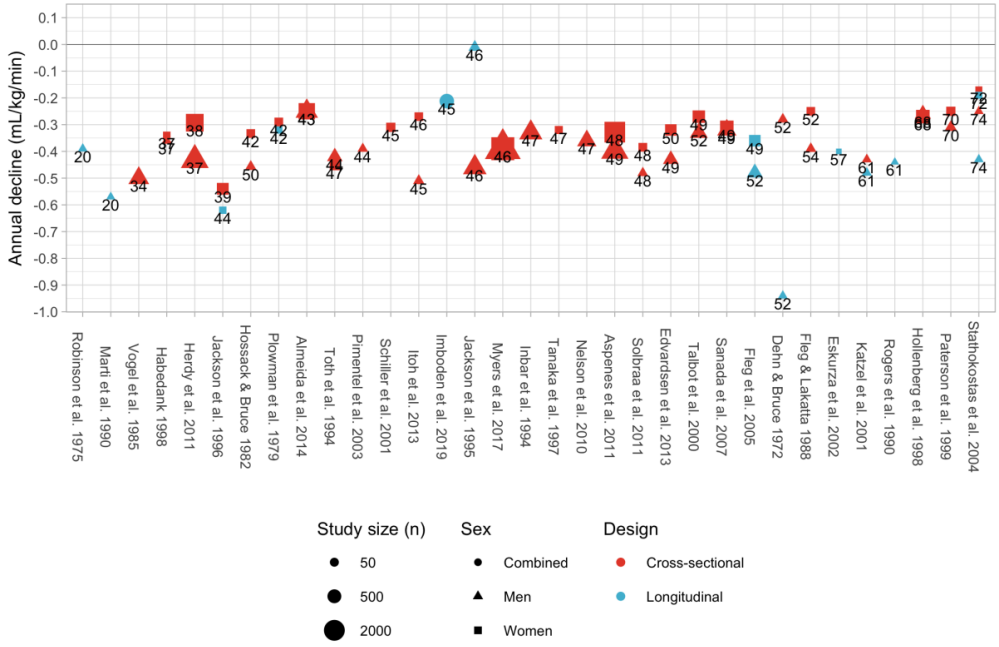

Figure 2. Overview of studies examining decline in directly measured VO_{2peak} by treadmill exercise in relation to age, sex and study design. Mean age depicted as the number corresponding to each point, increasing from left to right on the x-axis. In longitudinal studies age is given at baseline. Studies on athletes are not included. For several studies the annual decline is calculated from cross-sectional per-decade data or similarly available data. Where available data is collected from regression equations reported separately by sex.

The decline is somewhat similar across the wide variety of studies and reported to be about 0.25 to 0.50 mL/kg/min per year. Meta-analyses in men and women have shown annual declines of 0.40 mL/kg/min for both active and sedentary men,[97] and 0.44 and 0.35 and mL/kg/min for active and sedentary women, respectively,[98] corresponding to ~10% decline per decade. However, VO_{2peak} is shown to vary widely between different populations, with high values reported from Norwegian populations.[61,76,77,99–101] Two large longitudinal studies from the US,

one using directly measured VO_{2peak}[44] and the other estimating maximal METs from peak exercise,[43] have shown how the decline in CRF increases with higher age, while findings from cross-sectional studies[76,77] have shown relatively even declines of about 10% per decade higher age. Indeed, several studies have observed discrepancies between the cross-sectional and longitudinal decline in VO_{2peak},[44,79,85] which is also shown in Figure 2.

Studies examining temporal changes in populational CRF distribution have shown how CRF has declined on the populational level over the last decades in findings from several different populations, including the adult Swedish workforce,[102] US youth,[103] and children and adolescents from 19 different high-income and upper middle-income countries,[104] although some studies have shown stable trends as well.[105] Notably, the proportion of US youth aged 12 to 15 years with a good CRF declined from 52% to 42% between 2000 and 2012,[103] and pooled data from eight high- and upper-middle-income countries showed estimates of a 1.6% decline in CRF per decade from 1967 to 2016.[106] The somewhat discrepant findings across different designs and secular changes in CRF over the last decades signal the need for further large, *longitudinal* studies assessing age-related declines in VO_{2peak}.

1.5. Cardiorespiratory fitness as a marker of risk and disease

Over the last three decades, a wide variety of studies examining the associations between CRF and various health outcomes has been published. In 2009, Kodama et al. summarized the predictive value of CRF on CVD events and all-cause mortality showing, respectively, 15% and 13% lower risk per one MET higher CRF.[107] Inverse associations have also been shown between CRF and incidence of sub-types of CVD such as CHD, stroke, and heart failure, and other outcomes such as cancer, dementia, and disability.[17,108,109] A few studies have also found associations between changes in CRF and all-cause mortality.[110–114] However, the available evidence has dominantly been based on indirect measures of CRF, as opposed to directly measured VO_{2peak} by ventilatory gas analysis. In fact, at the start of planning this thesis, only a few cohorts had examined associations between VO_{2peak} and future events of CVD and/or mortality in healthy populations, showing inverse associations. One of the cohorts, based on the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), consists of a large sample of 2,682 men who performed direct assessment of VO_{2peak} by cycle ergometry between 1984 and 1989.[115] Several interesting studies have emerged from various sub-populations of this cohort on the association between VO_{2peak} and CVD and mortality end-points,[109,111,115–117] but the

limited age-span of 42 to 60 years in addition to including only men limits generalizability. Furthermore, VO_{2peak} was assessed by cycle-ergometry, which known to yield lower values,[118] and the cohort is based in an area known for its very high CVD risk.[115] Contemporary care may also have changed since the cohort was assembled in the late 1980s. The second cohort, based on The Baltimore Longitudinal Study of Aging (BLSA), measured VO_{2peak} directly during maximal treadmill exercise in 689 participants who were consequently assessed for future events of CHD.[119] Although they had a larger age-span then the KIHD cohort (mean 51.6, standard deviation (SD) 16.8) the cohort consisted only of men, and the number of events over a mean 13.4 years of follow-up was only 63. Lastly, a third cohort of 506 male veterans referred for exercise testing reported mortality outcomes based on a dichotomized measure of VO_{2peak} (over/under 16 mL/kg/min).[120] In a 2017 review of new evidence on the association between CRF and all-cause and disease-specific mortality since the 2009 meta-analysis by Kodama et al., Harber et al. indeed pointed out the under-representation of women, that many studies were performed on clinical populations referred for exercise testing, and that studies using direct measurement of VO_{2peak} by CPET was lacking.[108]

1.5.1. Cardiorespiratory fitness and cardiovascular risk factors

The relationship between cardiovascular risk factors and measures of CRF has been recognized for decades.[121] Furthermore, animal studies performing selective rat breeding by CRF status have suggested that CRF and cardiovascular risk factors share common genetic and molecular pathways,[122] and genetic studies indicate that genes associated with low CRF are inversely associated with cardiovascular risk factor levels.[123] Cross-sectional epidemiological studies have shown how CRF is inversely associated with traditional cardiovascular risk such as systolic and diastolic blood pressure (BP), unfavorable cholesterol levels, triglycerides, resting heart rate, and measures of obesity and glycemic control, both when assessed individually[76,124] and clustered as e.g. the metabolic syndrome or other measures of risk factor clustering.[76,124–132] Data from the Aerobics Center Longitudinal Study (ACLS) cohort have further shown CRF-dependent trajectories of lipids and lipoproteins[128] and BP[133] across the life-span. Associations between change in CRF and future incidence of unfavorable risk factor states such as hypertension, dyslipidemia, and metabolic syndrome, have been found in several studies,[134–136] including studies using directly measured VO_{2peak}.[137,138] There is also evidence from short-term RCTs showing how high-intensity exercise, producing higher VO_{2peak} increments compared to moderate intensity training,[139] may yield more beneficial changes in cardiovascular risk factors compared to moderate intensity training,[140,141] although some controversy exists.[142] However,

long-term randomized exercise trials are challenging, thus long-term effects of changes in exercise habits and CRF are preferably investigated by observational designs. Noteworthily, few thoroughly conducted studies have examined concurrent changes in CRF and changes in various cardiovascular risk factor levels. As summarized in Table 1, only one study used directly measured VO_{2peak} as the measure of CRF, and most studies have a limited age-span and predominantly consist of men.

Table 1. Studies assessing concurrent change in CRF and cardiovascular risk factors.

Population	Design	Findings
Blair et al. 1983.[143] 783 middle-aged men. [Only access to abstract]	Maximal treadmill time at two occasions between 1978 and 1981 (mean follow-up 1.6 years).	In multiple regression models increase in treadmill time was associated with rise in HDL (high-density lipoprotein), and decrease in total to HDL-cholesterol ratio and serum uric acid.
Knaeps et al. 2018.[144] 435 adult health-survey participants (65% men) mean age 56 years.	Maximal cycle-ergometry test with directly measured VO_{2peak} twice over mean 9.6 years in 2002-14.	Decrease in VO_{2peak} was associated with change in a clustered continuous cardiometabolic risk score and individual (waist circumference, fasting glucose, HDL, triglycerides, diastolic and systolic BP) cardiovascular risk factors.
Lamb et al. 2016.[145] 202 men and 106 women with type 2 diabetes, mean age 61 years.	Estimation of CRF by treadmill walk-test using sub-maximal heart rate two times four years apart.	Those increasing their CRF had a significantly lower constructed continuous metabolic syndrome sum of z-scores compared to those decreasing their CRF in age-and-sex adjusted and multi-adjusted analyses. For the individual components the analyses did not show significant findings besides waist circumference and systolic BP.
Lee et al. 2012.[135] Healthy participants (2,622 men and 526 women), mean age 42 years.	Maximal treadmill test estimating maximal METs in at least three surveys between 1979 to 2006.	Pearson partial correlation coefficients adjusted for age, sex baseline and change in percent body fat showed that change in CRF was inversely associated with change in systolic and diastolic BP, waist circumference, triglycerides, HDL and total cholesterol, but not with change in fasting glucose.
Rhéaume et al. 2011.[146] 132 previously healthy men (68) and women (64) mean age 35 years from the Quebec Familiy Study.	Sub-maximal cycle-ergometry estimated CRF mean 5.9 years apart between 1989 and 2001.	Change in CRF associated with change in HDL and a metabolic syndrome score after adjustment for visceral adiposity, age, sex and baseline level of risk factors. Associations were not significant for BP, insulin resistance, triglycerides, or inflammatory markers.
Sawada et al. 1993.[147] 3,305 healthy Japanese men mean age 32 years with normal baseline BP.	Cycle-ergometry estimated CRF using the Åstrand-Ryhming method in 1983 and 1988.	Changes in CRF was classified into improvement, unchanged, and deteriorated. The increase in BP in the group improving CRF was significantly lower than the other two groups after adjusting for initial BP, CRF, life-style variables and family history of hypertension.
Sternfeld et al. 1999.[148] General population sample of 1,777 black and white men and women aged 18-30.	Maximal treadmill test estimating maximal METs in 1985-86 and 1992-93	Modest correlations (Pearson) between change in CRF and change in total cholesterol, HDL, low-density lipoprotein, and triglycerides. In analyses adjusted for weight partial correlation coefficients only showed significant correlations to HDL.

1.6. Cardiac adaptations to exercise

Several mechanisms account for the enlarged stroke volume caused by endurance exercise, further associated with higher VO_{2max}. At the cellular and molecular level these alterations are characterized by enlargement of cardiomyocytes, changes in molecular expression improving contractility (e.g. change in contractile protein sub forms such as an increase in the α myosin heavy chain), and enhanced calcium handling improving LV relaxation.[149] On the macroscopic level, structural cardiac remodeling correlates strongly with exercise capacity,[150] and cardiac adaptations to even Olympic-level endurance exercise is known for a balanced LV remodeling, i.e. a combined LV volume and radius and mass / wall thickness increase preserving a low wall stress,[149,151] with increased maximal stroke volume and cardiac output.[152] The unchanged LV ejection fraction found in athletes shows how the LV end-systolic volume increases together with the end-diastolic volume,[151] but meta-analysis of exercise trials have shown that heart failure patients with reduced ejection fraction are able to increase their ejection fraction with endurance exercise.[153] Increased LV end-diastolic volume together with a functional decline in LV stiffness has been shown in RCTs of sedentary middle-aged men and women,[154] and after exercise in previously sedentary young adults.[152] Also, decline in constraints from the fibrous pericardium has been shown with endurance training, contributing to increased stroke volume and cardiac output associated with exercise training.[152] As for the LV, the left atrium (LA) also enlarges with long-term exercise training, as described in several studies on athletes.[155,156] Both the right atrium and ventricle also enlarge in response to endurance exercise, although they have not been subject to the same amount of research as the left-sided structures.[157]

1.6.1. The left atrium

The LA contributes to LV filling by acting as a reservoir during ventricular systole, and as a conduit and a booster pump during early and late diastole, respectively. Although the LA is enlarged in athletes, in the clinical context LA enlargement is included as a diagnostic criterion for LV diastolic dysfunction, due to its established function as a marker of increased LV filling pressures over time.[158–160] This stands in contrast to the normal diastolic function found in athletes.[161] Furthermore, LA enlargement is established as a marker of increased risk of CVD morbidity and mortality in clinical[162–164] and general population samples.[165] Doppler measures of diastolic dysfunction have also shown strong prognostic value in both general and diseased populations.[166,167] The diastolic dysfunction associated with LA enlargement,[168] and LA enlargement itself,[169,170] has traditionally been linked to a generally unfavorable cardiovascular

risk factor profile, which contrasts with the clinical characteristics one would expect with *athletic* LA enlargement. Although not established, one would expect a different prognosis for those with LA enlargement due to exercise training and/or a high CRF. However, both LA enlargement and an athletic history predicts atrial fibrillation (AF),[171,172] with increasing LA size having shown a linear relationship with AF prevalence in individuals with no obvious underlying disease[173] suggesting a linked pathophysiology. However, which is the chicken and which is the egg is not clear-cut, and LA enlargement as an underlying mechanisms for AF in athletes is still somewhat controversial.[174]. Although these findings in athletes may have implications for high-fit or very physically active non-athletic individuals, few studies have assessed associations between CRF, LA size, and diastolic function in the general population.[175] This represents another piece of the puzzle to disentangle the fine lines between pathological and physiological LA remodeling.

1.7. Summary of knowledge gaps and motivation

I. Numerous studies have evaluated the prognostic utility of CRF, but still the vast majority of evidence stems from studies using estimated CRF from maximal or submaximal treadmill or ergometer exercise without gas analysis, as opposed to directly measured VO_{2peak} by CPET. Furthermore, there is an underrepresentation of women, and most studies have been based on clinical populations referred for exercise testing.

II. Only one large study from the US has examined longitudinal changes in VO_{2peak}, which is of importance given the substantial variation in reference values across populations, and discrepancies in cross-sectional and longitudinal observations of age-related VO_{2peak}. Furthermore, few studies have examined longitudinal, concurrent changes in CRF and cardiovascular risk factors, and studies using directly measured VO_{2peak} from CPET are practically absent.

III. Despite knowledge on LA remodeling in athletes in relation to exercise status, there is a lack of studies addressing the relationship between CRF, LA remodeling and diastolic function in the general population. This is of particular relevance given the opposite prognostic implications in the available literature on having a high CRF versus an enlarged LA.

2. AIMS AND HYPOTHESES

The *general aim* of this thesis was to examine the prognostic significance of VO_{2peak} for CHD in healthy individuals, its longitudinal age-related decline, and associations to markers of cardiovascular risk.

2.1. Specific aims

2.1.1. VO_{2peak} and coronary heart disease

Aim was to study the prospective associations between VO_{2peak} and fatal and non-fatal CHD or coronary revascularization in a healthy low-risk cohort of both sexes. Furthermore, we aimed to investigate these associations separately for both acute and chronic CHD, and mortality, as well as the prognostic value of other CPET measures such as ventilatory efficiency equivalents for oxygen ($EqVO_2$) and carbon dioxide ($EqVCO_2$) and oxygen pulse. We hypothesized that low VO_{2peak} is a strong predictor of future CHD even in apparently healthy individuals.

2.1.2. Age-related changes in VO_{2peak} and associations to cardiovascular risk factors

Aim was to study longitudinal age-related changes in VO_{2peak} and the influence of intensity and volume of PA on these patterns. Furthermore, we aimed to investigate the association between change in VO_{2peak} with change of cardiovascular risk factor levels. We hypothesized that the longitudinal decline in VO_{2peak} was larger with higher age, and that better maintenance of VO_{2peak} was associated with favorable changes of cardiovascular risk factors.

2.1.3. VO_{2peak} and left atrial size

Aim was to study the cross-sectional association between LA volume, VO_{2peak}, and LV diastolic function in apparently healthy adults. Furthermore, we aimed to assess the association of LA volume to PA. We hypothesized that, in a healthy population, LA size is associated with VO_{2peak} and PA, but not with measures of LV diastolic dysfunction.

3. Material and Methods

3.1. The Trøndelag Health Study (HUNT)

The Trøndelag Health Study, abbreviated HUNT based on the Norwegian spelling "Helseundersøkelsen Trøndelag" (changed from "Helseundersøkelsen I Nord-Trøndelag" in 2019 after Nord-Trøndelag county was merged with Sør-Trøndelag county to form Trøndelag in 2018), is a population-based epidemiological study which started in 1984-86 (HUNT1).[176] Since then, additional surveys have been conducted in 1995-97 (HUNT2),[176] 2006-08 (HUNT3),[177] and 2017-19 (HUNT4). Participation rates in HUNT declined from 89% in HUNT1 and 70% in HUNT2, to 54% in both HUNT3[177] and HUNT4, which is similar to or higher than most participation rates reported in other epidemiological studies.[178] At each survey several sub-studies have been conducted, requiring participation in the main survey before inclusion.[177] Trøndelag and former Nord-Trøndelag county is located in the central part of Norway (Figure 3). The population of former Nord-Trøndelag was 137,223 by January 1st 2017,[179] living mostly in rural areas or small cities.[176] Education and income level is somewhat lower than national average,[176,180] and the population is predominantly Caucasian.

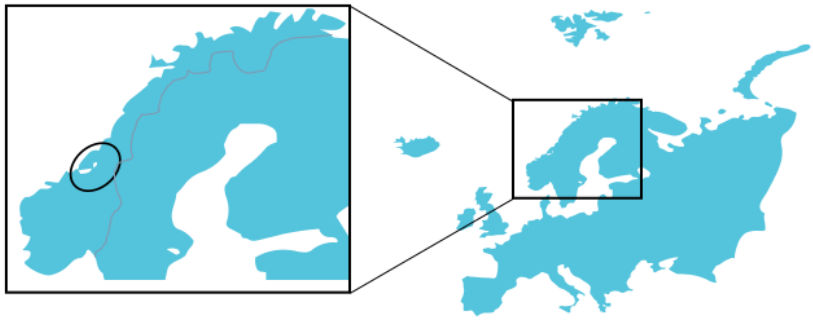

Figure 3. Geographical location of Trøndelag, Norway.

3.2. Study population and design

The three papers in this thesis is based on various selections of participants from sub-studies in HUNT3 and HUNT4, and specifically participants with overlap from the HUNT3 Fitness Study (paper I-III), the HUNT4 Fitness Study (paper II), and the HUNT3 Echocardiography Study (paper III). Description of the populations for the three papers is briefly summarized in Figure 4.

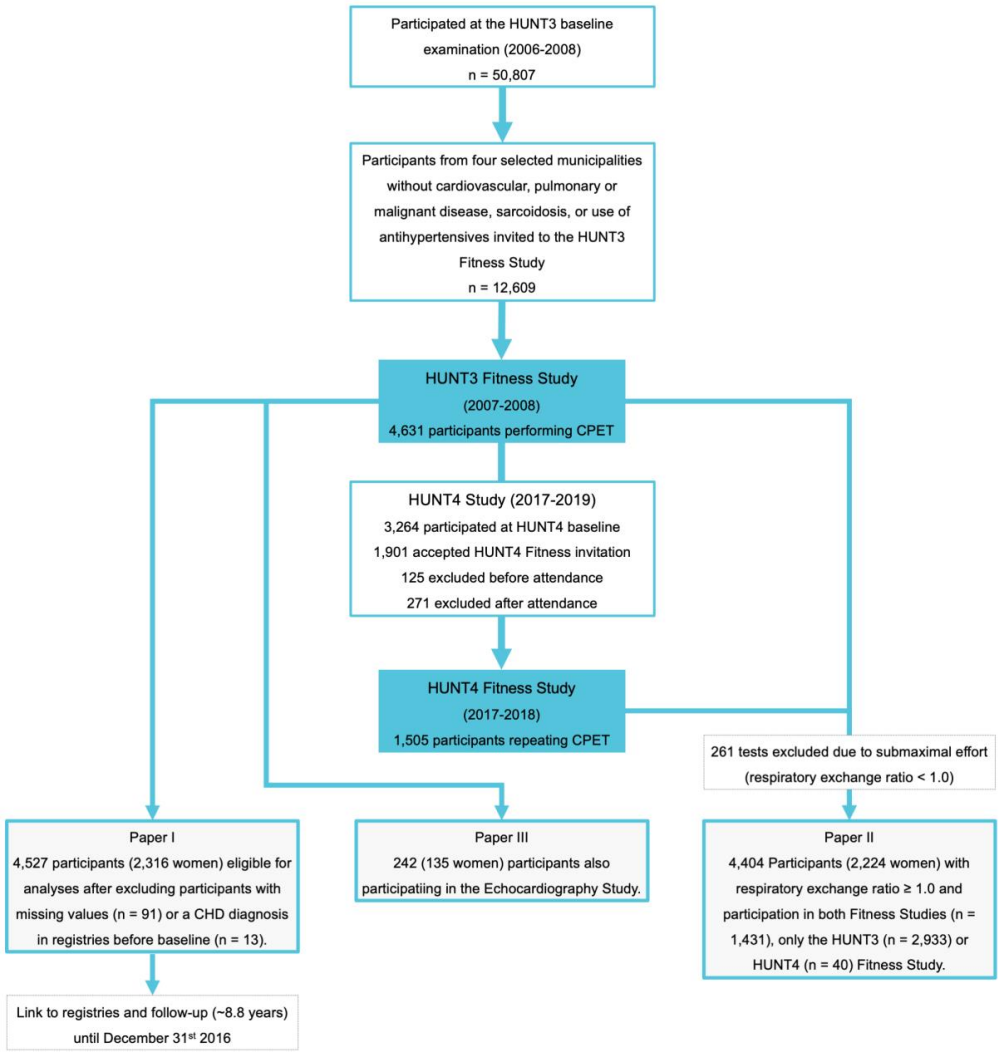

Figure 4. Overview of study populations in paper I-III.

3.2.1. Paper I

The *first paper (*corresponding aim in section *2.1.1.)* used a prospective observational design with basis in participants from the HUNT3 Fitness Study. Ninety-one of the 4,631 participants performing CPET had to be excluded due to missing information on one or more of the variables used in analyses, as well as 13 participants with a diagnose of CVD prior to participation in the HUNT3 Fitness Study (identified through registry data), yielding a total study population of 4,527. Ascertainment of end-points were based on information gathered from the Nord-Trøndelag Hospital Trust myocardial infarction registry, the regional health trust database on diagnoses and procedures, and the Norwegian Cause of Death Registry. Linkage between

HUNT and registry data is ensured through unique personal identification numbers. We used International Classification of Disease tenth edition (ICD-10) codes I20 (Angina pectoris), I21 (Acute myocardial infarction), I24 (Other acute ischemic heart disease), and I25 (Chronic ischemic heart disease) to define end-points of CHD. Information regarding coronary revascularization including percutaneous coronary interventions and coronary artery bypass graft surgery was based on relevant codes from Nomesco classification of surgical and radiological procedures. Information on cause and date of death was gathered from the Norwegian Cause of Death Registry. Death from CHD was based on underlying diagnosis of death (ICD-10 codes I20-I25), and death from CVD was based on underlying diagnosis of death (ICD-10 codes I00-I99).

3.2.2. Paper II

The second paper of the thesis (corresponding aim in section 2.1.2) used a longitudinal observational design utilizing repeated data (n=1,431) on VO_{2peak}, cardiovascular risk factors and PA from the HUNT3 and HUNT4 Fitness Studies, supplemented with data from participants only attending at the HUNT3 (n=2,933) or HUNT4 (n=40) Fitness Study in linear mixed model analyses.

3.2.3. Paper III

The third paper (corresponding aim in section 2.1.3) used cross-sectional data from participants in both the HUNT3 Fitness Study and the smaller HUNT3 Echocardiography Study, limiting the number of participants with overlap between the two studies to 242 (135 women). Participant overlap between the two sub-studies was limited due to being assigned different municipalities for inclusion with a common inclusion period in Namsos municipality only.

3.3. The HUNT3 Fitness Study

The HUNT3 Fitness Study was a sub-study of HUNT3 aiming to measure VO_{2peak} in a healthy, adult population, and to establish VO_{2peak} reference data.[76] The Fitness Study was conducted the same day as attending the HUNT3 baseline examination, and adults participating in HUNT3 reporting to be free from cancer, pulmonary disease, sarcoidosis, CVD, and antihypertensive medication (see section 3.7) were eligible for participation (n=30,588), whereas 12,609 of these were invited to participate as they lived in one of the four municipalities (Stjørdal, Levanger, Verdal, and Namsos) where the Fitness Study recruited participants. A total of 5,633

participants volunteered for exercise testing on a treadmill. 1,002 withdrew, did not finish of other reasons, or was excluded on basis of diagnoses or health issues not reported in the HUNT3 baseline questionnaire such as blindness, pregnancy, physical disabilities e.g. For complete information on reasons for exclusion see Aspenes et al.[76] In the end, 4,631 participants completed CPET having their VO_{2peak} established.

3.3.1. Cardiopulmonary exercise testing in HUNT3

The large and varied population participating in treadmill exercise testing called for a flexible and efficient exercise testing protocol. During a ten-minute warm-up the initial treadmill speed and inclination for the test was established guided by the Borg rating of perceived exertion scale (see section 3.6 for information on the Borg scale). When starting the test participants performed two submaximal steady-state levels before commencing the max test. Speed (0.5-1 $km \cdot h^{-1}$), inclination (1-2%), or a combination, was increased roughly every minute or when participants had stable oxygen uptake values over 30 seconds, and the test continued until voluntary exhaustion. Participants wore a tight face mask (Hans Rudolph, Germany) coupled to a MetaMax II mixing chamber gas analyzer (Cortex Biophysik Gmbh, Leipzig, Germany) using Metasoft 1 software for ventilatory gas analysis. Heart rate was measured by a Polar S610 or Polar RS300 (Polar, Kempele, Finland). Oxygen pulse was calculated the as VO_{2peak} in mL divided by heart rate at submaximal levels and peak exercise. Ventilatory efficiency equivalents was calculated as minute ventilation divided by carbon dioxide ventilation ($EqVCO_2$) or oxygen uptake in liters per minute ($EqVO_2$) at peak and steady state submaximal exercise. Procedures for calibration of the equipment were standardized. Two-point gas calibration towards known concentrations of carbon dioxide and oxygen was performed before the first test every day, as well as after every fifth test throughout the day. Volume calibration by a 3L standardized syringe (Calibration syringe D, Sensormedics, CareFusion, San Diego, CA, USA) was done before the initial test each day as well as after every third test. Ambient air was calibrated before each test. The MetaMax II analyzers were tested against a Douglas bag and iron lung and found both reliable and valid before commencing testing. Bland-Altman plots for test-retest of the MetaMax II was analysed in a previous study showing good repeatability, as did a coefficient of variability of 1.8%.[181]

3.4. The HUNT4 Fitness Study

The HUNT4 Fitness Study was a sub-study of HUNT4, and inclusion criteria were participation in the HUNT3 Fitness study, having a validated AF diagnosis (from previous work on the HUNT3 population[182]), self-reported AF in HUNT4, or participation in the HUNT3 Echocardiography Study. Furthermore, to be eligible all participants had to participate in the HUNT4 baseline examination mandatory for all HUNT participants. Participants from the HUNT3 Fitness Study in Namsos municipality (n = 343) was not re-invited to the HUNT4 Fitness Study as data collection in the HUNT4 Fitness Study ended before the HUNT4 baseline examination in Namsos was initiated.

Exclusion criteria in the HUNT4 Fitness study were limited to diseases or conditions associated with significantly elevated risk during exercise testing, in contrast to the HUNT3 Fitness study where stringent inclusion criteria ensured only healthy participants. In detail, exclusion criteria in the HUNT4 Fitness study were inability to walk uphill due to illness or physical limitations, uncontrolled hypertension >180/100, recent (last 6 weeks) heart failure admission, unstable angina, cardiac arrhythmia incompatible with safe exercise testing, severely elevated pulmonary artery pressure at echocardiography, recent (last 6 weeks) venous thromboembolism, symptomatic valvular heart disease, recent (last 4 weeks) myocardial infarction, recent cerebral infarction or haemorrhage (last 6 weeks), recent treatment for cancer (last 4 weeks), dementia, pregnancy, acute or chronic infectious contagious disease, or other serious illness incompatible with maximal exertion. A physician or a cardiologist was consulted in case of uncertainty regarding safety for exercise testing.

3.4.1. Cardiopulmonary exercise testing in HUNT4

The previously described procedure for exercise testing in HUNT3 was used for testing also in the HUNT4 Fitness Study, with a few notable exceptions regarding equipment and procedures. Gas analysis was performed with the Metalyzer (Cortex Biophysik Gmbh, Leipzig, Germany) portable mixing chamber system using Metasoft studio software. Calibration of volume using a standardized 3L syringe and ambient air were performed between each test. Sample line for gas analysis was changed every sixth test, and calibration towards gases with known content was done simultaneously as well as each morning. Participants with a physician-given diagnose of heart disease were tested with 12-lead electrocardiography (Custo med Gmbh, Ottobrunn, Germany) under surveillance of a physician. Heart rate was monitored by Polar heart rate

monitors (Polar, Kempele, Finland) for those without electrocardiography surveillance. Treadmill exercise was stopped at voluntary exhaustion, test abortion by the participant or the testing exercise physiologist, or by termination by the observing physician. Symptom and electrocardiography criteria for test abortion was developed in collaboration with a cardiologist complying to guidelines for exercise testing.[183] In both HUNT3 and HUNT4 the average of the three consecutively highest oxygen uptake values was defined as the VO_{2peak}. The NextMove core facility for exercise training and testing responsible for the study equipment validated the Metalyzer II systems used in HUNT4 against Douglas bag (mean bias 0.4 mL/kg/min, limits of agreement±6.0), and the test-retest repeatability coefficient for the Metalyzer II was 1.6 mL/kg/min. The Metalyzer II system used in HUNT4 was compared to the Metamax II used in HUNT3 (mean bias 1.4 mL/kg/min, limits of agreement±5.6 mL/kg/min). The same CPET protocol was used in HUNT3 and HUNT4, and effects of changing equipment and personell are thus expected to be low despite the ten-year follow-up.

In addition to CPET for assessment of VO_{2peak} a five-repetition sit-to-stand test, maximal squat-jump, and comprehensive transthoracic echocardiography were performed. A questionnaire regarding PA and sports participation was filled out by all participants, and participants with AF filled out a questionnaire with information on symptoms and quality of life regarding AF as well. All measurements in the HUNT4 Fitness Study were performed the same day, but median 44 (range: 5 to 270) days after inclusion in the HUNT4 baseline examination. Thus, weight measures for scaling of VO_{2peak} to body weight was based on measurements performed the same day as CPET.

3.5. The HUNT3 Echocardiography Study

The HUNT3 Echocardiography Study was a sub-study of HUNT3, including 1,296 participants from two municipalities (Steinkjer and Namsos) randomly selected for participation after participating at the baseline examination. Inclusion criteria were freedom from CVD, diabetes, and hypertension. Thirty participants were excluded due to cardiac pathologies which possibly could affect cardiac function. 265 participants were included in *both* the HUNT3 Fitness and Echocardiography sub-studies during data collection in Namsos municipality between June 9th and 19th 2008, and 242 of these had values for both echocardiographic measures and CPET available for analyses.

3.5.1. Echocardiographic acquisition

Transthoracic echocardiography was performed by a cardiologist experienced in echocardiography. Participants were examined in the left-lateral decubitus position using a Vivid 7 scanner with a phased-array transducer (M3S and M4S) (version BT06, GE Ultrasound, Horten, Norway). Recordings were performed during quiet respiration or breath-hold, and measurements represent an average of three cardiac cycles. Images were stored digitally and analyzed by the recording cardiologist.

3.5.2. Measurement of left atrial size and methodological considerations

LA size was quantified as maximal volumes using atrial-focused views from apical 4- and 2-chamber views in end-systole with tracing of the endocardial border,[184] excluding the pulmonary veins and the LA appendage. Start and stop of the trace were at the mitral annulus, with a straight line closing it. LA volume was calculated by the area-length and summation of disc methods[184] and subsequently indexed per square meter body surface area (LAVI) calculated by the formula by Dubois and Dubois. Mean difference between 4- and 2-chamber length was 0.36 cm (SD 0.28 cm). For 21 participants LAVI was estimated from one plane only.

LA size has commonly been measured by linear measures such as the anterior-posterior diameter in parasternal long axis view.[184] However, the LA does not enlarge symmetrically,[185,186] and biplane volume measurements correlates better with the (traditionally) more time-consuming echocardiographic three-dimensional quantification of volumes.[187] Further, LA volume is a better marker of CVD events than linear measures.[164] Also, atrial-focused two- and four chamber apical views, which avoid atrial foreshortening and maximize the LA area, contrary to ventricle-focused standard apical views, are important as atrial-focused views maximize LA volume quantification and improve agreement with image plane-independent three-dimensional measures.[188] The area-length and summation of disc methods for LA volume calculations are based on two-dimensional planimetry from the two apical views, having shown close agreement,[186,189] and are recommended in clinical use.[184] An advantage of the area-length method is the use of only one (the shortest) of two length measures, where a difference <0.5cm between the two atrial lengths indicates reliable measures.[184] Furthermore, although the summation of discs method has fewer geometric assumptions than the area-length method,[184] three-dimensional methods with less geometrical assumptions have

shown closer agreement to cardiac magnetic resonance (CMR) determined LA volumes than two-dimensional volumetric methods.[190,191]

3.5.3. Assessment of other echocardiographic measures

Mitral inflow pulsed-wave Doppler indices (early; E, and late; A) were assessed from apical 4-chamber with sample volume at the tip of the mitral leaflets together with assessment of E-wave deceleration time. The E/A ratio was calculated. Peak mitral annular longitudinal velocities were assessed from the septal and lateral wall by pulsed-wave tissue Doppler with sample volume localized to the basal part of the myocardium (near the insertion of the mitral leaflets). Peak velocities were measured at the outer contour of the Doppler spectrum using low gain settings. The average of the septal and lateral early (e') diastolic velocity was used for calculation of the ratio of the early mitral inflow to the early diastolic mitral annular velocity (E/e'). Pulmonary venous flow peak systolic (S) and peak antegrade diastolic velocity (D) were measured by analyzing their waveforms from pulsed wave Doppler with sample volume placed 1-2 cm into the upper right pulmonary vein, and the S/D ratio was calculated. When identifiable, peak velocity of the tricuspid regurgitation (TR) was measured by continuous Doppler. Assessment of diastolic function was based on current recommendations,[158] and following measures and cut-offs were used; mitral annular e' (septal e' <7 cm/s, lateral e' <10 cm/s), E/e' ratio >14, LAVI >34 mL/m^2, and peak TR velocity >2.8 m/s. LV diastolic dysfunction was present if more than half of the available parameters met these cut-off criteria. The number of participants with reduced ejection fraction was negligible, and thus we did not use a separate algorithm for assessing diastolic dysfunction in these subjects. Peak systolic mitral annular velocity was assessed by pulsed wave tissue Doppler similarly to e'. Tricuspid annular plane systolic excursion was measured at the tip of the tricuspid leaflets in M-mode. LV ejection fraction, global longitudinal end-systolic strain, and peak systolic tricuspid annular velocity were assessed as previously described.[192,193]

Reproducibility of a wide variety of measures in the HUNT3 Echocardiography Study has previously been published.[192,194] In addition, reproducibility of LA measures was calculated in a random sub-set of 145 participants by separate analyses performed by two experienced echocardiographers. Inter-rater coefficient of variation using separate echocardiographic datasets for LAVI was 12.1%, and for E, e' (average of septal and lateral), and E/e' 6.0%, 10.5%, and 7.9%.

3.6. Self-reported physical activity

Information on self-reported PA was based on a questionnaire specific to HUNT,[40] later coined "PAFID" (PA Frequency, Intensity, and Duration),[195] which has been used at the baseline examination in the HUNT1, HUNT3, and HUNT4 surveys (Table 2). The questionnaire has been validated showing good reliability[40] and to be valid compared to objective accelerometer measures.[40,195] A PA index calculated from the questionnaire correlated moderately with VO_{2peak} (Spearman r=0.48).[40] The correlation against accelerometer-measured light PA is reported to be low for PA volume/index calculations, while correlations against accelerometer measured moderate-to-vigorous PA has shown moderate correlations and the most valid results.[40,195] This may not be surprising, however, as the PAFID questionnaire asks specifically about bouted exercise as opposed to charting unplanned PA such as occupational, household or other incidental PA which may be best demarked by accelerometers and less by self-report. This also implies that "exercise", in the strict sense, is a more appropriate term for the information gathered in the questionnaires, but the PA terminology is commonly used in epidemiological studies. Notably, the question on intensity of PA provides information on *relative* intensity, contrary to the focus on absolute intensity in many studies[50] and questionnaires.

Table 2. The PA questionnaire in HUNT3 and HUNT4. Information in square brackets depicts values used for calculation of volume and weighted volume of PA.

Questionnaire component	Response
Frequency How often do you exercise? (average)	• Never [0] • Less than once a week [0] • Once a week [1] • 2-3 times a week [2.5] • Nearly every day [5]
Duration For how long do you exercise each time? (average)	• Less than 15 minutes [7.5] • 16 - 30 minutes [22.5] • 30 minutes - 1 hour [45] • More than 1 hour [75]
Intensity If you exercise as often as once or several times a week: How hard do you exercise?	• I take it easy, I don't get out of breath or break a sweat [low intensity, 0.5] • I push myself until I'm out of breath and break into a sweat [moderate intensity, 1] • I practically exhaust myself [high intensity, 2]

In all three papers, volume of PA was calculated as frequency multiplied by duration (minutes per session) according to values in square brackets in Table 2. Weighted PA volume was

calculated as PA volume multiplied by 1 for moderate intensity and 2 for high intensity, and in paper II times 0.5 for light intensity. Thus, the weighted PA volume was calculated with minor modifications from previous validated indexes based on the questionnaire,[40,196] to keep the scale in minutes of PA and to enable easy comparison to PA recommendations. In papers I and III light intensity was classified as inactive and thus a weighted volume of zero. Information on PA intensity was based on the intensity measure from the PAFID questionnaire for study I and II, while in paper III we used the Borg scale from the HUNT Fitness Study questionnaires, grading Borg 12 and 13 as moderate intensity and ≥14 as high intensity.[30,49] The HUNT Fitness Study questionnaire asked "On a scale from 6-20, how intense do you usually exercise?" with available responses from 6 to 20.[49] Adherence to PA recommendations was classified as equivalent to ≥150 weighted minutes of moderate and high-intensity PA (paper I-III).

3.7. Other self-reported information

The questionnaires in HUNT3 and HUNT4 include a large variety of questions. In both HUNT3 and HUNT4 participants received an initial questionnaire (Q1) per mail and were asked to fill out before attending the baseline examination, with an online response solution added in HUNT4. After submitting Q1 participants were handed further questionnaires based on age, sex and their responses regarding previous medical history e.g. in Q1. The information used in this thesis was asked in Q1 in both HUNT3 and HUNT4,[197] except the PA Borg intensity question used in paper III, which was asked on a questionnaire specific to the HUNT3 Fitness Study.

Information on smoking status (never, current, former, occasional, plus former occasional in HUNT4) was dichotomized to current regular or occasional smoker (yes/no; paper I-III). Pack-years of smoked cigarettes calculated at the HUNT baseline examination was based on several questionnaire items such as year starting and stopping, and number of daily cigarettes, as well as using self-reported information from previous HUNT surveys, if available (continuous variable; paper I). Snuffing status (never, current, former, occasional) was dichotomized to current snuffer (yes/no, paper I). Alcohol consumption in the last 12 months (always abstained, not intake last 12 months, once a month, 2-4 times a month, 2-3 times a week, 4 times or more a week; used for analyses in paper I) was dichotomized to alcohol intake >1 per week (yes/no; analyses in paper II and Table 1 in paper I). Family history of myocardial infarction or stroke in a first degree relative <60 years of age, was dichotomized to yes/no (paper I and II).

Information on previous medical history of diabetes in HUNT3 (paper I), a history of CVD (myocardial infarction, angina, heart failure, AF, cerebral hemorrhage or infarction), pulmonary disease (asthma or chronic obstructive pulmonary disease) or malignant disease (yes/no) in HUNT4 (paper II), and use of antihypertensives (yes/no; paper II) or cholesterol-lowering therapy (yes/no; paper II), was based on self-report in Q1. As it was a part of exclusion criteria for the HUNT3 Fitness Study, information on CVD (similar questions as for HUNT4 except "other heart disease" was asked instead of "AF"), pulmonary and malignant disease, sarcoidosis, and use of antihypertensive medications (all similar to HUNT4) was only used in identification of eligible participants in the HUNT3 Fitness Study and not for analyses (paper I-III). Further detailed information on questionnaires are available from the online HUNT Databank.[197]

3.8. Clinical measurements

Measurements were performed using standardized procedures by trained personnel in both HUNT3 and HUNT4. Height and weight were measured wearing light clothes without shoes, using a manual scale (Jenix DS-102 automated height and weight scale) in HUNT3 and an InBody 770 in HUNT4. Waist circumference was measured standing in a relaxed position with hands hanging down, at the height of the umbilicus, and hip circumference as the largest circumference around the hip. Body mass index (BMI) was calculated as kg divided by height in meters squared. BP was measured sitting to the nearest 2 mmHg by an oscillometry based Dinamap Carescape V100 in HUNT4 and Critikon 845XT in HUNT3, and the average of the last two of three measurements separated by one-minute breaks was used, or only the second if only two measurements were performed. Heart rate was measured simultaneously as BP, and the lowest of the available measurements was recorded as the resting heart rate.

3.9. Biochemical measurements

Non-fasting blood samples were drawn at both HUNT3 and HUNT4 for analyses of glucose (HUNT3), HbA1c (HUNT4), HDL cholesterol, total cholesterol, triglycerides, c-reactive protein, and creatinine. Analysis were performed by an Architect ci8200 in HUNT3 and HUNT4, although using slightly different methodologies (detailed information available from HUNT Databank[197]). Non-HDL cholesterol was calculated by subtracting HDL from total cholesterol (paper I), and total to HDL cholesterol ratio was calculated (paper II).

Classification to metabolic syndrome, dyslipidemia, and hypertension was used as an outcome measure in paper II, and some analyses were adjusted for dyslipidemia in paper I. Metabolic syndrome was defined as reaching at least three of the following thresholds;[198] waist circumference >88/102cm in women/men, triglycerides ≥1.70 mmol/L, HDL <1.3/1.0 mmol/L for women/men, elevated BP (systolic >130 or diastolic >85 mmHg or use of BP medication), and hyperglycemia using HbA1c ≥39 mmol/mol as the hyperglycemic criterion.[199] Dyslipidemia was defined as total cholesterol >7.0, or HDL or triglycerides according to the mentioned thresholds. Hypertension was defined as systolic and/or diastolic BPs >140/90 mmHg, respectively.

3.10. Statistical analyses

Statistical analyses were performed by using STATA15.1 (StataCorp, TX, USA) (paper I and III) and R (www.r-project.org) (paper II and III, and for other analyses and figures provided to this thesis).

3.10.1. Paper I

Age- and sex-specific quartiles of VO_{2peak} were generated by providing each gender-specific decade of age with a percentile based on their VO_{2peak} before categorizing in quartiles according to the calculated percentiles. The ten-year risk of myocardial infarction and cerebral stroke was calculated using the NORRISK2 risk prediction algorithm developed in a Norwegian population.[200] The model includes information on age, systolic BP and use of BP medication, total cholesterol, daily smoking, HDL cholesterol, and family history of CVD. The risk score was calculated, as described in detail by Selmer et al.,[200] with a simplification of family history of CVD where the HUNT questionnaire only charts ≥1 first degree relative with CVD events, as well as including participants at all ages, although the risk model is originally developed for the ages 45 to 74. Survival analysis by the Cox proportional hazards model was performed to assess the association between VO_{2peak} as a continuous variable and as quartiles to a primary composite end-point of diagnose or death from CHD, or coronary revascularization, as well as separate analyses for secondary end-points of acute and chronic CHD, and mortality. The Cox model is widely used in analysis of survival data, since it does not make assumptions regarding the survival distribution[201] and allows for inclusion of covariates for adjustment of confounders. Attained age, contrary to time-on-study, was chosen as time scale to reduce risk of bias.[202] The Cox model assumes proportional hazards, meaning that residuals (Schoenfeld residuals) are

unrelated to time in the model. This was tested by hypothesis testing without signs of violation (sex approached significant violation with p=0.08, but sex-stratified analyses were presented). Kaplan-Meier curves were produced to visualize the association of quartiles of VO_{2peak} to the primary end-point.

For the Cox models we adjusted for sex in one model (and inherently for age by attained age as the time scale in the models), and in a second model we adjusted for other variables also perceived as confounders based on a-priori knowledge (smoking status, alcohol use, and family history of CVD). In sensitivity analyses, further adjustment for other clinical variables and risk factors were included. Relationships between several clinical and demographic variables to the exposure and outcome variables were investigated using directed acyclic graphs to assess their status as confounders, mediators, or colliders. Cox analyses were also performed for oxygen pulse, $EqVCO_2$, and $EqVO_2$ with additional adjustment for BMI, since these CPET measures where not weight scaled.

We analyzed discrimination and reclassification of cardiovascular risk by adding VO_{2peak} to the variables from the NORRISK2 model. Harrels C index (C-statistic), which is an expansion of receiver operator characteristics to survival analysis,[203,204] net reclassification index (NRI), and integrated discrimination improvement (IDI) were calculated.[205] The C-statistic gives the probability that a person with event (CHD) is classified to a higher risk than a person without event,[206] thus assessing discrimination (the ability to correctly classify subjects to event or non-event). A C-statistic over 0.50 thus implies a better discrimination than by chance alone. The NRI is calculated as a net correct change of reallocating subjects to risk-categories based on addition of the new variable,[205,207] while the IDI is similar but does not apply the more or less arbitrary risk categories or thresholds.[205] The categories chosen for the NRI had to be based on a modification of the NORRISK2 categories,[200] since the original NORRISK2 risk categories (low, moderate, or high risk) differs according to age category, and we did not have the statistical power to perform these analyses stratified by age. For example, low risk is classified as <4% in age 45 to 54 years, but <12% in age 65 to 74 years. We chose cut-offs of <5% (low), 5-10% (moderate) and >10% (high) risk as an adaptation to the original NORRISK2 categories (see Supplemental Material in paper I for details).

3.10.2. Paper II

As the time between participation in the HUNT3 and HUNT4 Fitness Studies differed between subjects (mean 10.2 years, range 9.5 to 11.0), change in VO_{2peak} and change in CVD risk factors were scaled to ten-year change ((value HUNT4 – value HUNT3)×(10/time in years)). Mean ten-year changes in absolute, relative, and percentage declines of VO_{2peak} were presented by deciles of age and sex. Furthermore, age-related changes in VO_{2peak} were analyzed using a linear mixed effects regression model.[208] Models were fitted by maximal likelihood to allow assessment of model performance (trade-off between model fit and simplicity) by the Akaike information criterion, which is suited for exploratory analysis (identifying the best model), as it allows comparison between non-nested models.[209] As the linear mixed model allows imbalance in repeated measures we could include VO_{2peak} measurements from subjects only participating in HUNT3 as well. The mixed model approach handles dependence between repeated observations within the same subject by treating subjects as a random intercept.[210] Several models were fitted including age, sex, survey, weighted weekly minutes and intensity of PA, current smoking, alcohol use, and presence of CVD or pulmonary disease, and exploring for interaction between covariates and for quadratic or higher-order polynomials for age and weighted weekly minutes of PA. The best fit models with regression equations are presented in the Supplemental Material of paper II. Results from the linear mixed models were presented graphically, keeping continuous covariates at their mean and categorical covariates at representable proportions, unless otherwise specified.[211]

Concurrent changes in VO_{2peak} and cardiovascular risk factor levels were analyzed by linear regression models. Analyses were performed for HDL (n = 1,177), total cholesterol (n = 1,177), total to HDL-cholesterol ratio (n = 1,177), triglycerides (n = 1,204), systolic (n = 1,190) and diastolic (n = 1,189) BP, resting heart rate (n = 1,388), and waist circumference (n = 1,382). Participants reporting cholesterol or BP medication use in HUNT4 were excluded in analyses on lipids and BP, respectively. Based on a-priori assumptions on causal relationships we included adjustment for variables perceived as confounders (age and VO_{2peak} at HUNT3, sex, current smoking, and regular alcohol intake at HUNT3 and HUNT4, family history of CVD, and incident CVD between HUNT3 and HUNT4) in a first model. In a second model we included adjustment for weighted weekly minutes of PA at HUNT3 and HUNT4, and change and baseline value of weight. Effect modification based on sex, baseline VO_{2peak} (over/under age- and sex-specific averages based on a previously published reference equation[62]), age

(over/under 50 years), and BMI (over/under 30kg/m^2) were explored in stratified analyses. Assumptions regarding normality of residuals and heteroskedasticity were checked visually.

Logistic regression analyses were performed to investigate associations between change in VO_{2peak} as the predictor, with presence of metabolic syndrome, dyslipidemia and hypertension at HUNT4 as outcomes. Analyses were adjusted as for the previously described models, as well as for use of lipid-lowering drugs for the dyslipidemia model and BP medication for the hypertension model. Sensitivity analyses were performed excluding participants with medication use for the respective analyses. Age was included as a categorical variable in the analyses for metabolic syndrome, due to non-linear associations with age, but kept continuous in the other analyses.

3.10.3. Paper III

VO_{2peak} was expressed in mL/kg/min and as a percentage of predicted by age and sex ($VO_{2\%pred}$). $VO_{2\%pred}$ was calculated as (measured/predicted)×100. The predicted values were based on regression equations from a previous study based on the whole HUNT3 Fitness Study population (Men: VO_{2peak}=63.6-0.393×Age. Women: VO_{2peak}=51.6-0.328×Age).[62] Firstly, the influence of VO_{2peak} on top of previous variables shown to be independent predictors of LAVI[212,213] were explored by multiple regression. Differences in LAVI across sex and adherence to PA guidelines were tested by analysis of covariance. The associations between LAVI and VO_{2peak}/$VO_{2\%pred}$ were explored using multiple regression, including age and sex as covariates, while exploring for interaction between covariates and quadratic or higher-order polynomials for age and VO_{2peak}/$VO_{2\%pred}$. To allow for a more flexible modelling of VO_{2peak}, i.e. to let the regression coefficient for VO_{2peak} vary at different levels, we also fitted restricted cubic and linear spline models for $VO_{2\%pred}$ with knots set at 80%, 100%, and 120%. The best model fit was determined using the model with the lowest Akaike information criterion. Regression models were further fitted to assess associations between LAVI and other measures of diastolic function, and between measures of diastolic function and VO_{2peak}. Also, simple linear regression analyses for LAVI by VO_{2peak}, age, and weighted volume of PA, respectively, were performed. Results from the best fitted predictive model were shown graphically as predicted values of LAVI based on age and the measures of VO_{2peak}. Sensitivity analyses were performed by excluding participants with single-plane measurements of LAVI and differences in LA length between 2- and 4-chamber views >0.5cm, and analyses were performed with

LAVI measured both by the area-length method and summation of discs method, with main results presented for the former.

3.11. Ethics

All participants gave informed consent to participation in HUNT and in the mentioned sub-studies. The consent in HUNT is dynamic, meaning that information gathered and stored based on previous consent may be destroyed upon the participants' request at a later time. All studies in this thesis were approved by the Regional Committee for Medical Research Ethics (REC) in Central Norway (paper I: 2016/443, paper II: 2019/7243, paper III: 2018/929). The Norwegian Data Inspectorate approved the HUNT Study.

4. General Methodological considerations

The internal validity of epidemiological studies is generally challenged by issues regarding confounding, selection bias, and information bias.[214] Selection bias refers to biases arising from factors affecting participation or selection of participants to a study.[214] In practice, this means that the study sample in question may not be representable for the population one aims to make inferences about. Selection bias is a common challenge in cohort studies, e.g. due to self-selection for participation. In HUNT3, participants had higher socioeconomic position, less chronic disease, and lower mortality risk compared to non-participants.[215] Participation in exercise testing is expected to introduce some selection bias towards healthier, more physically active and fitter participants, as was indeed shown for the HUNT3 Fitness Study (see chapter 3.3).[76] The HUNT3 Fitness Study population was previously compared to the total HUNT3 population and to the proportion of the HUNT3 population reaching inclusion criteria for the Fitness study but not participating (non-participants). The total HUNT3 population had higher prevalence of cardiovascular risk factor clustering, and physical inactivity was more prevalent in both the total HUNT3 population and the non-participants.[76] In HUNT3, VO_{2peak} was higher than in other international cohorts,[61,99] which probably at least partly stems from this selection effect. However, it should be noted that the HUNT3 reference values were more similar to publications from other cohorts in the western[41] and south-eastern[41] parts of Norway than the international cohorts. Furthermore, in the papers included in this thesis, participants with CVD, pulmonary disease, cancer, sarcoidosis, or use of antihypertensives at baseline in HUNT3 were excluded, meaning that our results are not necessarily generalizable to populations with prevalent disease. However, the healthy sample reduces the risk of underlying disease confounding the associations between VO_{2peak} and end-points, thus strengthening the internal validity of the study, which is especially important for the results in paper I. Another strength is the wide age-range and balanced sex-distribution in the study sample increasing generalizability. Still, the mentioned caveats should be kept in mind when appraising the generalizability (external validity) of the results.

Confounding is a concept with overlap towards selection bias, as both concepts depicts mechanisms for bias in the relationship between exposure and outcome.[216] By definition, a confounder is a factor associated with the exposure, it is an extraneous risk factor for the outcome, and it is not affected by the exposure or outcome.[214] Not appropriately controlling for confounders may introduce spurious associations or bias in effect estimates. Residual

confounding due to imprecise and/or incomplete ascertainment of perceived confounders may also yield effect estimates placed somewhere between the true causal estimate and the unadjusted estimate.[214] On the other hand, inappropriate adjustment for a covariate may *cause* confounding or selection bias by introducing a collider. In practice, a collider is a variable which may lead to a spurious association between exposure and outcome by wrongfully adjusting for it (or in other ways conditioning on it). Over-adjustment of statistical models may thus cause confounding by adjustment for such (collider) variables, but may also underestimate the causal effect by inappropriately controlling for an intermediate variable (mediator).[217] Unnecessary adjustment, on the other hand, a concept different from over-adjustment, may affect precision.[217] These concepts were kept in mind when planning statistical analyses, and directed acyclic graphs were used to explore the best possible strategies for adjustment. We aimed to adjust only for clear confounders in the main models in paper I and II, to minimize the risk of introducing confounding from colliders, affecting precision, or underestimating the causal effect.[216] It should also be acknowledged that selection bias may lead to collider bias in associations between variables that influence participation in a study.[218]

Bias stemming from measurement error when trying to estimate the effect of an exposure is known as information bias.[214] The direct measurement of VO_{2peak} results in a low risk of information bias in measuring CRF, and also allows for mentioned objective criteria for a maximal effort, as opposed to other criteria such as reaching a percentage of age-predicted maximal heart rate or voluntary self-reported exertion. Ascertainment of PA exposure is challenging and susceptible to information bias and measurement error,[2] which is of special importance for paper II and III. However, established objective measurements of PA, such as doubly labelled water or accelerometer measures, lack relative intensity measures and clear-cut thresholds, as well as being impractical, which is especially true for the former. Accelerometers combined with heart rate monitoring answer several of these problems, but are also expensive and hard to implement in an epidemiological scale.[46] Thus, self-reported measures are often employed for practical reasons, as in HUNT, and therefore in this thesis. The PA questionnaire used in this thesis is proven valid and reproducible in line with other self-report PA measures,[40,195] as discussed in section 3.6, but self-reported PA has limitations. One mechanism likely to give bias is the tendency to report what is thought to be a favorable behavior, often named social desirability bias.[219,220] In line with this, high-intensity PA is commonly overestimated while sedentary time is underestimated.[220] Differences in reporting PA behavior have also been shown to vary with age and to be less accurate in adults >65 years,[220] which is

of interest given the trends with higher age described in our results. Furthermore, assessing light PA has generally proven to be more difficult, which has been explained by how unplanned activities such as walking, etc., are harder to remember (recall bias) than deliberate and planned activities,[2] and calculation of energy expenditure based on self-report is not very accurate with most correlations <0.60 compared to doubly-labelled water.[2,221] However, we expect misclassification of PA to be non-differential, meaning that we do not think classification of PA is associated with the outcomes in paper II or III. Such non-differential misclassification, as opposed to differential, generally causes underestimation of the associations and effect estimates, and not overestimations.[214] Similarly, measurement error and misclassification of other covariates used in analyses in paper I-III may affect estimates by residual confounding.

Further elaboration on methodological considerations specific to each paper are discussed where relevant in each of the following sections.

5. Results and Discussion

5.1. Paper I: VO_{2peak} and incident coronary heart disease

The prospective associations between directly measured VO_{2peak} and CHD was investigated in 4,527 apparently healthy participants from HUNT3 followed for a mean 8.8 years (40,060 person-years). During the course of follow-up, 147 participants met the primary end-point of diagnose or death from CHD, or coronary revascularization. VO_{2peak} was lower in those eventually reaching the primary end-point (n =147) compared to those not (Figure 5).

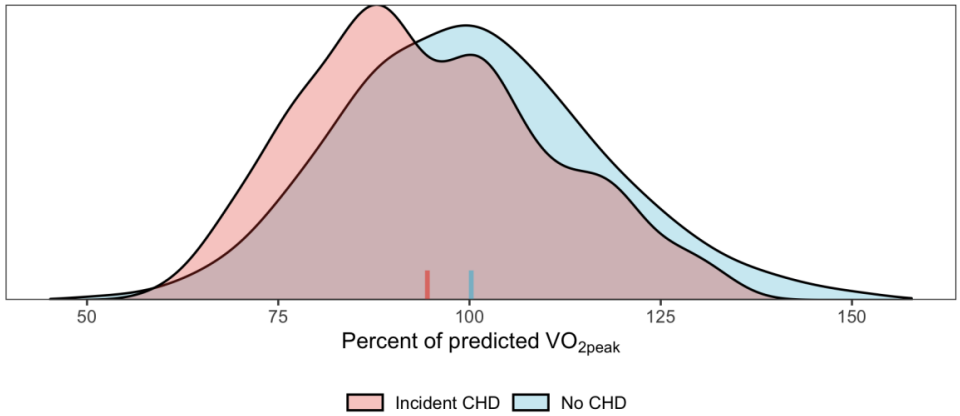

Figure 5. Distribution of $VO_{2\%pred}$ in those reaching and not reaching the primary end-point. The vertical bars at the x-axis corresponds to the mean for each distribution. $VO_{2\%pred}$ was calculated as described in section 3.10.3.

VO_{2peak} was strongly and inversely associated with the primary end-point, with 15% (95% confidence interval (CI) 7 to 23; hazard ratio (HR) 0.85 95% CI 0.77 to 0.93) lower risk per one MET higher VO_{2peak} in Cox proportional hazards analyses adjusted for age, sex, smoking status, alcohol use, and family history of CVD. Analyses by quartiles of VO_{2peak} showed a similar trend, with a ~50% lower risk of the primary end-point in the highest-fit quartile compared to the lowest-fit (Figure 6). However, in women the lowest risk was observed in the third quartile, compared to in the fourth in men, but the relatively low number of events and wide CIs merit conservative interpretations of these trends in women. The overall effect estimate for the primary end-point was somewhat attenuated in women (12%, 95% CI -8 to 27) compared to men (17%, 95% CI 8 to 25).

Furthermore, analyses for secondary end-points of myocardial infarction, chronic ischemic heart disease, coronary revascularization, and CVD mortality showed similar effect-estimates (Figure 6). All-cause mortality showed a weaker association than the other analyses with a 6% (95% CI -5 to 16) lower risk per one MET for the analysis adjusted for age, sex, smoking,

alcohol use, and family history of CVD. VO_{2peak} was also significantly and inversely associated with CHD in analyses stratified by high and low-medium baseline risk. Adding VO_{2peak} to the NORRISK2 risk prediction algorithm improved discrimination assessed by both the NRI (6.6% net improved reclassification, p=0.044) and the IDI (0.4% net improved probability estimates of event, p=0.019). However, the C-statistic did not improve by adding VO_{2peak} (change in C=0.003, p=0.43). Furthermore, risk for the primary end-point was 8% (95% CI 2-13) lower per mL/heart beat higher peak oxygen pulse, and the risk per unit higher peak $EqVO_2$ (3%, 95% CI 0-6) and $EqVCO_2$ (4%, 95% CI 0-8) was also significantly higher. Similar results were shown for submaximal values (see Supplemental Material in paper I).

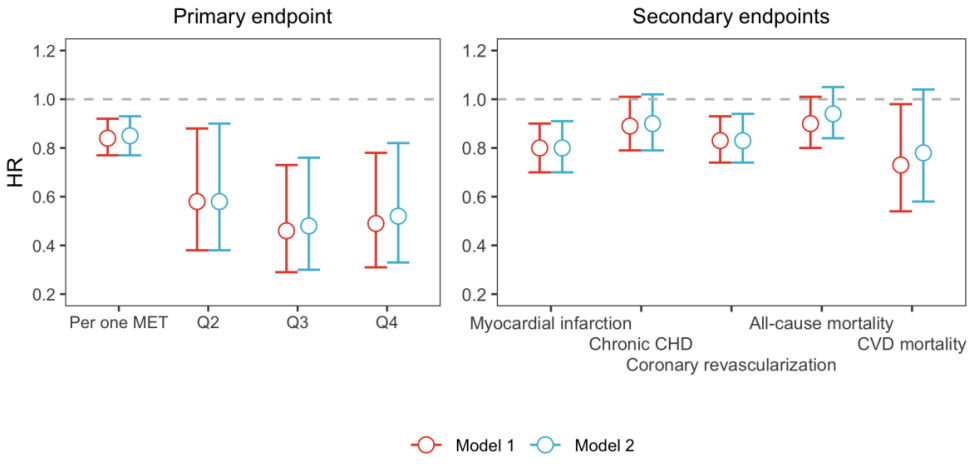

Figure 6. Overview of HR for primary and secondary end-points from paper I. Quartile 1 (Q1) serving as reference for quartile 2-4 (Q2-4), not shown. Secondary end-points shows results per one MET higher VO_{2peak}. Model 1 adjusted for age and sex. Model 2 as for the first model + smoking status, alcohol use, and family history of CVD.

5.1.1. VO_{2peak} and clinical end-points

The mean estimate of 15% (95% CI 12 to 18) lower risk of CHD/CVD per one MET higher CRF in the meta-analysis by Kodama et al.[107] was identical to the results for sexes combined in our study. For the 24 studies included in the CHD/CVD meta-analysis effect estimates ranged from 49% to 3%, all pointing in the same direction. Although there is a lack of studies examining these associations using directly measured VO_{2peak}, studies on participants free from known CHD from the KIHD study and the BLSA showed similar results. The KIHD study showed a 7% (multi-adjusted) and 13% (age-adjusted) lower risk per MET higher VO_{2peak} for non-fatal CHD,[109] and 13% and 16% lower risk for fatal CHD/stroke and CVD death, respectively.[222] In the study from the BLSA the risk for CHD was roughly 17% lower per one

MET (calculated from estimates given per SD higher VO_{2peak}).[119] Similar results were also shown in a study by Imboden et al. from the Ball State Adult Fitness Program Longitudinal Life-style Study published a few weeks prior to our publication of paper I. In that study, 4,137 men and women without prior malignancy or CVD, from a wide age-range, who self-referred to a fitness-program, or participants contributing in research studies between 1968 and 2016, were followed for CVD, cancer, or all-cause mortality.[223] Although not investigating CHD specifically, their results showed similar findings with 16.1% lower risk of CVD mortality per one MET higher VO_{2peak}, compared to 22% lower risk in our model 2. Importantly, contrary to most previous studies they also reported outcomes stratified by sex, with considerably lower and non-significant estimates for women compared to men (3.9% vs 20% lower risk per MET, respectively). The age and sex-adjusted HR for all-cause mortality in our study was very similar compared with the one from the Ball State cohort[223] (HR 0.90 and 0.88, respectively), and also relatively similar to the meta-analysis by Kodama (HR 0.87).[107] However, the estimate from model 2 in our study was somewhat lower compared to these estimates, with 95% CIs reflecting uncertainty of both direction and size of estimates (HR 0.94, 95% CI 0.84 to 1.05). This might not be so surprising given that malignant disease was the leading cause of death (48%), 2.5 times more prevalent than CVD (20%). Furthermore, relatively few died during follow-up (~2%), and thus it is likely that these trends as well as the low-risk and relatively young cohort with regard to CVD events, explain the somewhat weaker association to all-cause mortality compared to other studies.

A wide range of other studies has examined the predictive value of maximal or submaximal exercise estimated METs on mortality, CVD, and CHD outcomes with relatively consistent findings of inverse associations.[107,112,115,224–226] Some of the most relevant findings with focus on CHD outcomes will be discussed below. Gander et al. studied 29,854 men from the ACLS cohort without CVD or cancer at baseline and found a 20% risk reduction per one MET higher CRF for a composite CHD end-point similar to ours.[227] Similar findings have also been observed per MET regardless of measures of genetic risk of CHD by family history or a genetic risk score.[228,229] Harber et al. pointed out the relative lack of studies on CRF and mortality outcomes in women,[108] which is also true for studies on CHD end-points. As mentioned previously, Imboden et al. reported much weaker associations between VO_{2peak} and *CVD* mortality for women compared to men, representing the only study other than ours to report sex-specific outcomes by directly measured VO_{2peak}.[223] In a large study of 5,909 women and 20,728 men from the ACLS, both sexes showed similar effect estimates in comparisons of low,

medium, and fit groups for both total CVD, CHD, and myocardial infarction. However, precision was low for women, with non-significant estimates.[230] Balady et al. examined the predictive value of exercise capacity in 3,043 participants from the Framingham Study and found a 3% and 6% risk-reduction for CHD per MET in women and men, respectively, adjusted for risk factors included in the Framingham risk score.[231] The strategy for model adjustment included possible mediators of the relationship between CRF and CHD, which may explain the weak effect sizes reported compared to many other studies, especially for men. The findings were not significant for women. In analyses stratified by baseline ten-year Framingham risk scores the highest-risk group had 13% lower risk per MET compared to 6% in the lowest-risk group. Our estimates showed similar results for various levels of baseline risk, but the baseline risk was generally higher in the study from Framingham. A study from the ACLS has also shown a lower predictive ability of CRF for CHD in women compared to men, with no observed association per MET achieved.[232] In our paper II the adjusted (model 2) HR estimate of 0.88 (95% CI 0.73 to 1.08) was similar to the effect estimate for men, but the wide CI indicated that also a null-effect, or a much lower effect, was compatible with the present data. Ours and the cited studies all give estimates of a somewhat smaller, but still positive, effect in women. However, as described, precision is often too low to conclude due to lower number of included women and lower event-rates.

The meta-analysis by Kodama et al. showed a considerably stronger effect for intermediate vs. low CRF category compared to high vs intermediate (adjusted relative risk 1.47 vs 1.07, respectively) for CHD/CVD outcomes, and suggested the lowest CRF thresholds to reduce risk of adverse events in the range 5 to 9 METs. Other studies have also suggested similar cut-offs in the low-end spectrum of CRF for reducing risk.[113] A large study from the UK Biobank including over 70 000 participants of a wide age-range, both sexes, and a diverse ethnic background, found that the risk of CHD incidence was higher below roughly 7 METs, and that there was no obvious protective effect of having a higher CRF.[233] The estimates for myocardial infarction even suggested an increased risk in the high-end specter of the CRF, but precision was low and not significant in these estimates. The main limitation was the submaximal measures of estimated CRF making assumptions regarding predicted maximal heart rate in CRF calculation. Also, assumptions regarding the causal structure in adjustment for confounders was somewhat different, and they included adjustments for diet, which is a strength. Contrarily, our results indicate that VO_{2peak} is predictive of CHD also in a fit cohort, and that the effect is consistent within the high-fit portion when performing per one MET analyses in strata of high

baseline VO_{2peak} as well. Although our results also suggest that the largest effect is obtained by keeping clear of the lowest quartile, there still seems to be benefits to harvest with higher CRF, as shown in the higher quartiles for men, although there was a signal of increased risk in the fourth quartile of CRF in women. However, also here, precision was very low in these analyses for women, given the few events. Findings of protective effects of CRF on mortality have been shown even in the high-end of fitness in several cohorts.[233,234] It should be emphasized that comparing thresholds across studies does not necessarily make much sense given the varying normal values for VO_{2peak}.[61,99] E.g., 50-year-old men in the ~25th percentile in our population (see Supplemental Material in paper I) would be in the ~50th percentile in the FRIEND registry.[61]

5.1.2. The role of fitness in predicting cardiovascular risk

The American College of Cardiology and American Heart Association evaluated the value of including CRF in risk prediction to be uncertain in their 2013 guideline on assessing cardiovascular risk,[235] but several studies, including ours, have increased knowledge in the field since then. In our study, the reclassification indices NRI and IDI showed improved risk discrimination, although the C-statistic did not. Since each method has different weaknesses, and no method captures all important characteristics of a new risk marker, we chose to perform all these analyses, as has been recommended.[236] The C-statistic has been criticized for being too conservative in assessing the addition of new variables to prediction models,[206,237,238], and it has been advised against using it as a single assessment, since extremely strong associations could be needed to improve discrimination by the C-statistic.[237,238] The NRI, unlike the C-statistic, is sensitive to model miscalibration due to variations in absolute risk across populations, and sensitive to choice of risk categories.[207,239,240] We did not obtain information on non-fatal cerebrovascular disease, so investigating calibration of the NORRISK2 model to our study population was not possible. However, the HUNT population was included in the cohort used for establishing the NORRISK2 model, and also, if assuming the same ratio between CHD and stroke as observed in the original model population, the model was well-calibrated. The NRI and IDI calculations were based on a logistic model[205] contrary to a survival data based model.[240] Using a survival model may have strengthened the associations. Although some studies have failed to show an improved area under the curve statistic for prediction of CHD by adding METs estimated from maximal treadmill time to the Framingham risk score,[227] improved prediction of CVD/CHD by both the C-statistic and category-free NRI was shown by adding directly measured VO_{2peak} to conventional risk factors in the KIHD.[109] The latter cohort

is the only one previously showing improved prediction by adding directly measured VO_{2peak} to a risk prediction model, and this gap in knowledge increases the relevance of our findings. The lack of improvement in the C-statistic in our study may be due to a much lower event-rate compared to e.g. the KIHD. Several other studies have indeed shown improved discrimination, calibration and reclassification by adding other measures of CRF to existing models for predicting CVD/CHD or mortality risk assessed by NRI, IDI and goodness of fit.[109,222,241–245] Adding VO_{2peak} or other measures of maximal exercise capacity to established risk prediction algorithms thus seems to improve prediction of CVD/CHD, and even in our low-risk cohort, although the different results from the C-statistic and NRI/IDI in our results merit sober interpretations. Still, as recommended in the evaluation of new risk markers, the utility of the marker to improve clinical outcomes should be established, and cost-effectiveness should be taken into consideration.[236] CPET might be resource-demanding in primary prevention settings, and thus not easily implemented. With this in mind there are still some pieces to add to the puzzle before directly measured VO_{2peak} has a natural role in risk-prediction in primary prevention. However, both self-reported CRF and non-exercise estimated CRF from easily accessible clinical variables have shown promise in improving prediction of CVD,[242,246] implying that at least including *some* CRF measure to improve risk prediction should be possible in clinical practice, thus deserving further investigation.

5.1.3. Value of other CPET measures in apparently healthy individuals

There is considerable evidence for the prognostic value of peak oxygen pulse, $EqVCO_2$, and the oxygen uptake efficiency slope (similar to $EqVO_2$ but calculated as a in the equation $VO_2 = a \times \log_{10}V + b$, where V is minute ventilation) in heart failure and other cardiopulmonary conditions.[63,64,247,248] However, few studies have examined their relevance in healthy populations, largely due to the under-utilization of CPET in apparently healthy individuals. In addition to our findings, the relevance for peak oxygen pulse in predicting CVD, CHD, and all-cause mortality has previously been shown for men from the general population in the KIHD[249] and in male veterans,[120] although Fung et al. failed to show predictive value in a cohort of older adults.[250] The latter study by Fung et al. did not find a predictive value of the oxygen uptake efficiency slope on mortality neither,[250] representing the only identified study investigating ventilatory efficiency parameters in a non-clinical population. Combining several CPET parameters has also shown proof of improving prognostication in clinical populations,[66] but Laukkanen et al. failed to show improved predictive ability of CVD mortality in the KIHD when oxygen pulse was added to a risk score including VO_{2peak}.[249] Thus, the individual

prognostic value of these CPET measures and the value of adding several measures from a standard CPET in combination (e.g. VO_{2peak}, oxygen pulse, $EqVCO_2$, $EqVO_2$, heart rate recovery and more) to established risk prediction models in primary prevention is still also largely unknown. One study explored inclusion of other non-CPET exercise testing measures, such as maximal heart rate and electrocardiography information, showing improved prediction, especially in high-risk groups.[245]

5.1.4. Methodological considerations

Inclusion to the study was at a random time point for the participants, as it was as a part of a large health survey, as opposed to many studies using populations referred for exercise testing or to a preventive health examination. Such selection of study samples has a higher risk of confounding by subclinical disease despite no previous diagnose, since present symptoms or worry for disease may underlie study inclusion, increasing the risk of reverse causation. Such factors are less likely to play influential roles given the design and inclusion criteria of our study, as well as the sensitivity analyses performed, with exclusion of the first two years of follow-up, showing similar results. However, subclinical disease may still confound the relationship and yield some risk of differential misclassification (the CHD outcome may have affected measurement of exposure, VO_{2peak}, by subclinical disease at the time of measuring VO_{2peak}) despite the measures taken. Still, it is likely that subclinical disease and early atherosclerotic changes are important mediators on the path between a low VO_{2peak} and future CHD, and thus it may not make sense to try to exclude or control for patients with subclinical atherosclerotic disease, as VO_{2peak} is a strong predictor of future CHD stemming from such changes. This is especially relevant for prediction models where causal relationships are less relevant. Given the prospective ascertainment from hospital and national registries, the risk of misclassification of end-points was low.

5.2. Paper II: Age-related changes in VO_{2peak} and cardiovascular risk

Longitudinal changes in VO_{2peak} was based on repeated measures of VO_{2peak} mean 10.2 years apart in 1,431 participants. The mean annual decline in VO_{2peak} was 0.37 mL/kg/min in women and 0.53 mL/kg/min in men. Individual ten-year declines for women and men are shown as a scatterplots in Figure 7. The decline in both relative (mL/kg/min) and absolute (L/min) VO_{2peak} was higher with higher age in men, but levelled off in women from ~40 years age for relative and at ~60 years for absolute VO_{2peak}. Still, if considering a linear decline of relative VO_{2peak}

with age >40 in women the percentage decline increases by age, given the decreasing denominator (Figure 2 in paper II). In men the relative VO_{2peak} decline seems to increase and thus the percentage decline increases even more. Results from linear mixed models incorporating the 2,933 participants with measurements from HUNT3 only, and 40 participants with submaximal exertion in HUNT3 but not in HUNT4, showed similar results, with an accelerated decline with advanced age in men (Figure 3 in paper II).

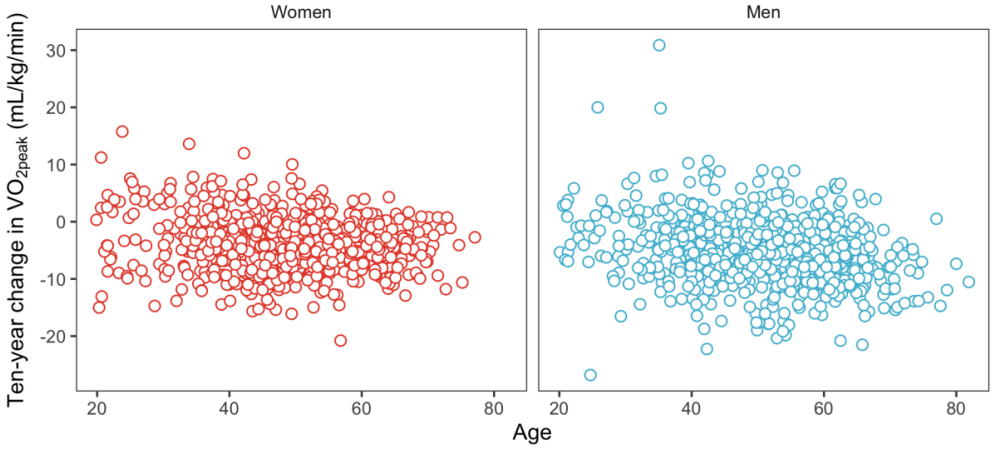

Figure 7. Ten-year change in VO_{2peak} (HUNT3 to HUNT4) for women and men by age at HUNT3.

Both descriptive and linear mixed effect model-based results showed that adherence to PA recommendations, but also levels of PA below recommendations, was associated with a better maintenance of VO_{2peak} compared to inactivity (Figure 4 and Supplemental Table 2 in paper II). However, the model-based results showed how the effect diminished with higher age, and this was especially pronounced for women. The effect of high-intensity PA on maintaining VO_{2peak} was clear for both men and women, but here as well the effect diminished with higher age for women (Figure 4 in paper I).

5.2.1. Age-related decline in VO_{2peak} – impact of study design

Our quantification of the longitudinal age-related decline in VO_{2peak} is based on the largest material reported as of now. The annual decline (-0.53 mL/kg/min for men and -0.37 for women) calculated from the two measurements separated by a decade was somewhat larger than the cross-sectional decline reported in the HUNT3 Fitness Study by Aspenes et al. (annual decline of -0.39 mL/kg/min for men and -0.33 for women).[76,251] The cross-sectional decline in the 7th decade of life from the same study was 13% for women and 11% for men, considerably lower than longitudinal declines of 14% and 16%, respectively (% of relative VO_{2peak}), and with

larger percentage declines for women in the cross-sectional material (in contrast to the patterns seen with the longitudinal data).

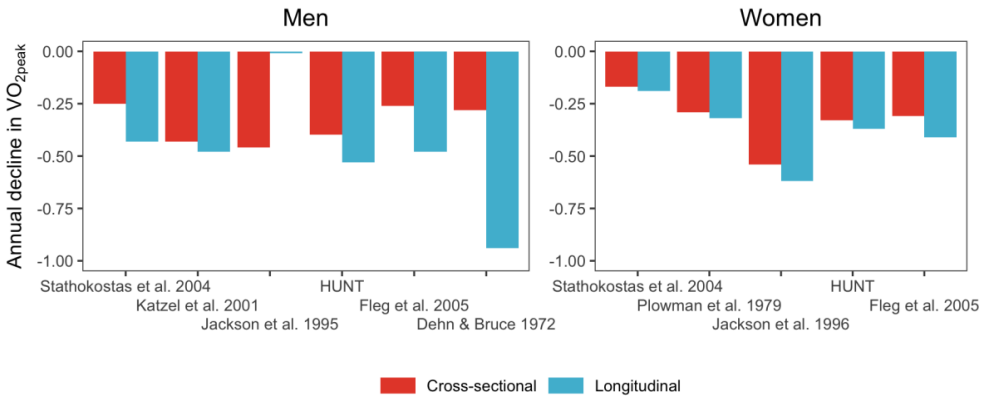

Figure 8. Comparison of age-related declines in VO_{2peak} in HUNT (Aspenes et al. 2011 and paper II) and other identified studies reporting both cross-sectional and longitudinal age-related declines in VO_{2peak}.[44,76,79,82,85,88,93,94] Values for Fleg et al. were extracted from figures at the mean age (50 years) and VO_{2peak} of the sample.

In the few studies reporting both longitudinal and cross-sectional declines in VO_{2peak}, the mean longitudinal decline is consistently larger than the cross-sectional declines (Figure 8).[44,76,79,82,85,88,93,94] The only exception from this pattern, the study by Jackson et al.,[94] may be explained by a large self-selection between the first and second measurement (~10% returned to repeated testing), as well as the the fact that the cohort itself consisted of National Aeronautics and Space Administration (NASA) employees who probably represent a highly selected and healthy group, especially given that they decreased their weight and increased their activity level during follow-up.

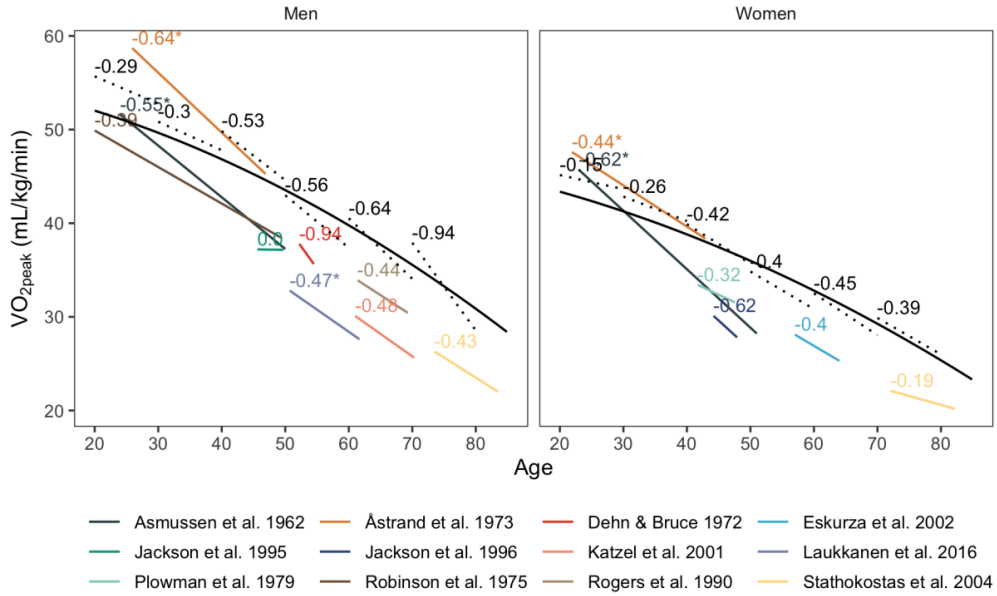

Figure 9. Overview of longitudinal declines in VO_{2peak} from various studies in non-athletes, combined with per decade mean longitudinal 10-year declines from paper II (abrupted black lines) and age-predicted VO_{2peak} from the linear mixed model in paper II (solid black line).[79,81–83,85,86,88,93,94,111,252,253] *Mean age at first measurement and length of follow-up for each study is depicted by the start and length of the given lines, respectively. The average annual change is denoted in text with corresponding colour as the given study. Presented data from the study by Robinson et al. 1975 are extracted from presented figures and may be somewhat inaccurate. The study by Plowman et al. 1979 reported values by different age groups but values were pooled due to low numbers in several groups. Annotation * = cycle ergometry.*

Longitudinal age-related declines in VO_{2peak} from paper II are visualized and compared to mean declines from other longitudinal studies in Figure 9,[79,81–83,85,86,88,93,94,111,252,253] excluding some with similar findings, for readability.[90] Several of the identified studies used more or less selected samples such as young university students (Robinson et al.[86]), physical education students (Åstrand et al.[252] and Asmussen et al.[253]) or selected workers (two studies by Jackson et al.[93,94]). Some have recruited more representative samples (Dehn & Bruce,[79] Eskurza et al.,[81] Katzel et al.,[82] Rogers et al.,[83] Stathokostas et al.[85]), but still almost all studies used a limited age span, and sample sizes were small, with 8 to 62 participants total or per sex, and most in the lower range. The study by Laukkanen et al. had a large sample size of 579 men, and although ~18% had ischemic heart disease with corresponding lower VO_{2peak} already at baseline, the decline was similar.[111] The study by Fleg et al., including 375 women and 435 men from the BLSA,[44] stands out among the other studies by presenting a representative sample in regard to age and sex distribution, allowing comparisons across age-groups. As illustrated in Figure 9, the estimated longitudinal declines vary, probably due to the described small sample sizes and very different inclusion criteria, but when comparing declines per decade of age from the BLSA study and ours, the trends in declines are similar with larger annual decline, with higher age

most pronounced in men (Figure 10). Similar trends have also been observed in athletes[254] and from maximal exercise-estimated METs in longitudinal ACLS data.[43]

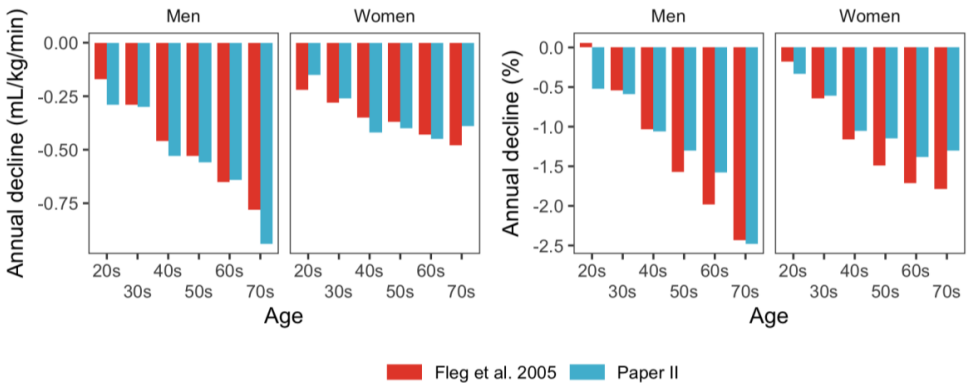

Figure 10. Comparison of longitudinal decline in relative VO_{2peak} and % of relative VO_{2peak} from the present study (descriptive) and Fleg et al. (model-based).[44]

A plethora of regression equations for predicting VO_{2peak} by age and sex have been constructed, and with few exceptions[78,255,256] they assume linear declines.[38,62,72,75,77,84,87,89,257–259] An overview of such equations is available in the reviews by Takken et al.[100] and Paap & Takken.[99] Given the larger observed declines at higher ages for men in longitudinal studies, this may seem like a simplification.[99] Some of the larger cross-sectional studies have reported declines over the 7th decade of life of between 5 and 19% (-0.10 to -0.61 mL/kg/min per year), with a mean 12% (-0.36 mL/kg/min per year),[34,44,61,76–78,84,92,260] and many show larger percentage declines in women than in men, or similar estimates. It seems cross-sectional patterns in age-related declines are more more or less accurate in women, but less so in men. Few large cross-sectional studies have sampled enough individuals >70 years of age to report declines in the eight decade of life as well, but some have reported values of 12 to 18%[34,44,84] which are also generally lower than the longitudinal findings for the same age-group (Figure 10).

The discrepancies between cross-sectionally and longitudinally measured declines in VO_{2peak} probably mainly stem from issues regarding selection and survivor bias (i.e. those living until older age most likely were healthier in their younger years than those becoming ill or dying at a younger age). In cross-sectional studies the most active part of the population will commonly be over-represented, as shown by e.g. Aspenes et al.,[76] and this may be especially true for men given the trends discussed above. Several studies have argued that longitudinal, paired observations are necessary to obtain reliable estimates on age-related declines in VO_{2peak}.[44,79,261] This will have an especially pronounced effect in older ages as the selection pressure due to

disease, ailments, and declining PA levels most likely will lead to super-healthy older adults participating in cross-sectional studies. In longitudinal studies this will also theoretically lead to some underestimation of the decline. Contrarily, highly active participants will, due to regression to the mean, on average be less active at a later follow-up measurement, with an expected larger concomitant decline in VO_{2peak}, which may lead to larger declines. Thus, given the strong association to health-outcomes for CRF and survivor bias it is very likely that an octogenarian in a cross-sectional study would have had a higher VO_{2peak} than the average 30-year-old in the same study when at the same age, as similarly pointed out by Fleg et al.[44]

5.2.2. Age-related decline in VO_{2peak} and physical activity

Linear mixed model results showed that the annual decline in VO_{2peak} for women at high age seemed to be higher for those doing high-intensity PA compared to moderate intensity and inactivity, while men performing high-intensity PA maintained their relatively higher VO_{2peak} compared to moderate intensity PA and inactivity. Both these patterns are in line with meta-analyses on cross-sectional declines in VO_{2peak} performed separately for endurance-trained and sedentary men and women, respectively, in that men doing high-intensity PA seem to maintain their relatively higher VO_{2peak}, while in women the relative difference between PA levels seems to diminish with age.[97,98] The meta analyses further showed that *percentage* declines were similar for endurance-trained and sedentary individuals across sex. Findings on athletes, masters athletes, and highly active populations have shown quite varying results with considerable span in estimates of longitudinal declines,[79,81,82,261,262] and have been shown to be highly influenced by the extent to which exercise is maintained over time in both sexes.[81,82,263] Athletes may show large longitudinal declines due to transitioning from high to low training volumes i.e. after finishing their career, but still show values of VO_{2peak} higher than expected for their age,[262] reflecting the mechanism causing underestimation of cross-sectional age-related declines (large longitudinal declines but high cross-sectional VO_{2peak}). Longitudinal declines in athletes may thus be exaggerated compared to in the general population.

Fleg et al.[44] showed that higher levels of PA was associated with a higher VO_{2peak} througout the life-span. However, they did not provide data on relative PA intensity, but quartiles of high-intensity absolute PA (≥6 METs). Although VO_{2peak} was higher for each higher quartile of high-intensity PA, their model on relative VO_{2peak} showed an interaction with age for the third quartile of high-intensity PA, indicating a sharper decline in VO_{2peak} with higher age compared to the other quartiles, meaning that the third quartile approached the second. This signal may

be equivalent to the differential effects of high and moderate intensity PA with age found in our results, although their use of absolute intensities makes these effects not directly comparable. The study from the ACLS by Jackson et al. examined longitudinal declines of maximal exercise-estimated METs on a large population of 3,429 women and 16,889 men performing median (range) 2 (2 to 23) tests each.[43] They studied the influence of life-style such as PA and weight on CRF changes, and similarly showed that PA was associated with better maintenance of VO_{2peak} across the age-span. In the models represented from the BLSA, ACLS, and us, it is important to note that individual predictions are based on PA being the same across the age-span, which is often not accurate, as shown both in the US and in Norway.[38,264] In relation to this, it has been proposed that the accelerated decline in VO_{2peak} is primarily related to reductions in PA,[261] but estimates from our models suggest that this is not the only explanation, since the curvilinear shape of age-related decline was still evident, although to a lesser extent, with higher intensity PA. Similar findings were reported by Fleg et al.[44] Still, it seems clear that reductions in PA habits will augment the inevitable decline seen with aging. Our focus were not on changes in weight, thus we omitted weight measures from our models, while e.g. Jackson et al. included BMI.[43] This likely explains the earlier onset of CRF decline in our study compared to Jackson et al., as assuming a fixed BMI does not consider the rise in BMI expected in early adulthood.[265]

5.2.3. Methodological considerations

It is worth noting that repeated measurements to describe age-related changes may be influenced by the time period of measurement and the subject's birth cohort, as well as the individual's age, as nicely explained by Twisk.[266] Distinguishing effects of aging from period and cohort effects is not straight-forward, and to overcome such challenges one would need a multiple longitudinal design[266] where repeated measurements are performed on several age-cohorts at several repeated occasions. These factors may affect estimates of longitudinal changes in VO_{2peak}, since CRF has changed over time on the populational level.[102] However, the accelerated longitudinal design (i.e. a wide span of age categories followed over a shorter time period) used in paper II has obvious advantages, such as lower cost due to describing changes over the adult age-span in a relatively short time, ten years as opposed to a life-time. The shorter follow-up probably gives a lower loss to follow-up as well,[267] which generally has been considerable in longitudinal studies. Stathokostas et al. included 441 participants in the original sample, and 62 men and women (14%) repeated testing 10 years later. Similarly, the studies on men[94] and women[93] by Jackson et al. both had a ~90% loss to follow-up, and

Laukkanen et al. ~80%.[111] Unfortunately, loss to follow-up was not described in the BLSA study.[44] Some of the smaller studies have managed lower loss to follow-up rates between 23% and 53%.[79,82,252] In our study, 32% of the original sample repeated CPET, and the loss to follow-up is thus in the mid-range compared to the other studies, but low considering the large study size. There was a general trend towards a favorable risk profile in HUNT3 for those returning for a new test in HUNT4, compared to the whole HUNT3 Fitness population at baseline, including a higher baseline VO_{2peak} (Supplemental Table 1 in paper II). Similar trends were seen for the risk profile for the 2,933 participants participating only in HUNT3 compared to those performing repeated testing in HUNT4 (n = 1,471) (data not shown). This suggests some selection bias towards repeating CPET in HUNT4.

Linear mixed model analyses as performed in paper II, by Fleg et al., and by others[43,44] is unbiased under the assumption of missing at random. However, the loss to follow-up is, as mentioned, not random, and such models will thus still include some bias. This bias is most likely towards underestimation of age-related declines, since the cross-sectional estimates are generally overestimated, especially in the older age groups, as previously discussed. Stathokostas et al.[85] described their estimates of change over time as descriptive of those undergoing "successful healthy aging", and the same assumption could probably be extended to interpretation of data on CRF in aging in general. Further studies should attempt methods such as inverse probability of censoring-weighting to overcome such issues.[268] Still, this would not address the practically inevitable selection bias arising from voluntary exercise testing. Some studies have used strict criteria for VO_{2max} when reporting reference values,[77,181] although it has been documented that traditional VO_{2max} criteria may be too strict for older adults.[29] Using strict and not age-adjusted VO_{2max} criteria may underestimate the decline in CRF with age, as it is possible that it will favour older adults who are able and used to pushing themselves hard during PA.

Another limitation is that the two cross-sectionally reported measures of PA at HUNT3 and HUNT4 only captures "snapshots" of participants' PA behavior in the follow-up of the study, and several measurements over time would have been preferable, although not feasible. Keeping these limitations of PA assessment (chapter 4) in mind when interpreting observational findings as ours, the results are nevertheless generally in line with findings from short-term RCTs examining the effect of high-intensity exercise on VO_{2peak},[142,269,270] and in a recently published large RCT on five years of exercise-training in older adults >70 years of age.[32]

5.2.4. Change in VO_{2peak} and risk factors

Analyses of concurrent change in VO_{2peak} and cardiovascular risk factors showed a consistent association between a one-unit (mL/kg/min) better maintenance, or improvement, in VO_{2peak} and favorable change of all risk factors when adjusted for confounding variables in the first model (see section 3.10.2). In a second model, the included adjustment for PA and weight attenuated the effect estimates to being significant only for HDL, total cholesterol to HDL cholesterol ratio, resting heart rate, and waist circumference. Results from both models are shown in Figure 11. Change in VO_{2peak} was also significantly and inversely associated with incidence of metabolic syndrome (odds ratio 0.86, 95% CI 0.83 to 0.90), dyslipidemia (odds ratio 0.92, 95% CI 0.89 to 0.94), and hypertension (odds ratio 0.95, 95% CI 0.92 to 0.98) in HUNT4.

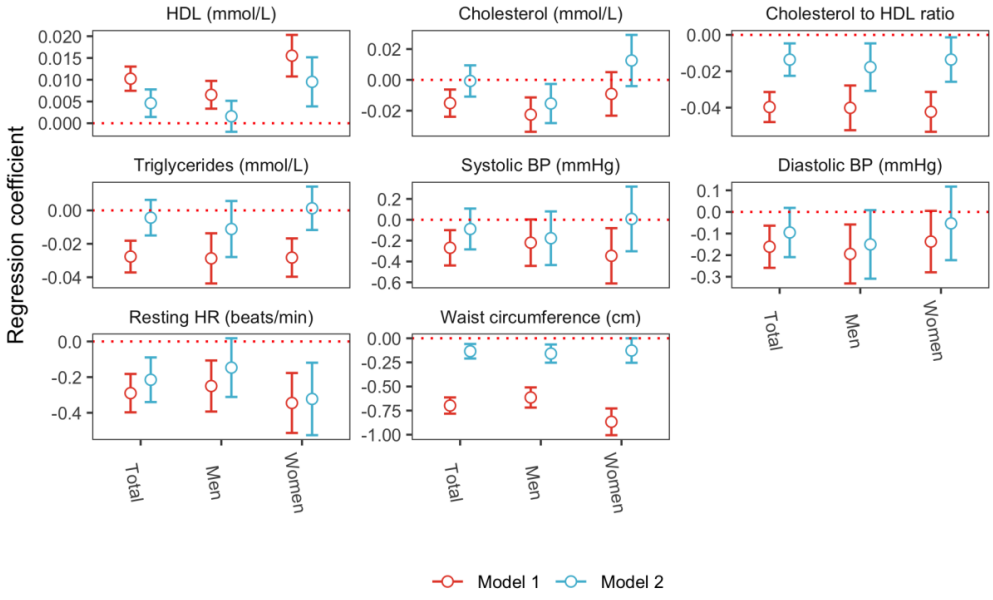

Figure 11. Model estimates and 95% CIs from linear regression analyses for change in cardiovascular risk factors by one-unit change in VO_{2peak} (mL/kg/min) for total sample, men, and women for model 1 (adjusted for age at HUNT3, sex, VO_{2peak} at HUNT3, current smoking and regular alcohol intake at HUNT3 and HUNT4, family history of CVD, and incident CVD between HUNT3 and HUNT4) and 2 (model 1 + weighted PA volume at HUNT3 and HUNT4 and weight at HUNT3 and weight change).

The study by Knaeps et al. on Flemish adults is, to our knowledge, the only previous study assessing changes in directly measured VO_{2peak} in relation to changes of cardiovascular risk factors.[144] Their results were similar, showing significant associations between changes in VO_{2peak} and changes in individual and clustered risk factors. The included adjustment for changes in dietary habits strengthened their findings. As listed in Table 1 a few other studies

have assessed changes in estimated CRF measures with changes in cardiovascular risk factors as well. The most notable is the study by Lee et al. assessing concurrent changes in maximal exercise-estimated METs and individual risk factors, showing significant adjusted partial correlations for all measures except glucose in 3,418 healthy adults (17% women).[135] They also showed how change in CRF was inversely associated with risk of incident hypertension, metabolic syndrome, and hypercholesterolemia over 6 to 7 years follow-up, in line with our findings. Several other studies have used similar approaches, investigating associations between change in CRF and incidence of hypertension and dyslipidemia with generally similar conclusions,[134–138] although Carnethon et al. failed to show strong associations for change in CRF with similar risk factor end-points in analyses adjusted for BMI and weight change in a population of 2,478 men and women 18 to 30 years.[271] Sternfeld et al. performed analyses on changes in CRF with changes in lipids in 1,777 participants from the same cohort.[148] They found significant partial correlation coefficients only for HDL when adjusting for weight. Although these were young adults, the results are similar to ours where all effect estimates were attenuated after adjustment for weight and PA, and several analyses were no longer statistically significant. Changes in weight seems to be an important contributor to the changes in cardiovascular risk factors seen with changes in CRF. However, the discrepancy to the findings by Knaeps et al. mentioned above, where associations were significant for all risk factors when adjusting for weight change, even when using a much smaller sample than ours, is intriguing. The decline in VO_{2peak} over the ten years was small (average ~3% compared to ~10% in our study), which may have some influence. However, contrary to in our analyses and the findings by Lee[135] and Sternfeld[148], Knaeps et al., and some others,[146,147] included adjustments for the baseline value of the outcome variable in their model, which is controversial.[216,272,273] Decades ago, Frederic Lord pointed out the paradoxical effects of adjusting for baseline variables in non-randomized groups,[274] since coined "Lord's paradox". Years later, Glymour et al.[273] used directed acyclic graphs and showed how baseline adjustment in analyses of change may introduce collider bias when the baseline measure of the outcome and the exposure variable are associated and the outcome variable has some degree of measurement error, eliciting the underlying mechanism of the effect observed by Frederic Lord. Causal inference is based on prior knowledge and assumptions of relationships between different variables,[216] and thus given the assumptions made for our models, we believe an adjustment for baseline would give stronger, but more likely confounded, effect estimates (by introducing a collider). However, the causal interrelationships between several of these variables are not straight-forward, and we do not claim that our effect estimates are totally unbiased or unconfounded, but they are our best

attempt at such. VO_{2peak}, weight and PA are strongly connected entities interacting in a complex manner, whereas e.g. both VO_{2peak} and weight may influence PA behavior, and not only the other way around. Both measures of weight and PA may thus be perceived as confounders and mediators, depending on the *interpreted* assumptions. As discussed in chapter 4 this may lead to over-adjustment, with risk of underestimating true effects or introduction of bias. Therefore, we chose to adjust for weight and PA in a separate model. Supplementing with analyses of changes in risk factors with changes in VO_{2peak} scaled to fat-free mass would have increased the understanding of these relationships, but information on body composition was not available from HUNT3.

5.2.5. Mechanisms for decline in VO_{2peak}

Our studies were not designed to investigate mechanisms behind declines in VO_{2peak}, but other studies have elaborated on such relationships, which will be briefly covered. Ogawa et al. found that changes in stroke volume, maximal heart rate, and arteriovenous oxygen difference explained ~50%, 25% and 25% of the decline in VO_{2max} in a cross-sectional study on 110 healthy subjects,[275] while the thirty-year longitudinal follow-up of the five participants from the Dallas bed rest study found that declines in heart rate and arteriovenous oxygen difference contributed to declines in VO_{2peak}, but that cardiac output was unchanged.[276] Ogawa et al. also showed that body composition plays a central role as stroke volume was not lower at higher age for sedentary subjects when scaled to fat free mass,[275] and others have also found that changes in stroke volume may be due to reductions in fat-free mass.[277] This underscores the importance of changes in body composition with aging. The BLSA study[44] added to this understanding by showing how the oxygen pulse decline accelerated with higher age, in line with the $VO_{2peak,}$ whereas the maximal heart rate had a linear decline, which is also supported by other studies.[261,278] Reduced oxygen extraction, narrowing the arteriovenous oxygen difference, can partly be explained by reductions in lean body mass,[80,254] but intrinsic changes in skeletal muscle has also been suggested as a possible cause.[261]

5.3. Paper III: VO_{2peak} and left atrial size

The study sample of 242 participants had a mean $VO_{2\%pred}$ of 95%, and LAVI was mean 32.6 mL/m^2 with 39% having values higher than the threshold for enlargement of 34 mL/m^2.[184] A significant sex-difference in LAVI was heavily attenuated and no longer significantly different after adjusting for VO_{2peak}. VO_{2peak} significantly predicted LAVI on top of predictors reported

in previous studies (LV mass index, LV end-diastolic volume, E/e', sex and age (VO_{2peak}: beta=0.16, 95% CI, 0.04–0.28, p=0.02). Also, PA significantly predicted LAVI with roughly 1 mL/m^2 larger LAVI per 100 weekly weighted minutes of PA (0.9 mL/m^2 95% CI 0.3 to 1.5). One mL/kg/min higher VO_{2peak} was associated with 0.2 mL/m^2 higher LAVI when adjusted for age and sex, and 10% higher $VO_{2\%pred}$ was associated with 0.8 mL/m^2 higher LAVI (95% CI 0.3 to 1.3). There was an interaction between VO_{2peak}/$VO_{2\%pred}$ and age signifying a larger LAVI with higher age and higher VO_{2peak}/$VO_{2\%pred}$. The models for VO_{2peak} and $VO_{2\%pred}$ explained 9% and 10% (R^2 0.09 and 0.10) of the variance in LAVI, respectively. Other interactions or quadratic functions of age or VO_{2peak}/$VO_{2\%pred}$ did not improve predictions, nor did models including spline functions for VO_{2peak}/$VO_{2\%pred}$ or age. In stratified analyses by sex, the same model on $VO_{2\%pred}$ predicted LAVI best for men, but for women none of the models significantly improved LAVI prediction. Prediction of LAVI based on the model including $VO_{2\%pred}$ is shown in Figure 12, illustrating the higher LAVI with higher values of $VO_{2\%pred}$ and age in a nomogram, adding to the figures presented in paper III.

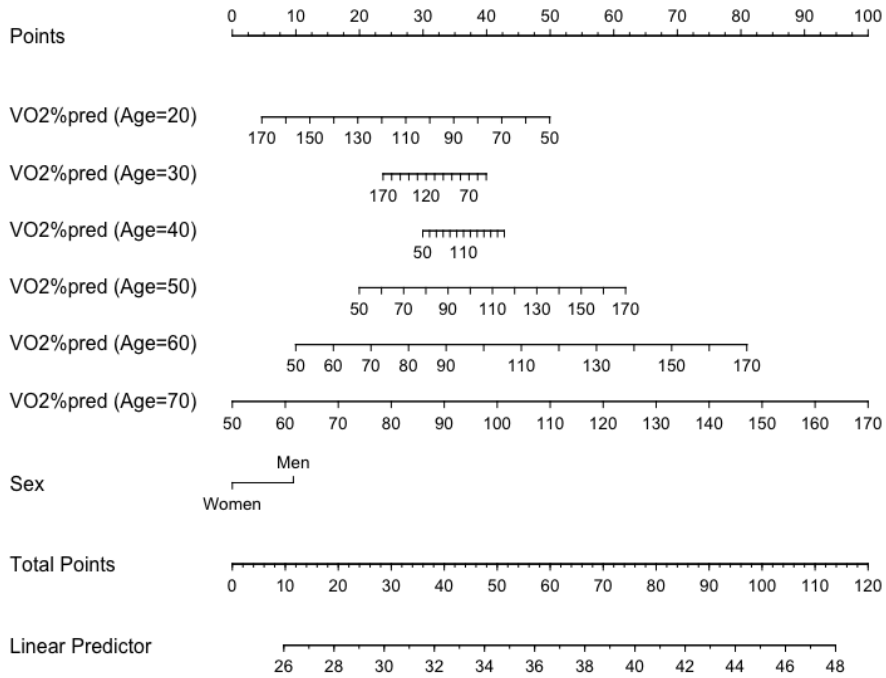

Figure 12. Nomogram for prediction of LAVI based on $VO_{2\%pred}$, age and sex. To calculate predicted LAVI; sum up points for the interaction term of interest and sex and use the total points to get the linear predictor of LAVI by drawing a vertical line.

Diastolic dysfunction, as defined by the 2016 guidelines,[158] was only present in three participants, and LAVI did not show significant associations to other measures of diastolic

function (septal and lateral e', E/e', maximal velocity of tricuspid regurgitation, pulmonary vein systolic and diastolic velocities and their ratio, or mitral deceleration time). In line with the lack of association to measures of diastolic dysfunction, an enlarged LAVI and $VO_{2\%pred}$ higher than predicted was not associated with unfavorable levels of traditional cardiovascular risk factor levels, as shown in exploratory analyses below. Analysis of covariance (adjusted as for model 2 in paper I, see section 3.10.1) was performed to assess cardiovascular risk factor levels for three groups of participants with I) LAVI >34 mL/m^2 and $VO_{2\%pred}$ >100, II) LAVI >34 mL/m^2 and $VO_{2\%pred}$ <100, and III) LAVI <34 mL/m^2 (Figure 13). Those with LAVI <34 mL/m^2 and those with LAVI >34 mL/m^2 and $VO_{2\%pred}$ <100 had significantly higher resting heart rate, waist circumference, and cholesterol to HDL ratio, as well as lower HDL cholesterol, compared to those with LAVI >34 mL/m^2 and $VO_{2\%pred}$ >100 (all p ≤0.003) Also, those with LAVI >34 mL/m^2 and $VO_{2\%pred}$ <100 had significantly higher systolic BP and waist circumference than those with LAVI <34 mL/m^2 (both p ≤0.03). Other comparisons did not reach statistical significance at p<0.05.

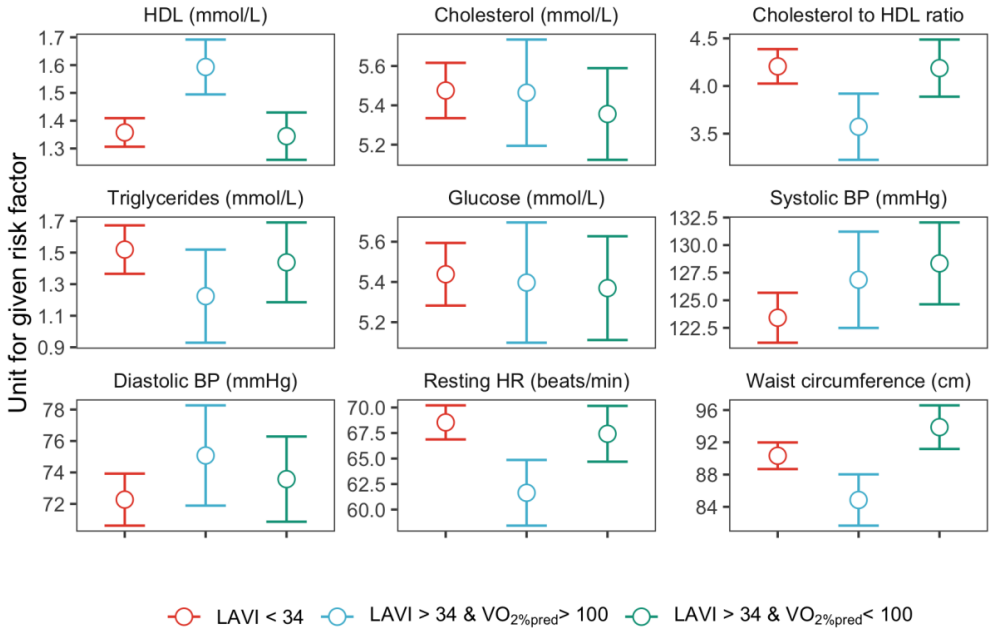

Figure 13. Predictions (mean and 95% CIs) based on analysis of covariance models for given cardiovascular risk factors comparing participants with LAVI <34 mL/m^2 (red, n = 132). LAVI >34 mL/m^2 and $VO_{2\%pred}$ >100 (blue, n = 37), and LAVI >34 mL/m^2 and $VO_{2\%pred}$ <100 (green, n = 51). Analyses adjusted for age, sex, current smoking, alcohol consumption and family history of CVD. One more participant in the LAVI <34 mL/m^2 group and two more participants in the LAVI >34 ml/m^2 and $VO_{2\%pred}$ <100 group had missing variables in analyses for lipid and glucose measures. HR; heart rate.

5.3.1. *VO_{2peak} and left atrial size in non-athletic populations*

Our results showed a significant association between VO_{2peak} and LAVI, and the effect was stronger with higher age. The explained variance was only modest (9-10%). Several studies have shown LA enlargement in athletes,[155,156,212] and a larger LA is associated with more years and volume of training in both non-elite and elite athletes,[212,279] as well as in recreational marathon runners.[280] Furthermore, both combined atrial areas, and LA diameter and volume have been shown to correlate with exercise capacity in athletes.[281–285] The relationship between CRF, LA size, and diastolic function is less studied in general population samples. Previously, 1,678 men and 1,247 women from the ACLS, who received an echocardiogram based on clinical indication as well as maximal exercise-estimated CRF, were studied.[175] LAVI was largest in the quartile with highest fitness. In men and women one MET higher exercise capacity was associated with 0.77 and 0.88 mL/m^2 larger LAVI, respectively, which is quite similar to 0.20 mL/m^2 higher LAVI per mL/kg/min higher VO_{2peak} in our study. Furthermore, LAVI was positively associated with E/e' only in low-fit individuals, paralleling our observations of normal diastolic function despite enlarged LAVI, as well as observations in another study that E/e' was an independent predictor of LAVI in non-athletes, but not athletes.[286] Similarly, athletes show enhanced early transmitral flow reflecting supra-normal diastolic function accompanied with lower LA contraction (active) contribution to LV filling at rest.[161,287] Arruda et al. compared LAVI in patients with systolic and diastolic dysfunction with healthy controls (all groups mean age 63 to 66 years and 30% to 47% women).[288] LAVI was higher in systolic > diastolic dysfunction > controls. As expected, LAVI was inversely correlated to VO_{2peak} both for those with systolic and diastolic dysfunction, but in line with our findings, a positive correlation (r = 0.36) was observed for the healthy controls.

5.3.2. *Fitness as a two-faceted risk factor for left atrial enlargement*

An unfavorable cardiovascular risk profile has been found in combination with LA enlargement in several studies,[169,170] and cardiovascular risk factors are associated with other measures of LV diastolic dysfunction as well.[193,289] However, the positive association between VO_{2peak} and LAVI, combined with normal LV diastolic function and a favorable cardiovascular risk profile, suggests another pathophysiological pathway[168–170] compared to concentric LV remodeling due to e.g. LV hypertrophy or diabetes,[159,290] and reduced exercise capacity.[290] Of course, other causes for LA dilation also exists, such as e.g. mitral valve disease. The mechanisms for enlargement of the LA is mediated by increasing wall stress by volume or pressure overload.[159,160,291,292] LA pressure is higher during acute exercise and may confer even higher

wall stress on the atria due to the mitral valve being closed for an increased proportion of time, as the shortening of the diastolic time is more pronounced than the systolic as heart rate goes up.[291] High CRF is associated with larger circulating blood volumes, and larger circulating blood volumes is associated with LA enlargement despite normal diastolic function.[293] Similarly, dilation of the inferior cava is common in athletes.[294] Our findings suggest that age is an effect-modifier in the relationship between VO_{2peak} and LA size, likely in the role of being an indirect marker of years of endurance training and thus cumulative stimulus. This may be interpreted as suggestive of cumulative and possibly irreversible changes on the LA in relation to chronic exercise, and our findings have some support by studies on athletes which show that cumulative years of endurance exercise is a predictor of LAVI enlargement,[212,295] and that LA dilation exist also in former athletes.[296,297] Importantly, LA fibrosis in response to endurance exercise has been shown in animal studies[298] and master athletes.[299] Enlargement and fibrosis of the LA has implications for AF development by providing substrate for arrhythmias,[292] and may provide the link between high exercise volumes and AF development shown in observational studies.[171,300]

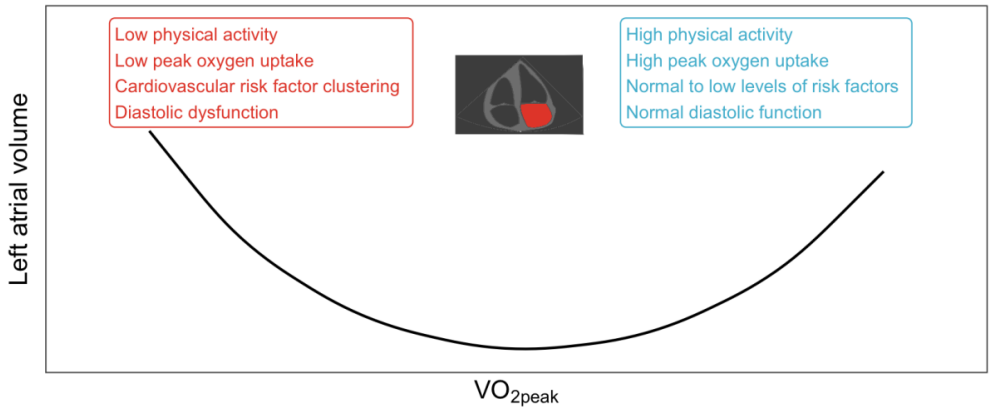

Figure 14. Conceptual illustration of two different paths to LA enlargement.

Borlaug summarized the notion of two different pathophysiological pathways to LA dilation in a previous editorial by stating that "*LA dilation proceeds via unique pathways in fit and unfit people, ostensibly through repeated exercise-induced volume loading in fit individuals and probably through elevated LA afterload from high LV filling pressures in the unfit*".[301] This implies that the sample under investigation determines if the relation between LA size and CRF observed in a given study will be positive or negative, since the association seems to follow a U- or reverse J-shape (Figure 14). Subjects with CVD or related risk factors and increased LV filling pressures pertain on the left side of the curve, while healthy subjects with a normal to

beneficial cardiovascular risk profile stay on the right side. The material in paper III included individuals free from CVD, pulmonary disease, cancer, and use of antihypertensives who volunteered to exercise testing, and thus the sample relates best to the right side of Figure 14. It is worth mentioning that the $VO_{2\%pred}$ in our study was based on predicted values from the same healthy population,[62,251] meaning that the sample's VO_{2peak} is expected to be higher than in a *truly representative* sample of the population. Interestingly, Pathak et al. observed that in low-fit obese individuals with AF an increase of CRF ≥2 METs over four years lowered LAVI from 38 to 32 mL/m^2 and led to significantly better arrhythmia-free survival, while those with <2 METs gain in CRF had only 1.2 mL/m^2 decline of LAVI.[302] This supports the understanding shown in Figure 14 by showing that moving from the far left of the curve in $VO_{2\%pred}$ and rightwards (improving $VO_{2\%pred}$) is associated with decreasing LA size. Conversely, increasing VO_{2peak} by high-intensity exercise training in men with a high baseline VO_{2peak} has been shown to increase LA volumes in exercise trials of just six weeks,[303] and changes in LA volumes have been shown to be dynamic on/off season in elite soccer players.[304] Similarly, McNamara et al. found, in an RCT of 53 previously healthy men and women (mean age 53 years) with normal BMI and BP measures, that LA maximal and LV end-diastolic volumes increased significantly over 10 months in the high-intensity exercise group compared with control, but from 10 months to completion of the trial at two years, only LAVI increased further with a resulting increase in the LA to LV volume ratio.[305] Furthermore, they found similar LA to LV volume ratio, but higher absolute volumes, in athletes. Although endurance training is generally thought to yield balanced remodeling of all chambers,[295] ours and the findings by McNamara[305] indicate that this might not always be true. Still, despite the changes in LA volumes in the exercise versus control group, in the mentioned study, no signs of significant electrophysiological remodeling were shown. This is similar to observational findings in athletes where the number of premature ventricular and atrial contractions was similar across training volumes despite differences in LA size.[306]

The prognostic significance of LA enlargement in relation to high CRF or training status is not yet established, although the U-shaped relationships suggest a favorable prognosis for those pertaining to the right side of the curve in Figure 14 compared to the left side. A CMR study of patients with AF and large LAs showed that those with low CRF had more LA tissue remodeling (fibrosis) measured by CMR late gadolinium enhancement, and the highest-fit individuals had lower probability of AF recurrence, although it should be mentioned that none of the participants had very high CRF values.[307]

Our results did not show significant associations between $VO_{2peak}/VO_{2\%pred}$ and LAVI in stratified analyses for women. However, the abovementioned large study by Brinker et al. showed similar relationships for both sexes.[175] Thus, our lack of association in women may stem from random sampling of somewhat lower-fit women compared to men, with only 26% of women having VO_{2peak} higher than predicted for age and sex compared to 47% of men. Studies on athletic women have shown enlarged LA in line with findings in men,[308,309] although a study from Sweden showed less LA enlargement in athletic women compared to men, while LA volumes were similar for the non-athletic controls across sex.[310] Differences in LA volume by sex is generally thought to be accounted for when indexing to body-surface area.[311,312] In line with this, the observed, significant sex-difference in LAVI in our material disappeared after adjusting for VO_{2peak}.

5.3.3. Methodological considerations

Reference values and cut-offs for LA volumes have been debated and changed.[184,313,314] Although this study was not designed to assess normal values for LA size, it is noteworthy that both present (34 mL/m^2)[184] and previous (28 mL/m^2)[315] recommended upper limits of normal, as well as cut-offs argued in a recent meta-analysis (24 mL/m^2),[313] do not seem suited to distinguish pathological remodeling in our study. However, the mean LAVI values presented in our study (32.6 mL/m^2) fit well with values found in the NORRE Study (two SD range from 19.3 to 41.5 mL/m^2 and mean 29 mL/m^2) of normal echocardiograms in >700 participants.[316] Similar values have also been reported from a large Italian cohort,[213] and recently a large study, including 2,008 adults balanced by age, sex and ethnicities, proposed an upper normal limit of LAVI of 41 mL/m^2.[312] Differences across studies may stem from both the methods used and demographic characteristics. Several previous studies have used standard apical projections optimized for LV investigations, which are known to underestimate LA volumes,[188] and different methods (area-length, method of discs, prolate-ellipsoid, e.g.) for quantifying LA volumes by two-dimensional echocardiography are known to give different estimates. The area-length model used in this thesis has shown the largest values,[186,189,317,318] with the method of discs with relatively close agreement, but rather large discrepancies towards the prolate-ellipsoid method.[186,189] Measurement of LA volumes by CMR might have reduced measurement error and strengthened the observed associations due to superior reproducibility and being less dependent on imaging angles and body habitus.[150,191] Furthermore, data on LA function by e.g. LA strain has shown potential in detecting LV diastolic dysfunction at an early

stage in its development.[319] However, neither CMR imaging or echocardiographic measures of LA function were available in our study.

As our results suggest, fitness of different samples may also explain differences across populations. The sentence in the 2015 cardiac chamber quantification recommendations by Lang et al., which describes that "*LA volume can be increased in elite athletes, which needs to be taken into account to avoid misinterpretation as abnormal*",[184] may be expanded to include precautions for fit individuals from the general non-athletic population as well. Similarly, this topic is only touched upon with a few words regarding athletes in the latest recommendation for evaluation of LV diastolic dysfunction.[158] Nistri et al. previously addressed that individuals' athletic status should be taken into account to avoid misinterpreting LAVI as enlarged,[213] which we add to, further suggesting that this should be especially emphasized in fit, *older* adults.

Normalization of cardiac structures to body size may also yield differences across study samples. The normalization of cardiovascular measures to body size may be challenging, and the ratiometric approach has been argued to be suboptimal.[320,321] Although normalized values should yield body size independent values,[320] studies show that LAVI significantly correlate with obesity.[322] Also, differences in height across populations has been proposed to explain differences in LAVI across samples.[323] The ratiometric scaling of VO_{2peak} to body weight and LA volume to body surface area[320] should therefore be considered, given that fit individuals are expected to have a higher proportion of fat-free mass compared to unfit individuals, which may affect the relationships between LAVI and VO_{2peak}. Furthermore, changes in body composition are expected with aging, making this even more complex. Differences between sex may partly stem from differences in lean body mass, although training status may also differ across sex.[295] Scaling LA volume to body surface area may have limitations, but unfortunately, measures of body composition were not available in HUNT3.

5.4. Clinical implications

This thesis provides novel insights by using directly measured VO_{2peak} to investigate associations to markers of cardiovascular risk and disease in a large cohort of apparently healthy adults. As we showed in paper I, VO_{2peak} has the potential to help predict future events of CHD and improve risk prediction of CHD even in asymptomatic and healthy adults. These findings

encourage further studies which should investigate practical implementation of VO_{2peak} in risk stratification in clinical practice.

In paper II we described the longitudinal age-related decline in VO_{2peak} in a large cohort with a balanced distribution by sex and adult age-groups. Our study reinforces findings from smaller, more selected studies, as well as a somewhat larger study from the US,[44] showing that the decline in VO_{2peak} with higher age is larger than estimated from cross-sectional studies for men in particular, while estimates seems to be rather similar with both designs for women. The clinician should consider the expected age-related decline from longitudinal studies when following patients over time at older age to reduce the risk of falsely interpreting declines in CRF as abnormal. When aiming to assess age-related changes in CRF, researchers should keep in mind the non-linear relationships expected with age, acknowledge weaknesses of the cross-sectional as well as the longitudinal design, and how this may explain differences in estimates between studies. The results from paper I and II indicate that clinicians should encourage a lifestyle best suited for maintaining VO_{2peak} with age, as better maintenance of fitness is related to lower cardiovascular risk.

In paper III we show that high CRF and LA dilation, markers traditionally associated with opposite implications for future prognosis, are positively associated with each other. Information on individuals' CRF status may assist the clinician in correctly interpreting prognosis, as an enlarged LA associated with high CRF may reflect a lower risk than classically associated with LA enlargement. However, prospective studies to establish prognosis in this patient group is needed.

5.5. General and concluding remarks

The required composite function of several organ systems to yield a high CRF is reflected by CRF being a powerful marker of numerous health outcomes such as CVD, dementia, cancer, disability, surgical risk, and mortality.[17] Although not fully understood, several plausible mechanisms link CRF to a favorable risk profile, including altered autonomic tone, improved endothelial function, lower levels of inflammation, and lower amount of central adiposity with improved lipid and glycemic profile.[17,324] Findings from this thesis show how changes in CRF is associated with changes in cardiovascular risk factors, further building up under these mechanisms. Besides aging itself, it is hard to think of such a strong and readily available

prognostic marker of future health, and several have argued that CRF should have a more prominent place in clinical evaluation, given its prognostic value.[17,325] Although CRF is tightly linked to PA behavior, as discussed, heredity is thought to explain roughly 50% of the variation in CRF and in its response to exercise training.[326–328] Twin-studies have shown that genetic factors affect PA behavior as well.[329] Furthermore, genetic and molecular mechanisms for CRF and cardiovascular risk are linked.[122,123] Thus, although the link between PA and CRF is thoroughly established, findings for the trait CRF should not be translated to be synonymous with PA *behavior*. CRF could theoretically only be a marker of a genetically more robust cardiovascular system,[325] or a product of genetical background and "cumulative" environmental and life-style influence over many years such as dietary choices, PA behavior, and weight control, explaining its strong relation to health outcomes. Thus, increasing CRF one MET by PA probably does not accurately reflect the health-benefit of having one MET higher CRF in epidemiological studies. Still, the relatively large effect sizes and consistent findings across the literature, despite a wide variety of study designs, support the benefit of increasing CRF, making CRF not only a marker of risk but also a potential target for intervention to reduce risk through e.g. promoting PA and a healthy life-style.

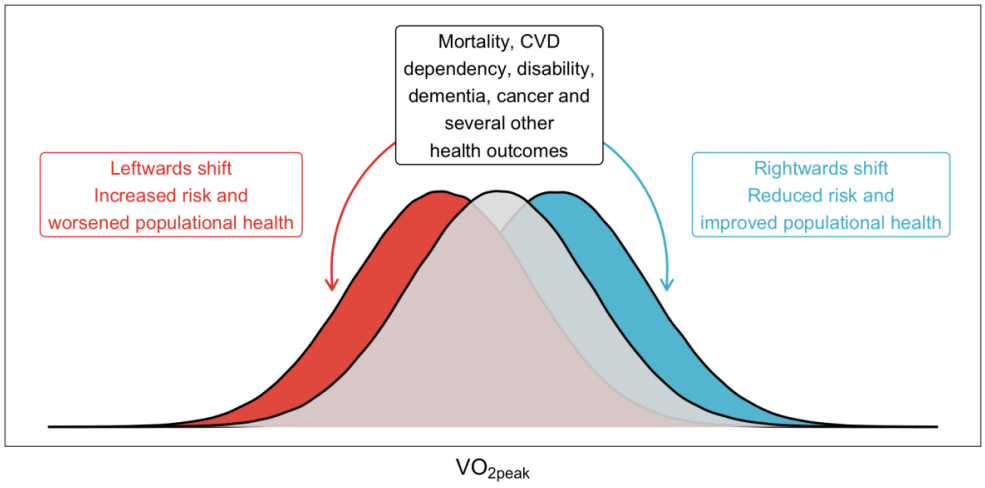

Figure 15. Illustrating the impact of populational shifts in VO_{2peak} distribution on population health.

Population strategies, shifting the distribution of a risk factor for the whole population, are more effective than high-risk strategies for prevention of disease, as the latter only influences those at highest risk which contribute to a minority of total adverse events.[330] E.g., the favorable effect of maintaining or increasing CRF on cardiovascular risk factors observed in paper II were relatively small on the individual level. However, increasing CRF on the population level, even

to a very small extent, may have a considerable impact on overall health (Figure 15). If we were only able to make the many move a *little* more over the life-course the potential for improving health outcomes through improvements in CRF at the population level would most likely be huge.

6. CONCLUSION

This thesis reinforces and further expands prior knowledge on the relationship between CRF and cardiovascular health and disease. By considering patients' VO_{2peak}, clinicians may improve several aspects of clinical decision making. Firstly, VO_{2peak} may help identify individuals at risk for future adverse events, by improving future risk prediction. Next, information on fitness status may have the potential to help discriminate pathological and physiological cardiac remodeling, and LA remodeling in particular, even for non-athletic subjects. Furthermore, knowledge on the expected longitudinal change in VO_{2peak} with aging may assist clinicians in avoiding wrongful conclusions regarding abnormal VO_{2peak} trajectories. Lastly, this thesis reinforces what is already largely known on the value of promoting improvement and maintenance of CRF for maximizing health benefits for the individual and the society.

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PAPER I

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PAPER II

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Age-related change in peak oxygen uptake and change of cardiovascular risk factors. The HUNT Study

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ABSTRACT

Background: Large longitudinal studies on change in directly measured peak oxygen uptake (VO_{2peak}) is lacking, and its significance for change of cardiovascular risk factors is uncertain. We aimed to assess ten-year change in VO_{2peak} and the influence of leisure-time physical activity (LTPA), and the association between change in VO_{2peak} and change in cardiovascular risk factors.

Methods and results: A healthy general population sample had their VO_{2peak} directly measured in two ($n = 1431$) surveys of the Nord-Trøndelag Health Study (HUNT3; 2006–2008 and HUNT4; 2017–19).

Average ten-year decline in VO_{2peak} was non-linear and progressed from 3% in the third to about 20% in the eight decade in life and was more pronounced in men. The fit linear mixed models including an additional 2,933 observations from subjects participating only in HUNT3 showed similar age-related decline. Self-reported adherence to LTPA recommendations was associated with better maintenance of VO_{2peak}, with intensity seemingly more important than minutes of LTPA with higher age. Adjusted linear regression analyses showed that one mL/kg/min better maintenance of VO_{2peak} was associated with favorable changes of individual cardiovascular risk factors (all $p \leq 0.002$). Using logistic regression one mL/kg/min better maintenance of VO_{2peak} was associated with lower adjusted odds ratio of hypertension (0.95 95% CI 0.92 to 0.98), dyslipidemia (0.92 95% CI 0.89 to 0.94), and metabolic syndrome (0.86 95% CI 0.83 to 0.90) at follow-up.

Conclusions: Although VO_{2peak} declines progressively with age, performing LTPA and especially high-intensity LTPA is associated with less decline. Maintaining VO_{2peak} is associated with an improved cardiovascular risk profile.

Cardiovascular disease (CVD) is a burden to societies and healthcare systems globally despite the reduction in CVD mortality over the last decades,[1,2] and strategies for population-level prevention of CVD should have high priority. Low cardiorespiratory fitness (CRF) is a strong predictor of morbidity and mortality from both CVD and other causes.[3,4] Furthermore, it is a predictor of dependence,[5] which is of interest given the aging populations in most countries. The growing knowledge of CRF as a powerful composite health measure in both clinical and apparently healthy populations was highlighted in the 2016 recommendations for cardiopulmonary exercise testing (CPET) by the European Association for Cardiovascular Prevention & Rehabilitation and the American Heart Association.[6] To exploit the potential of CRF in both preventive and clinical settings knowledge about reference values and normal age-related changes in CRF is needed.

In a sub-study of the third wave of the Nord-Trøndelag Health Study (HUNT3, 2006–2008) peak oxygen uptake (VO_{2peak}) was assessed by CPET in 4631 apparently healthy men and women, establishing a large reference material on VO_{2peak}.[7] Reference values from the Norwegian HUNT population and several other populations have shown that normal CRF values differ widely across various populations.[7–9]

Knowledge on the age-related decline in CRF is important for follow-up of patients in lifestyle interventions and for identification of

Abbreviations: ACLS, Aerobics Center Longitudinal Study; BMI, Body mass index; BP, Blood pressure; CARDIA, Coronary Artery Risk Development in Young Adults; CPET, Cardiopulmonary exercise testing; CRF, Cardiorespiratory fitness; CVD, Cardiovascular disease; HbA1c, Glycosylated hemoglobin; HDL, High-density lipoprotein cholesterol; HR, Heart rate; HUNT, Nord-Trøndelag Health Study [Helseundersøkelsen Nord-Trøndelag]; LTPA, Leisure-time physical activity; PA, Physical activity; VO_{2peak}, Peak oxygen uptake; WC, Waist circumference.

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abnormal trajectories in CRF for possible intervention. However, large studies have traditionally been confined to investigate this by cross-sectional designs which seem to underestimate the age-related decline observed when VO_{2peak} are investigated serially.[10,11] To our knowledge only two large individual-level longitudinal studies examining CRF in the whole adult population-span have been conducted.[11,12] Of these, only one measured CRF as VO_{2peak} by CPET.[11] The differences in cross-sectional and longitudinal findings, and lack of rigorous longitudinal studies emphasize the need for further studies.

Given the strong prognostic value of CRF for future health outcomes it is not unexpected that both CRF and change in CRF have been associated to favorable levels of CVD risk factors.[7,13] However, no studies have examined the association between change in directly measured VO_{2peak} and concurrent change in CVD risk factor levels in a large adult population.

Therefore, our aims were to use novel follow-up data from the HUNT study (HUNT4, 2017–2019) on VO_{2peak} to assess I) the age-related change in VO_{2peak} in an apparently healthy population sample after ten years, II) the influence of intensity and volume of leisure-time physical activity (LTPA) on change in VO_{2peak}, and III) the association between change in VO_{2peak} and change in CVD risk factors.

Methods

Study population

The study population includes 4404 participants from the HUNT3 Fitness Study, of whom 1471 also participated in the HUNT4 Fitness Study (Fig. 1). Exclusion criteria in the HUNT3 Fitness Study were presence of CVD, malignant, or pulmonary disease, or use of antihypertensive medication,[7] while exclusion criteria for the HUNT4 Fitness Study were disease or disability prohibiting exercise testing (see exhaustive list in Supplemental Methods).

Ethical approval for the current study and the HUNT4 Fitness Study itself was obtained from the Regional Committee for Medical Research Ethics (2019/7243, 2017/911).

CPET in HUNT4

The treadmill protocol used in HUNT4 was similar to HUNT3, which have been described previously.[7] In short, participants performed a 10-min warm-up followed by a stepwise protocol starting with two submaximal levels of 3 and 1.5 min, respectively, before inclination (1–2%) and/or speed (0.5–1 km/h) was increased every minute until voluntary exhaustion. Continuous gas analysis was done with the MetaLyzer II (Cortex Biophysik Gmbh, Leipzig, Germany) mixing chamber system with participants wearing an oro-nasal mask (Hans Rudolph V2, US) tested for leakage. VO_{2peak} was defined as the highest oxygen uptake averaged over 30 s (three 10 s measurements), and are presented as absolute (L/min) and relative (mL/kg/min) values. Observations were excluded from the analyses ($n = 227$, HUNT3; $n = 34$, HUNT4) if the respiratory exchange ratio was <1.0, indicating a submaximal effort, which is in line with previous studies.[9] Forty participants had a submaximal effort in HUNT3 but not in HUNT4. Peak heart rate (HR) was defined as the highest HR recorded during exercise. Further information regarding CPET, criteria for maximal oxygen uptake, calibration procedures, and reproducibility of measures are available in Supplemental Methods.

Clinical and biochemical measurements

Detailed information on collection of these measures have been described for HUNT3.[14] Briefly, weight was measured wearing light clothes without shoes, height standing relaxed, and waist circumference (WC) horizontally at umbilical level in a relaxed standing position with arms hanging. Body mass index (BMI) was calculated. Blood pressure (BP) was measured sitting using standardized methods to the nearest 2 mmHg by an oscillometry-based Dinamap Carescape V100 in HUNT4 and Critikon 845XT in HUNT3. The average of the last two of three measurements were used. Resting HR was measured during BP measurements and defined as the lowest of three measures. Non-fasting blood samples were analyzed for high-density lipoprotein (HDL) and total cholesterol, triglycerides, glucose (HUNT3 only), glycosylated hemoglobin (HbA1c; HUNT4 only), c-reactive protein, and creatinine. Total-cholesterol to HDL-cholesterol ratio was calculated.

Self-reported measures

Smoking status (never, former, regular, occasional, plus former occasional in HUNT4) was dichotomized to current occasional or regular smoker (yes/no), snuffing (never, former, regular, occasional) was dichotomized to current occasional or regular snuffer (yes/no), and alcohol intake ("About how often during the last 12 months did you drink alcohol?") was dichotomized to more than once per week (yes/no). Information on family history of CVD (stroke or myocardial infarction <60 years of age in first-degree relative), and information on previous cardiac, pulmonary, and malignant disease was also based on self-report questionnaires. Information on LTPA was gathered by validated questionnaires[15,16] in the baseline examinations of HUNT3 and HUNT4 with questions regarding frequency (never, less than once per week, once per week [1], 2–3 times [2.5] per week, or roughly every day [5]), duration of exercise each session (less than 15 min [7.5], 15–29 min [22.5], 30–60 min [45], or over 60 min [75]), and intensity as low ("I take it easy, I don't get out of breath or break a sweat"), moderate ("I push myself until I'm out of breath or break into a sweat"), or high ("I practically exhaust myself") intensity. Weekly minutes of LTPA was calculated based on values in brackets. Never or less than once per week of exercise was interpreted as inactive (no regular/weekly LTPA). Weighted weekly minutes of LTPA were calculated where low, moderate, and high intensity was weighted as 0.5, 1, and 2 multiplied by the weekly minutes of LTPA (with inactive as 0). Adherence to LTPA recommendations[17] was defined as ≥75 min high intensity LTPA or ≥150 min moderate intensity LTPA.

Statistical analyses

Time between participation in the HUNT3 and HUNT4 Fitness Studies was mean 10.2 years (range 9.5 to 11.0), and change in VO_{2peak} and CVD risk factors were therefore scaled to ten-year change ((value HUNT4 – value HUNT3) * (10/time in years)). Descriptive data on mean change in VO_{2peak} is presented by age group and sex for subjects participating at both examinations ($n = 1431$). Analyses on changes in VO_{2peak} with age were further performed by linear mixed-effects regression models using the lme4[18] package in R, and fitted by maximal likelihood to assess model performance by the Akaike information criterion. This design handles dependency of observations within participants[19] and allows imbalance between HUNT3 and HUNT4 participation, meaning that those only participating in HUNT3 ($n = 2,933$) or HUNT4 ($n = 40$) still could contribute with information to the model. We included age, sex, survey, weighted weekly minutes and intensity of LTPA, current smoking, alcohol use, and presence of CVD or pulmonary disease in the models. Time was modelled as participant age. We explored for interaction between model covariates and polynomials for age and weighted weekly minutes of LTPA. The final regression equations are available in Supplemental Methods. Presented figures were produced keeping continuous covariates at their mean (unless otherwise specified) and categorical covariates at representable proportions.

We performed linear regression analyses with change of traditional cardiovascular risk factors (HDL-cholesterol, total cholesterol, total cholesterol to HDL-cholesterol ratio, triglycerides, systolic and diastolic BP, resting HR, and WC) as the outcome and change of relative VO_{2peak} as

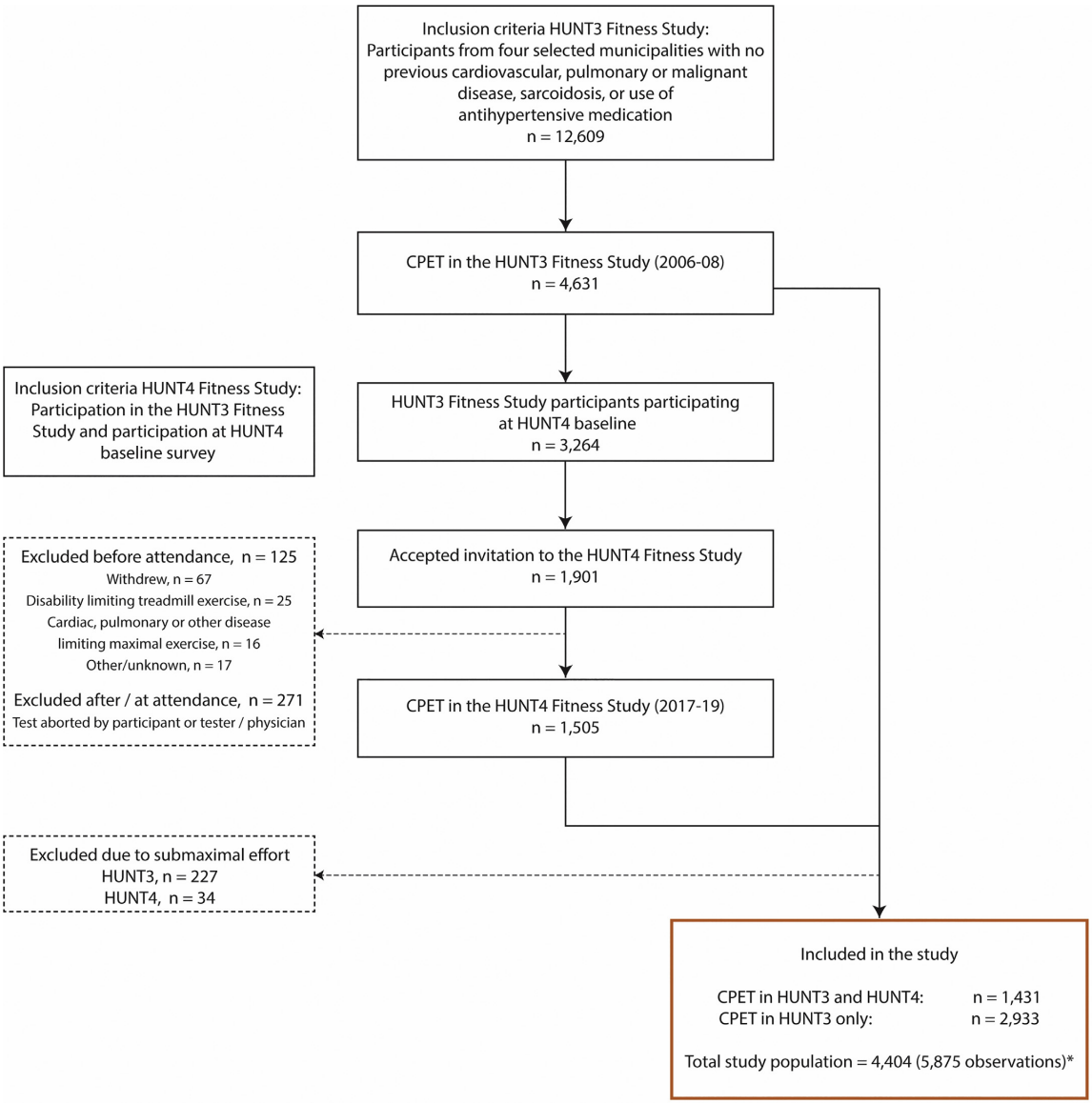

Fig. 1. Flow chart of the study population. *Forty participants had submaximal effort in HUNT3 but not in HUNT4.

predictor for the participants participating at both examinations. Analyses were adjusted for age and VO_{2peak} at baseline (HUNT3), sex, current smoking and regular alcohol intake at baseline and follow-up (HUNT4), family history of CVD, and incident CVD between baseline and follow-up in one model (model 1), plus weighted weekly minutes of LTPA at baseline and follow-up, and change and baseline value of weight in a second (model 2). Stratified analyses by sex, baseline VO_{2peak} (over/under age- and sex-specific averages), age (over/under 50 years), BMI (over/under 30 kg/m^2) were also performed. In analyses of systolic and diastolic BP and lipids we excluded participants with use of medication due to elevated BP or lipids, respectively. Assumptions regarding normality of residuals and heteroskedasticity were checked visually.

We also performed logistic regression analyses with presence of metabolic syndrome, dyslipidemia, and hypertension in HUNT4 as the outcome and change of relative VO_{2peak} as predictor for those participating at both examinations. Analyses were adjusted as for model 1 and model 2 plus adjustment for cholesterol and BP medication for dyslipidemia and hypertension, respectively, and adjustment for cholesterol medication for metabolic syndrome. Sensitivity analyses were performed by excluding those with medication use. Due to non-linear associations for age, age was included as a categorical covariate in the

analyses for the metabolic syndrome. Definitions of metabolic syndrome, dyslipidemia and hypertension were based on established criteria (see Supplemental Methods). All statistical analyses were performed using R (www.r-project.org).

Results

Baseline characteristics, stratified by sex, from HUNT4 is shown in Table 1, and the age distribution among participants in HUNT3 and HUNT4 in Fig. 2A. Sex was balanced within age groups at both surveys. General characteristics from HUNT3 for all HUNT3 Fitness Study participants ($n = 4404$) and the participants repeating CPET in HUNT4 ($n = 1471$), as well as the repeated measures in HUNT4, are presented in Supplemental Table 1. Notably, adherence to physical activity (PA) recommendations was higher in HUNT4 (37%) than HUNT3 (28%), with the same trend of higher adherence for all age groups (except similar for age > 80 years). Active smoking declined from 13 to 3% from HUNT3 to HUNT4. VO_{2peak} data by deciles of age are shown in Supplemental Fig. 1.

Age-related change in VO_{2peak}

Average ten-year decline in VO_{2peak} was 3.7 mL/kg/min (10%) in women and 5.3 mL/kg/min (12%) in men, ranging from mean 3% decline in those between 20 and 30 years of age at baseline to about 20% in those between 70 and 80 years of age at baseline (sexes combined). Average ten-year change in VO_{2peak} by sex and deciles of age for both absolute (L/min) and relative (mL/kg/min) VO_{2peak} is shown in Fig. 2B–C, demonstrating an accelerated decline with advanced age for men compared to women. Percentage decline in absolute VO_{2peak} was similar for men and women at lower ages, while the same trend of accelerated decline in men was seen with higher age (Fig. 2D). Similar trends in decline in VO_{2peak} were seen when excluding participants with CVD between baseline and follow-up (Supplemental Fig. 2). The results from the linear mixed model showed the same non-linear pattern (Fig. 3). Both the descriptive data and model-predicted results indicate that the accelerated decline among men is most pronounced for absolute VO_{2peak}. In women the age-related decline in relative VO_{2peak} was more linear. Sensitivity analyses regarding the age-related decline in VO_{2peak} excluding participants not reaching true VO_{2max} did not make notable changes to the results.

Table 1
General characteristics of the HUNT4 study population by sex.

	Women*	Men*
N (%)	743 (50.5%)	728 (49.5%)
Age (years)	59.1 (11.6)	60.5 (11.7)
Weight (kg)	69.7 (11.3)	84.8 (11.3)
Height (cm)	166 (5.7)	179 (6.3)
BMI (kg/m^2)	25.4 (3.9)	26.4 (3.1)
Waist circumference (cm)	90 (12)	96 (11)
Hip circumference (cm)	97 (6)	102 (5)
Resting heart rate (beats/min)	66 (11)	64 (11)
Systolic blood pressure (mmHg)	128 (19)	132 (17)
Diastolic blood pressure (mmHg)	73 (9)	78 (10)
HDL (mmol/L)	1.63 (0.37)	1.32 (0.30)
Cholesterol (mmol/L)	5.70 (1.1)	5.42 (1.1)
Cholesterol/HDL ratio	3.65 (1.0)	4.29 (1.2)
Triglycerides (mmol/L)	1.33 (0.7)	1.67 (1.0)
HbA1c (mmol/mol)	33.5 (3.8)	34.7 (5.0)
Creatinine (μmol/L)	67.1 (10.2)	81.8 (13.1)
C-reactive protein (mg/L)	1.92 (4.1)	1.63 (2.3)
VO_{2peak} (mL/kg/min)	33.8 (7.6)	40.5 (9.3)
VO_{2peak} (L/min)	2.34 (0.5)	3.41 (0.8)
Oxygen pulse (ml/beat)	13.6 (2.7)	19.9 (4.2)
Respiratory exchange ratio	1.11 (0.05)	1.11 (0.05)
Peak heart rate (beats/min)	172 (14)	171 (15)
Current smoker, n(%)	25 (3.4%)	19 (2.6%)
Regular alcohol intake, n(%)	200 (27%)	256 (35%)
Physically active, n(%)	264 (36%)	281 (39%)
Metabolic syndrome, n(%)	102 (14%)	129 (18%)
Cardiac disease, n(%)	30 (4%)	83 (11%)
Pulmonary disease, n(%)	19 (2.6%)	26 (3.6%)

Abbreviations: SD = standard deviation; BMI = body mass index; HDL-cholesterol = high-density lipoprotein cholesterol; HbA1c = glycosylated hemoglobin; VO_{2peak} = peak oxygen uptake; Physically active = adherence to physical activity recommendations.
* Values are mean (SD) or n (%).

LTPA and change in VO_{2peak}

Descriptive data for mean change of relative VO_{2peak} from HUNT3 to HUNT4 showed a clear trend towards lower decline in VO_{2peak} with adherence to PA recommendations and with higher intensity of LTPA (Supplemental Table 2). Specifically, adherence to LTPA recommendations in HUNT4 was associated with a 9.0% ten-year decline compared to a 15.9% decline in those being inactive. A higher number of weekly minutes of LTPA did not show the same clear pattern of higher VO_{2peak} in the descriptive data. Similar trends were seen when stratified by sex, although low numbers in some strata when stratifying by both LTPA and sex (data not shown). Results from the linear mixed model showed similar findings with a better maintenance of VO_{2peak} with better adherence to LTPA recommendations (Fig. 4A). Seventy-five minutes of high-intensity LTPA was associated with a similar age-related decline in VO_{2peak} as for 150 min of moderate intensity. However, age affected this relationship for men with high-intensity being associated with better maintenance of VO_{2peak} at higher age (Fig. 4B). With higher age the lines for predicted relative VO_{2peak} for 75 and 150 min of LTPA converge within intensity categories, indicating that the relative effect on VO_{2peak} of intensity increases with higher age compared to minutes of LTPA. In both HUNT3 and HUNT4 the percentage of participants performing high-intensity LTPA was lower with higher age.

Change in VO_{2peak} and change of CVD risk factor levels

Linear regression analyses showed significant associations between change in relative VO_{2peak} and favorable changes of HDL- and total cholesterol, total-cholesterol to HDL-cholesterol ratio, triglycerides, systolic and diastolic BP, resting HR, and WC (Table 2; Model 1). After further adjustment for weighted volume of LTPA, weight at baseline, and weight change (Model 2), significant associations to change in VO_{2peak} were seen for change of HDL, total-cholesterol to HDL-cholesterol ratio, resting HR and WC. Results were similar in analyses stratified by sex, age, baseline VO_{2peak}, and BMI (Supplemental Fig. 3), although effect estimates were generally higher for those with high BMI (>30 kg/m^2) for lipid and BP measures.

Odds ratios from logistic regression analyses for the association between per one unit (mL/kg/min) lower decline in VO_{2peak} and presence of the metabolic syndrome in HUNT4 was 0.86 (95% CI 0.83 to 0.90; Model 1) and 0.93 (95% CI 0.89 to 0.98, Model 2). For present dyslipidemia in HUNT4 the odds ratio was 0.92 (95% CI 0.89 to 0.94, Model 1) and 0.96 (95% CI 0.93 to 0.99, Model 2), and for present hypertension 0.95 (95% CI 0.92 to 0.98, Model 1) and 0.96 (95% CI 0.93 to 1.00, Model 2). Sensitivity analyses excluding those with medication use gave similar results.

Discussion

Our long-term follow-up data of VO_{2peak} in adults demonstrate a non-linear decline with higher age, and the progressive decline is more pronounced in men. Performing LTPA according to recommendations was associated with better maintenance of VO_{2peak}, and for both sexes high-intensity LTPA was associated with maintaining a higher VO_{2peak} with aging compared to moderate intensity. Better maintenance of VO_{2peak} was associated with a more favorable change in individual CVD risk factors and less CVD risk factor clustering at follow-up.

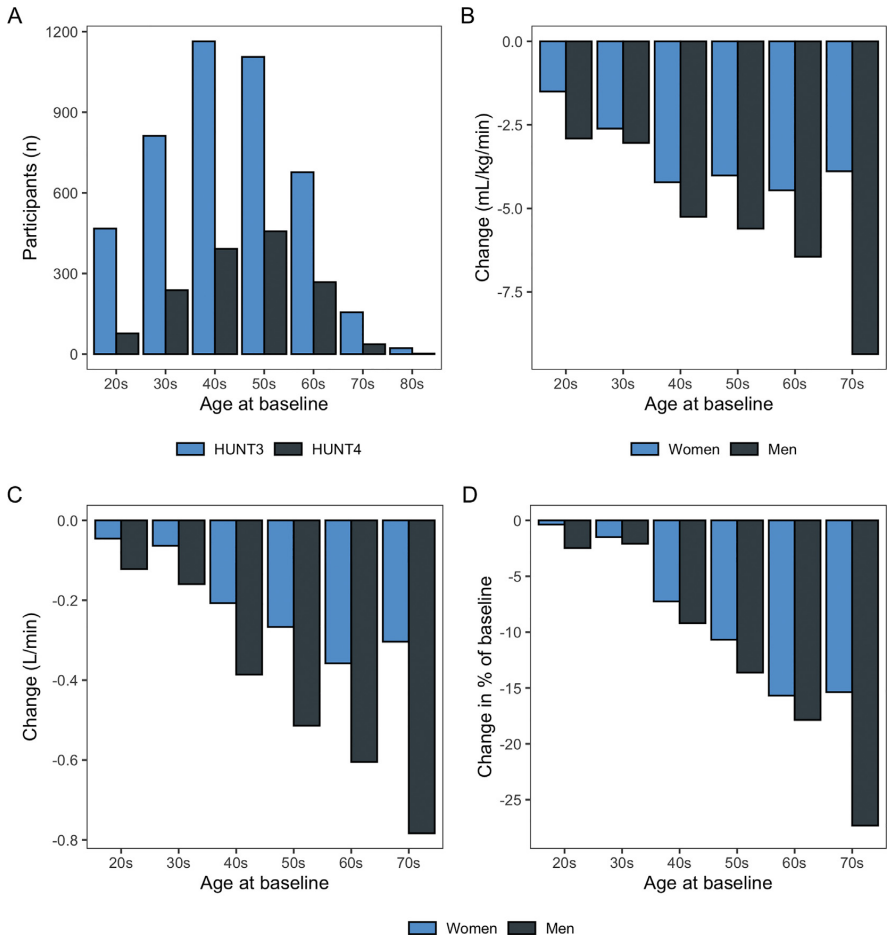

Fig. 2. Age distribution among participants in HUNT3 and HUNT4 (A), and decline in peak oxygen uptake as mL/kg/min (B), L/min (C) and percentage of baseline absolute peak oxygen uptake (D).

Age, LTPA, and change in VO_{2peak}

The progressive decline in VO_{2peak} with advanced age is in line with previous observations.[11,12] Fleg et al. assessed longitudinal changes in VO_{2peak} based on 2302 CPETs performed in 810 healthy volunteers (375 women) and reported an accelerated decline with higher age that was more pronounced in men.[11] The decline progressed from 5% per ten years in 30-year-old men to nearly 25% in 70-year-old men, comparable to our findings. The results for women were also similar, with a somewhat lower decline compared to men, and the percentage decline seemed to level off after the sixth decade. A study from the Aerobics Center Longitudinal Study (ACLS) using maximal treadmill exercise estimated CRF showed similar patterns.[12] Smaller longitudinal studies (~10 to 60 participants) with varied inclusion criteria have also shown relatively similar annual reductions in VO_{2peak}.[10] In our study the age-related peak in absolute VO_{2peak} occurs at about 25 to 30 years of age, similar to the findings by Fleg et al.[11] The decline in relative VO_{2peak} is evident from the early 20's. However, the study from the ACLS cohort showed a decline in relative CRF from about 35 years of age, but their models were conditioned for BMI providing a likely explanation for the different trends as weight change affects these interrelations, and weight increase at these ages is well established.[20] Longitudinal changes in VO_{2peak} scaled to fat-free mass support these interpretations.[11] Given weight being a decisive component in relative VO_{2peak} and the age-related changes in body weight/body composition, we decided not to condition for weight in models to predict age-related change in relative VO_{2peak}. Although our findings imply that the decline in VO_{2peak} in at least some sense seem to reflect inevitable physiological aging, performing LTPA, and especially high-intensity LTPA, may slow the decline. Both weekly minutes and intensity of LTPA was associated with higher VO_{2peak}, but notably high intensity was associated with a slower decline in VO_{2peak} by higher age compared to moderate intensity. The difference between the predicted lines of VO_{2peak} for 75 and 150 min of LTPA became smaller with higher age, while the difference between moderate and high-intensity LTPA was still large, especially in men. This may suggest that increasing intensity is more efficient than increasing minutes of LTPA, especially for older male individuals. Thus, these longitudinal observational data over a decade provide novel insights supporting findings from previous short-term randomized controlled trials showing superior effect of high-intensity compared to moderate intensity exercise on VO_{2peak}.[21] LTPA was also associated with higher CRF in previous longitudinal studies, however these studies did not report differential effects of LTPA intensity on the age-related decline.[11,12]

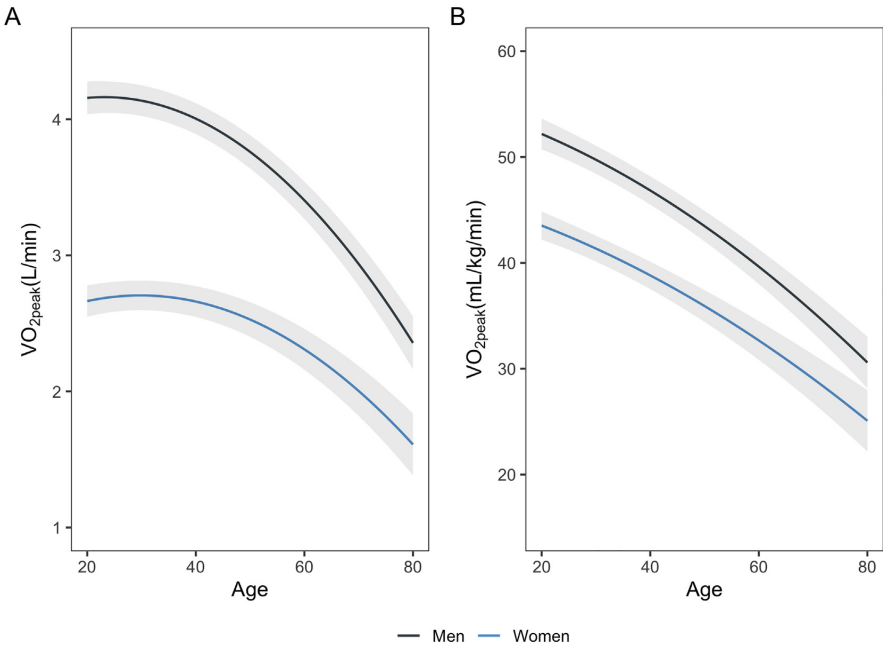

Fig. 3. Age-related change in absolute (A) and relative (B) peak oxygen uptake (VO_{2peak}) by sex with associated 95% confidence intervals.

Concurrent change in VO_{2peak} and CVD risk factors

Results from the CARDIA (Coronary Artery Risk Development in Young Adults) study showed a significant inverse association between change in CRF and development of the metabolic syndrome, but not with development of hypertension or hypercholesterolemia.[22] However, the 2478 participants were all relatively young (<30 years), and CRF was estimated by maximal treadmill time, which may explain some of the discrepancy to our study. Few other studies have examined the *concurrent change* of CRF and CVD risk factors, but another investigation from the CARDIA study showed how change in CRF was associated with change in HDL-, LDL- and total-cholesterol, and triglycerides, while only the association with HDL-cholesterol was significant after adjustment for weight change.[23] These findings are similar to ours. Similarly, a study from Belgium showed an association between change in VO_{2peak} over ~10 years and change in individual and clustered cardiovascular risk factors in 425 adults.[24] Further, they showed that the effect of moderate-to-vigorous PA on CVD risk factors was mediated by VO_{2peak}. A study from the ACLS cohort found significant associations between change in CRF and development of hypertension, dyslipidemia, and metabolic syndrome in 3148 participants, as well as significant correlations between change in CRF and change in the individual levels of blood lipids, BP, and WC.[25] In our models adjusted for LTPA, weight and weight change the associations were attenuated retaining only clear associations between VO_{2peak} and HDL-cholesterol, total-cholesterol to HDL-cholesterol ratio, resting HR, and WC. Clearly, PA and weight reduction are keys in CRF improvement and general CVD risk reduction, and attenuation of these associations is thus in line with what one would expect.

Strengths and limitations

The repeated measures of VO_{2peak} by gold standard CPET in a large, free-living, and at baseline apparently healthy population sample is the main strength of the study. As part of a large population study we had access to high-quality measurements of measured and self-reported covariates for analyses. As for all studies performing voluntary exercise testing, selection bias is an issue, and as previously reported the HUNT3 Fitness Study participants were slightly leaner, more physically active, and had a favorable CVD risk profile compared to non-participants.[7] Survivor bias (from death or diagnoses leading to study exclusion), in particular between HUNT3 and HUNT4, affects the population returning to testing in HUNT4. The differences at HUNT3 between those repeating testing in HUNT4 and the whole HUNT3-population were small, but with trends towards healthier returning participants and a higher baseline VO_{2peak}. Time of measurements (period effects) and birth cohort (cohort-effects) may influence studies on the normal aging-process of CRF such as ours due to e.g. societal changes. This may also explain some of the reported discrepancy between cross-sectional and longitudinal decline in CRF.[10,11] Self-reported LTPA at baseline and follow-up as the sole information on LTPA for a decade follow-up of change in VO_{2peak} is another limitation, and self-reported measures of PA has previously been shown to be less accurate in individuals >65 years compared to younger individuals,[26] which may affect the observed relationships between LTPA and VO_{2peak}. Also, levels of LTPA was not matched on energy expenditure in the comparisons in this study. The observational data also precludes firm conclusions regarding cause and effect, especially noteworthy for the analyses on change in VO_{2peak} and change in CVD risk factors adjusted for weight and LTPA, as these factors and VO_{2peak} are closely related entities without a straightforward causal relation. That mentioned, these longitudinal observational data over ten years support randomized trials indicating a relation between CRF response and lowering of CVD risk factors.

Clinical implications

Given that many of the health-benefits from PA seem to be mediated through CRF as shown in several studies, PA recommendations should

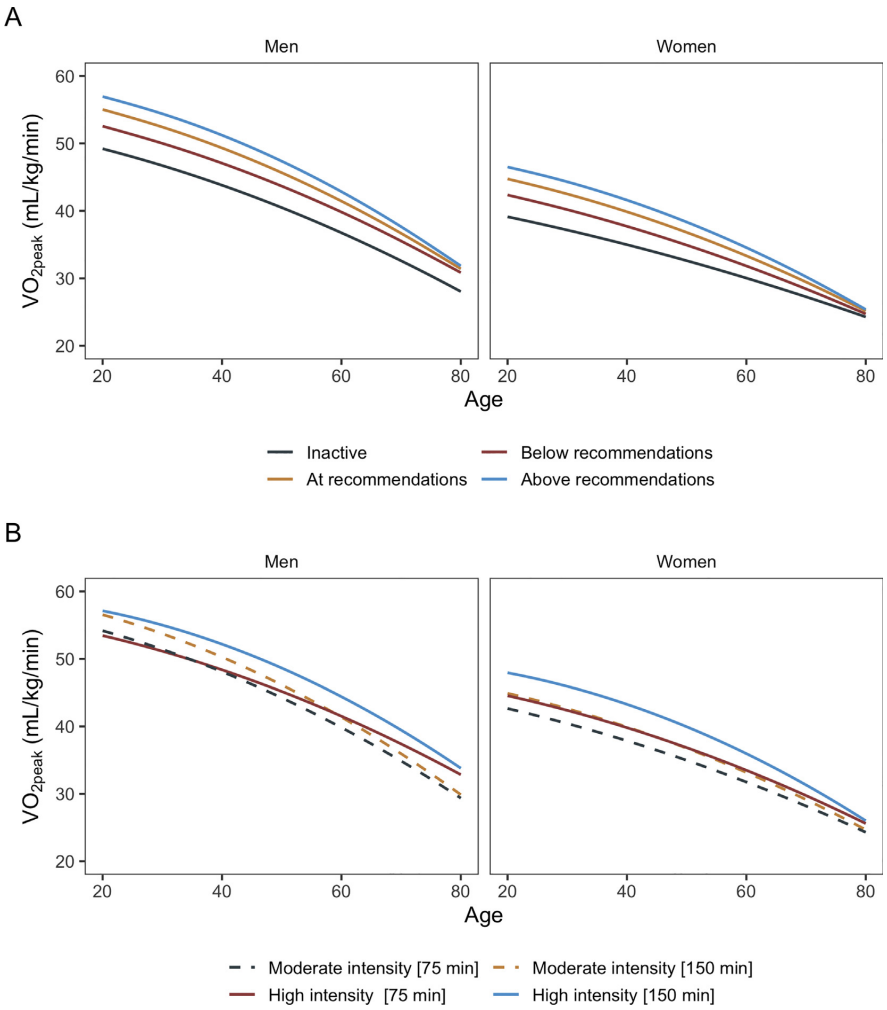

Fig. 4. Age-related change in relative peak oxygen uptake (VO_{2peak}) by measures of leisure-time physical activity (LTPA) for women and men by A) adherence to physical activity recommendations (inactive = 0, below recommendations = 75, at recommendations = 150, and above recommendations = 225 weighted weekly minutes of LTPA), and B) 150 and 75 weekly minutes of LTPA of moderate and high intensity.

Table 2
The associations of one mL/kg/min lower decline of peak oxygen uptake (VO_{2peak}) with change in different cardiovascular risk factors.

	Model 1†			Model 2‡		
Risk factor (dependent)*	Beta	95% CI	p	Beta	95% CI	p
HDL-cholesterol	0.010	0.008, 0.013	<0.001	0.005	0.001, 0.008	0.004
Cholesterol	−0.015	−0.024, −0.006	<0.001	−0.001	−0.011, 0.009	0.9
Cholesterol/HDL-cholesterol ratio	−0.040	−0.048, −0.031	<0.001	−0.014	−0.02, −0.005	0.003
Triglycerides	−0.028	−0.037, −0.018	<0.001	−0.004	−0.015, 0.006	0.4
Systolic blood pressure	−0.27	−0.44, −0.1	0.002	−0.088	−0.28, 0.11	0.4
Diastolic blood pressure	−0.16	−0.26, −0.0634	0.001	−0.095	−0.21, 0.02	0.10
Resting heart rate	−0.29	−0.40, −0.18	<0.001	−0.21	−0.34, −0.10	<0.001
Waist circumference	−0.70	−0.78, −0.61	<0.001	−0.13	−0.21, −0.06	<0.001

Abbreviations: CI = confidence interval; VO_{2peak} = peak oxygen uptake; HDL = high-density lipoprotein.

* Change in relative VO_{2peak} was used as independent variable in the models.

† Model 1: adjusted for age at baseline, sex, VO_{2peak} at baseline, current smoking and regular alcohol intake at baseline and follow up, family history of CVD, and incident CVD between baseline and follow-up.

‡ Model 2: adjusted as for model 1 plus weighted volume of physical activity at baseline and follow-up, and weight at baseline and change in weight between baseline and follow-up.

emphasize the importance of performing PA known to increase or maintain CRF. Results from this study suggest that, especially with higher age, performing high-intensity LTPA may be more beneficial on VO_{2peak} than increasing the weekly minutes of LTPA. Although this study presents observational data, the value of maintaining a high VO_{2peak} is supported by the association between maintaining VO_{2peak} and a more favorable CVD risk profile.

The provided longitudinal data on age-related decline in VO_{2peak} may assist clinicians in interpreting trajectories of CPET data to identify abnormalities, although caution should be taken when comparing different populations.

Conclusions

Our study shows that VO_{2peak} declines progressively with age, but performing LTPA, and especially high-intensity LTPA, may slow the decline. Maintaining a higher VO_{2peak} is also associated with favorable changes of CVD risk factors. These findings may have implications for future PA recommendations, and should ease and further encourage regular assessment of LTPA and CRF by clinicians involved in preventive medicine.

Authors contributions

Conceptualization: JL, HD, UW, and BN. Data curation: JL, HD, SA, UW, and BN. Formal analysis: JL, ØS and BN. Funding and acquisition: BN and UW. Investigation: All authors. Methodology: JL, ØS and BN. Project administration, recources and supervision: HD, UW and BN. Resources: UW and BN. Visualization: JL. Writing original draft: JL. Writing, review & editing: all authors. co All authors gave final approval of the manuscript, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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There are no relations to industry associated with this work.

Declaration of Competing Interest

The Authors declare that there is no conflict of interest.

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We recognize the invaluable effort by employees and students from our research group throughout the data collection process at the HUNT4 Fitness Study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pcad.2020.09.002.

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SUPPLEMENTAL METHODS AND RESULTS

Manuscript title: Age-related change in peak oxygen uptake and change of cardiovascular risk factors. The HUNT Study.

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SUPPLEMENTAL METHODS AND RESULTS

The HUNT4 Fitness Study

The HUNT4 Fitness Study recruited participants having participated in the HUNT3 Fitness Study ~10 years earlier, as well as participants with validated (HUNT3)[1] or self-reported atrial fibrillation (HUNT4), and participants from the HUNT3 Cardiac Ultrasound Study.

Participants were invited to the test-station on a separate day from the HUNT4 baseline examination (median 44 days (range 5 to 270) later), and after giving informed consent to participation a medical symptom-based structured interview was performed to assure safety of CPET. A physician was consulted if there was any doubt regarding safety of participation. Height and weight (wearing light clothes without shoes) was measured before a sit-to-stand chair test and rate of force development test by squat jump on a force platform prior to comprehensive transthoracic echocardiographic examination (the HUNT4 Fitness Study was co-organized with the HUNT4 cardiac ultrasound study) and answering self-reported questionnaires regarding leisure-time physical activity habits and sporting history. Clinical and biochemical measures were done at the HUNT3/HUNT4 main test stations, while weight used for scaling of VO_{2peak} was measured at the participation day in the HUNT4 Fitness Study.

Exclusion criteria for the HUNT4 Fitness Study

Uncontrolled high blood pressure (>180/100 mm Hg), recent heart failure admission (<6 weeks), unstable angina, serious cardiac arrhythmia, pulmonary hypertension, recent deep venous thrombosis or pulmonary embolus, symptomatic valvular or other serious heart disease including pacemaker or implantable cardioverter defibrillator, recent acute myocardial infarction (last 4 weeks), active cancer treatment (last 4 weeks), chronic or acute contagious

infectious disease, diagnosed dementia, pregnancy, or restrictions to physical activity ordered by a physician.

Cardiopulmonary exercise testing

For participants with preexisting heart disease, or other conditions suggesting the need for extra surveillance, a physician monitored the test including a 12-lead electrocardiogram (ECG). Absolute and relative ECG and symptom criteria for test abortion was established in collaboration with a cardiologist. Healthy participants not requiring ECG monitoring had their heart rate continuously monitored by a heart rate sensor coupled to a sport watch (Polar Electro OY, Finland).

The criteria for reaching a true maximal oxygen uptake (VO_{2max}) were the combination of a plateau in oxygen consumption (< 2mL/kg/min increase) despite increasing work load and a respiratory exchange ratio ≥1.10. The criteria for VO_{2max} was met in 60% of participants (71% in HUNT3).

Volume and ambient air calibration were performed between each test. Volume calibration was performed using a standardized 3L syringe. The sample line for the gas analysis was changed for every sixth test, and gas calibration towards gases with known content was done at the same time as well as each morning.

The test-retest repeatability coefficient for the Metalyzer II used in HUNT4 was 1.6 mL/kg/min. In an in-house study at our lab the Metalyzer II systems used in HUNT4 has been validated against Douglas bag showing a mean bias of 0.4 mL/kg/min (limits of agreement ± 6.0) higher VO_{2peak} for the Metalyzer II, and the Metalyzer II used in HUNT4 has also been validated against the Metamax II used in HUNT3 showing a mean bias of 1.4 mL/kg/min (limits of agreement ± 5.6 mL/kg/min). Data on reproducibility of the HUNT3 Metamax II measures have been reported previously.[2]

Regression equations

Final linear mixed effects models.

Abbreviations: Age2 = quadratic term of age; Intensity = LTPA intensity; Paweightwk = weighted weekly volume of physical activity, Paweightwk2 = quadratic term of Paweightwk; Cardvascpulm_disease = cardiovascular or pulmonary disease; Smkcurrent = current smoking

Relative:

VO2kg~Age + Sex + Survey + Age2 + Intensity + Paweightwk + Paweightwk2 + Cardvascpulm_disease + Smkcurrent + Alcohol + Sex*Age2 + Sex*Intensity + Age2*Intensity + Age2*Paweightwk + Age*Paweightwk2 + Intensity*Paweightwk2 + Age2*Paweightwk2 + Age*Alcohol + Sex*Age2*Intensity + Age2*Intensity*Paweightwk2 + Sex*Intensity*Paweightwk2 + Sex*Age2*Paweightwk2 + Sex*Age2*Intensity*Paweightwk2 + (Random intercept for subject)

Absolute**:**

VO2litres~Age + Sex + Survey + Age2 + Intensity + Paweightwk + Paweightwk2 + Cardvascpulm_disease + Smkcurrent + Alcohol + Sex*Age2 + Sex*Intensity + Age2*Intensity + Age2*Paweightwk + Sex*Paweightwk + Intensity*Paweightwk2 +Age2*Paweightwk2 + Sex*Paweightwk2 + Age*Smkcurrent + Sex*Age2*Intensity + Sex*Age2*Paweightwk + Age2*Intensity*Paweightwk2 + Sex*Intensity*Paweightwk2 + Sex*Age2*Paweightwk2 + Sex*Age2*Intensity*Paweightwk2 + (Random intercept for subject)

Definitions of metabolic syndrome, dyslipidemia, and hypertension

Metabolic syndrome was defined as reaching minimum three of the following risk factor thresholds;[3] waist circumference (>88/102cm in women/men), triglycerides (≥1.70 mmol/L), HDL-cholesterol (<1.3/1.0 for women/men), BP (systolic >130 or diastolic >85 mmHg or use of BP medication), and hyperglycemia (using HbA1c ≥39 mmol/mol as the hyperglycemic criterion[4] due to plasma-glucose levels not being measured in HUNT4). Dyslipidemia was defined as total cholesterol >7.0, or HDL-cholesterol or triglycerides according to the provided thresholds. Hypertension was defined as systolic and/or diastolic BPs >140 and/or >90 mmHg, respectively. Thus, metabolic syndrome, dyslipidemia, and hypertension was based on clinical measures performed in the study and not diagnoses.

Supplemental References

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Supplemental Tables

Supplemental Table 1. General characteristics of the study population by participation in HUNT3 baseline (n=4,404), HUNT3 values for those participating in HUNT4 (n=1,471), and HUNT4 participants.

	HUNT3	HUNT3*	HUNT4
n	4,404	1,471	1,471
Age (years)	47.7 (13.3)	49.7 (11.7)	59.8 (11.7)
Weight (kg)	77.5 (13.8)	76.9 (13)	77.2 (13.6)
Height (cm)	173 (9.05)	173 (8.87)	172 (8.99)
BMI (kg/m^2)	25.9 (3.56)	25.7 (3.34)	25.9 (3.55)
Waist circumference (cm)	90.1 (10.9)	89.2 (10.2)	92.8 (11.5)
Hip circumference (cm)	102 (6.98)	102 (6.41)	99.6 (5.92)
Resting heart rate (beats/min)	66 (10.6)	64.7 (9.93)	64.9 (10.9)
Systolic blood pressure (mmHg)	127 (15.4)	128 (15.2)	130 (17.9)
Diastolic blood pressure (mmHg)	73 (10.5)	73.3 (10.3)	75.4 (9.6)
HDL-cholesterol (mmol/L)	1.39 (0.35)	1.43 (0.35)	1.48 (0.37)
Cholesterol (mmol/L)	5.44 (1.06)	5.51 (1.03)	5.56 (1.07)
Cholesterol/HDL ratio	4.13 (1.23)	4.07 (1.18)	3.96 (1.13)
Triglycerides (mmol/L)	1.5 (0.96)	1.47 (0.96)	1.5 (0.89)
Glucose (mmol/L)	5.37 (1.29)	5.32 (1.17)	NA
HbA1c (mmol/mol)	NA	NA	34.1 (4.48)
Creatinine (µmol/L)	78.8 (12.9)	79.9 (12.3)	74.3 (13.9)
C-reactive protein (mg/L)	1.85 (4.4)	1.55 (3.04)	1.78 (3.33)
VO_{2peak} (mL/kg/min)	40.5 (9.33)	41.7 (9.19)	37.1 (9.12)
VO_{2peak} (L/min)	3.14 (0.90)	3.2 (0.89)	2.87 (0.85)
Oxygen pulse (mL/beat)	17.5 (4.9)	17.9 (4.9)	16.8 (4.7)
Respiratory exchange ratio	1.13 (0.06)	1.13 (0.06)	1.11 (0.05)
Peak heart rate (beats/min)	180 (14.4)	179 (13.1)	171 (14.4)
Current smoker, n (%)	796 (18%)	187 (13%)	44 (3.0%)
Regular alcohol intake, n (%)	787 (18%)	329 (22%)	456 (31%)
Physically active, n (%)	1232 (28%)	441 (30%)	545 (37%)

Values are mean (SD) or n (%).

*Values from HUNT3 for the selection of HUNT3 participants participating in HUNT4

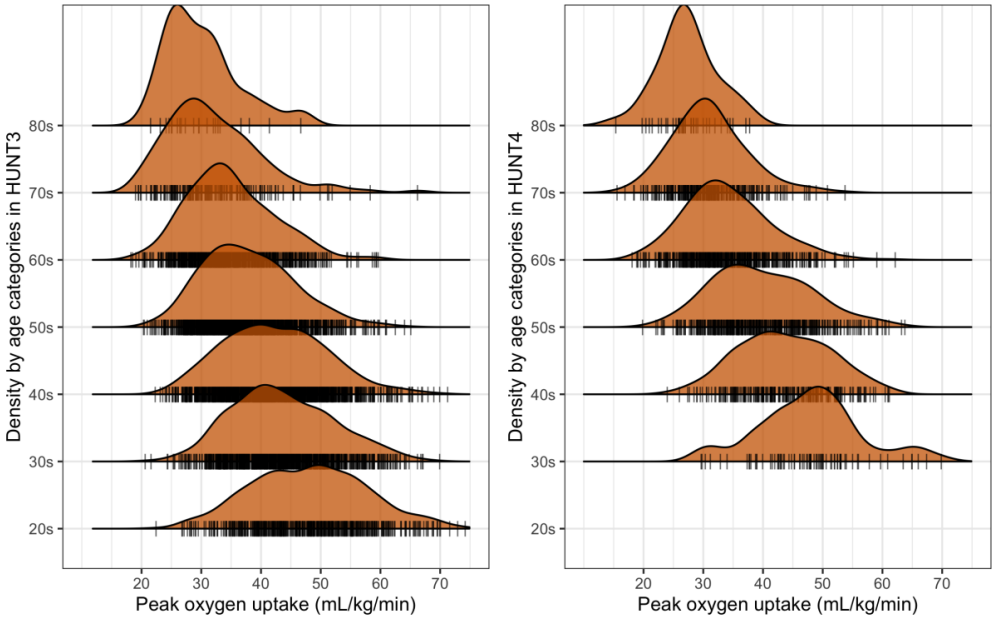
Abbreviations: HUNT3 / HUNT4 = third / fourth wave of the Nord-Trøndelag Health Survey; BMI = body mass index; HDL-cholesterol = high-density lipoprotein cholesterol; HbA1c = glycosylated hemoglobin; VO_{2peak} = peak oxygen uptake; Physically active = adherence to physical activity recommendations.

Supplemental Table 2. Effect of different measures of LTPA on ten-year change in relative peak oxygen uptake (mL/kg/min) from baseline to follow-up.

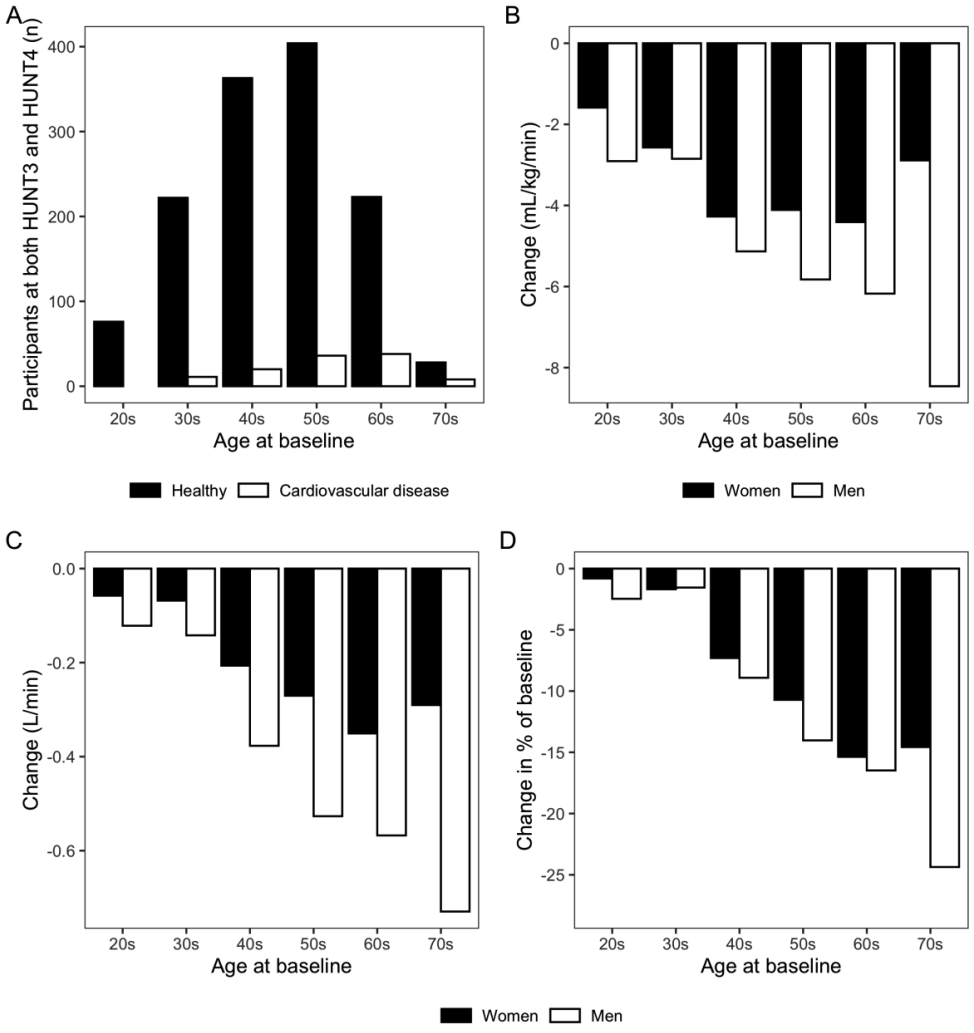
LTPA	n	Baseline VO_{2peak}	Follow-up VO_{2peak}	Change	Change (%)
Inactive*	80	38.7	32.6	-6	-15.9
Below recommendations*	808	40.8	36.1	-4.6	-11
Above recommendations*	529	44.1	40	-4.1	-9
Low intensity*					
1-75 minutes	98	38.5	33.2	-5.1	-12.4
75-150 minutes	106	38.1	32.5	-5.6	-14.7
>150 minutes	95	38.8	33.1	-5.6	-13.7
Moderate intensity*					
1-75 minutes	132	41.2	36.4	-4.7	-11.6
75-150 minutes	389	42.3	38.1	-4	-9.1
>150 minutes	437	43.7	39.5	-4.2	-9.3
High intensity*					
1-75 minutes	10	44.4	38.5	-5.8	-11.4
75-150 minutes	36	46.9	44	-2.8	-5.4
>150 minutes	34	49.1	46.6	-2.5	-4.6
Change in LTPA intensity†					
High to M/L intensity	77	50.2	43.8	-6.3	-12.7
M/L to high intensity	48	45.1	43.1	-1.9	-4.1
High to high intensity	27	52.1	46.7	-5.3	-9.9
M/L to M/L intensity	1073	41.4	36.8	-4.5	-10.7

* Self-report LTPA information from the fourth wave of the Nord-Trøndelag Health Study
† Self-report LTPA information from the third and fourth wave of the Nord-Trøndelag Health Study
Abbreviations: LTPA = leisure-time physical activity; VO_{2peak} = peak oxygen uptake, M/L = moderate or low.

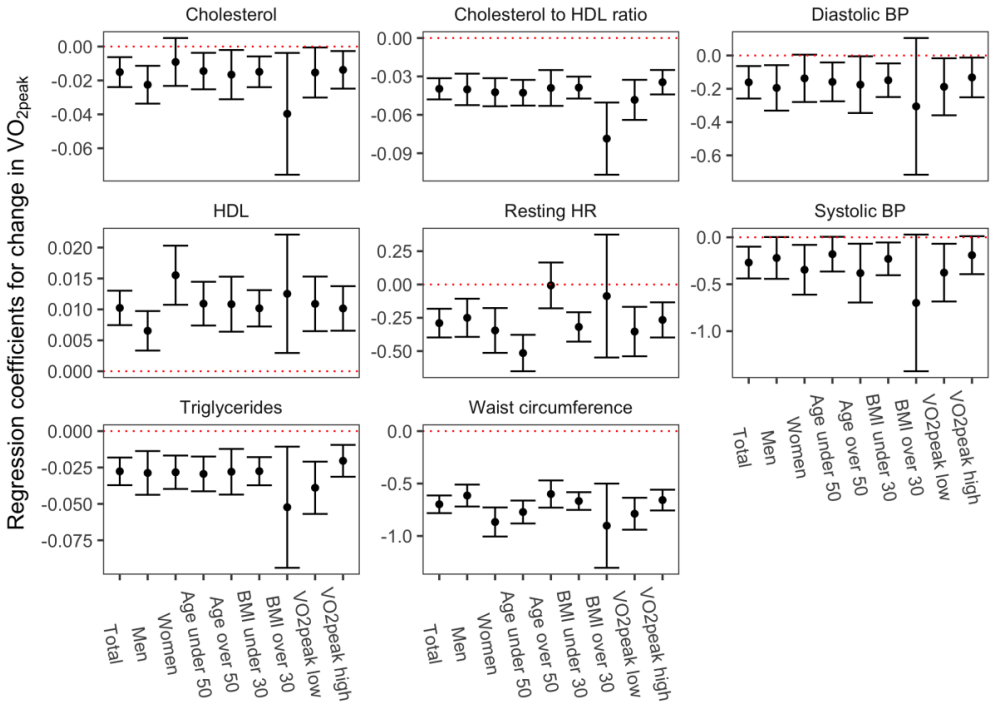
Supplemental Figures



Supplemental Figure 1. Raw data from the third (HUNT3) and fourth (HUNT4) wave of the Nord-Trøndelag Health Study showing probability density plots by age groups with individual observations plotted as vertical bars on the x axis.



Supplemental Figure 2. Age distribution among those participating in both HUNT3 and HUNT4 grouped by presence of self-reported cardiovascular disease occurring between HUNT3 and HUNT4 (A), and decline in peak oxygen uptake as mL/kg/min (B), L/min (C) and percentage of baseline absolute peak oxygen uptake (D) for participants without cardiovascular disease at both HUNT3 and HUNT4 (excluding 113 participants with cardiovascular disease).



Supplemental Figure 3. Regression coefficients with 95% confidence intervals for the effect of one unit change in relative VO_{2peak} (mL/kg/min) on change in shown cardiovascular risk factors for total sample, and compared to analyses stratified by sex, age (over/under 50 years), baseline BMI (over/under 30 kg/m^2), and baseline VO_{2peak} (over/under the age- and sex-specific predicted). Confidence intervals not encompassing zero indicates p-value <0.05. Analyses are adjusted as for model 1 (age at baseline, sex, VO_{2peak} at baseline, current smoking and regular alcohol intake at baseline and follow-up, family history of cardiovascular disease, and incident cardiovascular disease between baseline and follow-up).

PAPER III

Letnes JM, Nes B, Vaardal-Lunde K, Slette MB, Mølmen-Hansen HE, Aspenes ST, Støylen A, Wisløff U, Dalen H. Left Atrial Volume, Cardiorespiratory Fitness, and Diastolic Function in Healthy Individuals: The HUNT Study, Norway. *J Am Heart Assoc.* 2020;9(3).

ORIGINAL RESEARCH

Left Atrial Volume, Cardiorespiratory Fitness, and Diastolic Function in Healthy Individuals: The HUNT Study, Norway

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***Background*—**Left atrial (LA) size and cardiorespiratory fitness (CRF) are predictors of future cardiovascular events in high-risk populations. LA dilatation is a diagnostic criterion for left ventricular diastolic dysfunction. However, LA is dilated in endurance athletes with high CRF, but little is known about the association between CRF and LA size in healthy, free-living individuals. We hypothesized that in a healthy population, LA size was associated with CRF and leisure-time physical activity, but not with echocardiographic indexes of left ventricular diastolic dysfunction.

***Methods and Results*—**In this cross-sectional study from HUNT (Nord-Trøndelag Health Study), 107 men and 138 women, aged 20 to 82 years, without hypertension, cardiovascular, pulmonary, or malignant disease participated. LA volume was assessed by echocardiography and indexed to body surface area LAVI (left atrial volume index). CRF was measured as peak oxygen uptake (VO_{2peak}) using ergospirometry, and percent of age- and-sex-predicted VO_{2peak} was calculated. Indexes of left ventricular diastolic dysfunction were assessed in accordance with latest recommendations. LAVI was >34 mL/m^2 in 39% of participants, and LAVI was positively associated with VO_{2peak} and percentage of age- and-sex-predicted VO_{2peak} (β (95% CI) 0.18 (0.09-0.28) and 0.10 (0.05-0.15)), respectively) weighted minutes of physical activity per week (β [95% CI], 0.01 [0.003–0.015]). LAVI was not associated with other indexes of left ventricular diastolic dysfunction. There was an effect modification between age and VO_{2peak}/percentage of age- and-sex-predicted VO_{2peak} showing higher LAVI with advanced age and higher VO_{2peak}/percentage of age- and-sex-predicted VO_{2peak} as presented in prediction diagrams.

***Conclusions*—**Interpretation of LAVI as a marker of diastolic dysfunction should be done in relation to age-relative CRF. Studies on the prognostic value of LAVI in fit subpopulations are needed. **(*J Am Heart Assoc*. 2020;9:e014682. DOI: 10.1161/JAHA.119.014682.)**

Key Words: echocardiography • endurance training • exercise • heart • physical activity

Left atrial (LA) volume is established as a strong prognostic marker of future cardiovascular events in high-risk populations.[1–3] The strong association between LA volume and left ventricular (LV) diastolic dysfunction[4,5] has resulted in LA volume becoming one of the diagnostic criteria for LV diastolic dysfunction, with a cutoff of 34 mL/m^2 defining pathological enlargement.[6]

Contrary to this, LA enlargement is often observed among endurance athletes,[7] in whom it is regarded a physiological adaptation.[8] Similarly, LA enlargement has been observed in recreational runners,[9] and higher lifetime training hours in nonelite athletes are linked to LA enlargement.[10] LV diastolic dysfunction, however, is associated with low cardiorespiratory fitness (CRF)[11]; and low CRF has a well-established link to mortality.[12] Thus, enlarged LA in both patients with cardiac disease and endurance athletes represents a paradox as these are populations with different prognoses.

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Accompanying Data S1, Tables S1, S2, and Figures S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014682

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Clinical Perspective

What Is New?

- In previously healthy men and women participating in the HUNT (Nord-Trøndelag Health Study), left atrial size was positively associated with higher cardiorespiratory fitness, but not with indexes of left ventricular diastolic dysfunction.
- The association between cardiorespiratory fitness and left atrial enlargement was stronger with higher age.

What Are the Clinical Implications?

- Cardiorespiratory fitness should be considered when left atrial size is included in clinical decision making.

Although LA size has been studied thoroughly in athletes and patients with cardiac diseases, studies from healthy populations, and particularly studies on the associations between CRF and LA size in general free-living populations, are scarce. Furthermore, as many patients examined by echocardiography do not have a well-defined medical and cardiac history, a universal threshold of 34 mL/m^2 defining LA enlargement may be unfavorable. Still, the current recommendations for assessment of LA volume and diastolic function only include a short precaution about the interpretation of LA volume in athletes.[6,13] However, it is likely that one should be cautious also when assessing LAVI and diastolic function in nonathlete subjects with high CRF and/or a long exercise training history. To avoid misinterpretation of normal LA as pathologically enlarged with following misdiagnosis, overtreatment, and unnecessary worry for patients, knowledge on the association between LA size, CRF, and diastolic function in healthy, free-living, nonathletic subjects is needed.

Therefore, we aimed to investigate the association of LA volume with CRF and LV diastolic function in a healthy, free-living population. Second, to better allow for generalization of the results to populations in whom measurements of CRF are not available, we aimed to assess the association of LA volume with leisure-time physical activity (PA). We hypothesized that in a healthy, free-living population, LA volume is associated with CRF and leisure-time PA, but not with echocardiographic indexes of LV diastolic dysfunction.

Methods

The data from HUNT (Nord-Trøndelag Health Study) used in this study are available on application to the HUNT Data Access Committee in accordance with the policy on data availability (further information and contact information: https://www.ntnu.edu/hunt/data).

Study Population

The subjects of this cross-sectional study participated in the third wave of HUNT (HUNT3). HUNT3 was performed between October 2006 and June 2008, and all inhabitants of the county, aged ≥20 years, were invited, with 50 821 individuals (54%) participating. HUNT has been comprehensively described elsewhere.[14] Subjects free from hypertension or use of hypertensive medications, pulmonary or cardiovascular disease, cancer, and sarcoidosis were invited to cardiopulmonary exercise testing as a part of the HUNT3 Fitness study, a substudy of HUNT3. The HUNT3 Echocardiography study[15] was another substudy with similar inclusion criteria as the HUNT3 Fitness study, and 242 participants contributing in both the HUNT3 Echocardiography study and the HUNT3 Fitness study compose the population in this study. Data collection took place from June 9 to 19, 2008, in the town of Namsos, Norway.

Previously unknown minor pathological features were revealed in 6 study participants, including possible history of angina pectoris (n=2), aortic regurgitation (n=1), LV hypertrophy (n=2), and other (n=1). These individuals were not excluded from the analyses.

The study was approved by the Regional Committee for Medical Research Ethics (2018/929) and conducted according to the second Declaration of Helsinki. Informed consent was obtained from all participants. The study participants could withdraw from the study at any time.

Self-Reported Questionnaires and Clinical Measurements

Questionnaires on medical history, clinical examinations, and blood sampling for biochemical analyses were performed in the HUNT3 baseline examination, and detailed information on the sampling of these data has been reported earlier.[14] Information on PA (frequency, duration, and intensity of leisure-time PA) was gathered from validated questionnaires.[16] PA was then calculated as volume of moderate-to-vigorous PA per week (PA volume), where relative vigorous intensity PA was weighted double in accordance with current recommendations.[17] Information on PA intensity was given on the scale of rated perceived exertion, ranging from 6 (no exertion at all) to 20 (maximal exertion). Rated perceived exertion 12 and 13 were graded as moderate intensity, and rated perceived exertion >13 was graded as vigorous intensity. PA was also classified as adherence or nonadherence to PA guidelines[17] on the basis of these calculations of minutes of PA per week.

Echocardiography

Transthoracic echocardiograms were recorded by one physician (H.D.) highly experienced in echocardiography. Participants were examined in the left-lateral decubitus position

using a Vivid 7 scanner with a phased-array transducer (M3S and M4S) (version BT06; GE Ultrasound, Horten, Norway). All measurements were averaged over 3 cardiac cycles. Echocardiographic data were stored digitally and analyzed subsequently by the same physician echocardiographer.

For quantification of LA volume-specific views, the LA was recorded from apical 4- and 2-chamber views in end systole with tracing of the endocardial border (Figure 1).[13] Mean difference between 4- and 2-chamber length was 0.36 cm (SD, 0.28 cm). LA volume was calculated by the area-length (A-L) method and by the summation of disc method, and subsequently indexed per square meter body surface area LAVI (left atrial volume index). Mitral inflow peak early (E) and late velocity and early filling mitral deceleration time were assessed from apical 4-chamber view by pulsed-wave Doppler. Peak velocity tricuspid regurgitation was measured by continuous Doppler. Mitral annular peak systolic and peak early diastolic (e') longitudinal velocities were assessed by pulsed-wave tissue Doppler. The ratio of the early mitral inflow/the early diastolic mitral annular velocity (E/e') was calculated.

Assessment of diastolic function was based on latest recommendations.[6] The following parameters and corresponding cutoffs were used; mitral annular e' (septal e' <7 cm/s, lateral e' <10 cm/s), E/e' ratio >14, LAVI >34 mL/m^2, and peak tricuspid regurgitation velocity >2.8 m/s. LV diastolic dysfunction was present if more than half of the available parameters met these cutoff criteria.

Data on the reliability of most of the measurements have been comprehensively described previously.[15] Reproducibility for LAVI was calculated in a random subset of 145 study participants by separate analyses performed by 2 physicians experienced in echocardiography (H.D. and H.E.M-H.). Shortly, coefficient of variation for LAVI was 12.1%, and the test-retest coefficients of variation for separate echocardiographic data sets for E, e' (average of septal and lateral), and E/e' were 6.0%, 10.5%, and 7.9%, respectively.[15]

Cardiorespiratory Fitness

Peak oxygen uptake (VO_{2peak}) was measured by ergospirometry during walking or running on a treadmill using an individualized protocol to voluntary exhaustion while wearing a tight face mask (Hans Rudolph, Germany) connected to a portable mixing chamber gas-analysis system (MetaMax II; Cortex, Leipzig, Germany). Reliability of the cardiopulmonary exercise testing measurements has been published

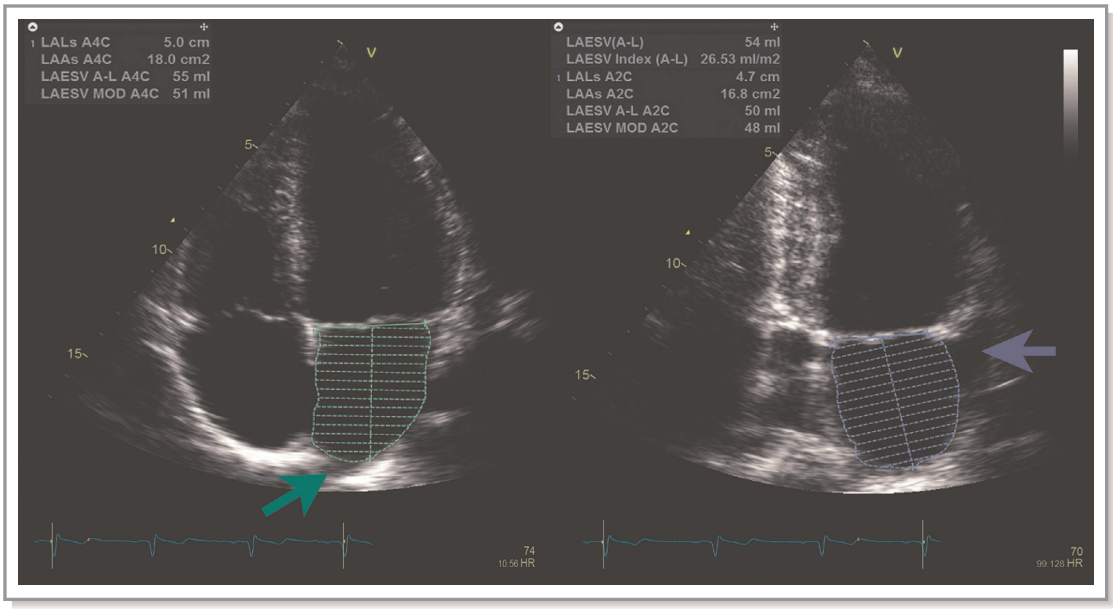

Figure 1. Left atrial (LA) assessment. LA volume was measured from B-mode recordings in apical 4-chamber view (**left**) and 2-chamber view (**right**). Tracing was done from one side at the mitral annular level following the endocardial border around the atrium and to the opposite site at the mitral annular level. The contour was closed at the mitral annulus with a straight line. The area of the atria in the specific view is annotated LAAs in the figure. Pulmonary veins (green arrow) and LA appendage (blue arrow) were excluded from the tracings. LA length was measured in both views, illustrated by the central line in the 2 tracings (annotated as LALs in the figure). LA volume was measured by the area-length method (annotated as LAESV A-L in the figure) and the summation of disks method (annotated as LAESV MOD in the figure).[13]

previously.[18] Test-retest correlation of oxygen uptake was 0.99 ($P<0.001$), and the coefficient of variation was 1.8%.

Statistical Analyses

VO_{2peak} is expressed in milliliters of oxygen consumed per kg body weight per minute and as percentage of predicted from age and sex ($VO_{2\%pred}$). Age- and sex-predicted VO_{2peak} was calculated by previously published sex-specific regression equations of age (men: $VO_{2peak}=63.6–0.393\times age$; women: $VO_{2peak}= 51.6–0.328\times age$)[19] based on a larger sample of the HUNT Fitness study.[18] $VO_{2\%pred}$ was calculated by dividing measured VO_{2peak} by predicted VO_{2peak} and multiplying by 100. We included VO_{2peak} and $VO_{2\%pred}$ in a multiple regression model in addition to other echocardiographic variables shown to be independent predictors of LAVI[10,20] to assess if CRF improved prediction. Furthermore, linear regression was used to assess associations between LAVI and VO_{2peak}, $VO_{2\%pred}$, PA volume, and age. We tested between-group differences of LAVI for sexes and for adherence or nonadherence to PA guidelines using an ANCOVA. We used multiple regression to predict LAVI on the basis of

Table 1. Baseline Characteristics of the 242 Participants by Sex

	Men		Women	
Characteristics	Mean	SD	Mean	SD
Clinical characteristics				
n	107	. . .	135	. . .
Age, y	49	13	48	14
Waist circumference, cm	93	8	87	10
Waist/hip ratio	0.91	0.05	0.86	0.07
Weight, kg	85	11	71	12
BMI, kg/m^2	26.4	3.1	25.6	3.9
Body surface area, m^2	2.05	0.14	1.79	0.14
Systolic BP, mm Hg	129	13	122	15
Diastolic BP, mm Hg	76	9	70	10
Resting HR, bpm	66	10	69	10
Current smoker, %	21.7	. . .	19.2	. . .
Physical activity adherence, %	43.7	. . .	40.3	. . .
Total cholesterol, mmol/L	5.5	0.9	5.4	1.1
HDL cholesterol, mmol/L	1.3	0.3	1.5	0.3
Glucose, mmol/L	5.6	1.1	5.2	0.8
Cardiopulmonary exercise testing variables				
Peak oxygen uptake, mL/kg per min	43.8	7. 7	33.3	7.0
$VO_{2\%pred}$	99	18	92	16
Peak RER	1.14	0.07	1.13	0.07
Peak HR, bpm	181	13	181	14
General echocardiographic indexes				
LV end-diastolic volume, mL	110	22	85	18
LV end-diastolic diameter, mm	52	5	49	5
LV ejection fraction, %	57	6	58	6
Mitral annular S', cm/s	8.6	1.4	8.2	1.4
TAPSE, cm	2.9	0.5	2.8	0.4
Tricuspid annular S', cm/s	13.1	2.2	12.8	2.1
Global longitudinal LV end-systolic strain, %	−17.1	2.1	−18.5	2.1

BMI indicates body mass index; BP, blood pressure; bpm, beats per minute; HDL, high-density lipoprotein; HR, heart rate; LV, left ventricular; RER, respiratory exchange ratio; S', peak mitral annular systolic velocity (by pulsed-wave tissue Doppler); TAPSE, tricuspid annular plane systolic excursion; $VO_{2\%pred}$, percentage of age- and sex-predicted peak oxygen uptake.

VO_{2peak} and $VO_{2\%pred}$, age, and sex, and explored for statistical interaction. The Akaike information criterion was used to assess model performance. The resulting regression models were used to predict LAVI on the basis of the mentioned variables. Linear regression was also performed to assess associations between LAVI and other echocardiographic indexes of diastolic function, as well as between $VO_{2peak}/VO_{2\%pred}$ and diastolic function indexes. Sensitivity analyses were performed with exclusion of measurements from those with 1-plane LAVI measurements only and for those with difference between 4- and 2-chamber atrial length of >0.5 cm. $P<0.05$ was considered statistically significant, and P values and CIs are based on robust SEs. Analyses were conducted using R Studio Version 1.2.1335 (R Foundation for Statistical Computing, Vienna, Austria) and STATA 15.1 (StataCorp, TX).

Results

General characteristics of participants are presented in Table 1 and demonstrate the low cardiovascular risk profile of the population, as well as the normal profile of echocardiographic parameters of LV structure and systolic function. VO_{2peak} was 5% lower than predicted by age and sex, and adherence to PA guidelines was high (40.3%). Echocardiographic LV diastolic function indexes are shown in Table 2. By the A-L method, LAVI was a mean of 32.6 mL/m^2 (SD, 6.7 mL/m^2), and 39% of participants had LAVI above the cutoff of 34 mL/m^2. Three participants (1.2%) met criteria of diastolic dysfunction. Men had significantly higher LAVI compared with women (mean difference, 2.9 [95% CI, 1.1–4.6] mL/m^2; P=0.001), but not after adjusting for VO_{2peak} (mean difference, 1.4 [95% CI, −0.6 to 3.4] mL/m^2; P=0.17). VO_{2peak} independently predicted LAVI in a multiple regression model including LV mass index, LV end-diastolic volume, E/e', sex, and age (model: R^2=0.16, $P<0.0001$; VO_{2peak} β=0.16 [95% CI, 0.04–0.28], P=0.02).

Table 2. Echocardiographic Indexes of LV Diastolic Function by Sex

	Men		Women	
Variables	Mean	SD	Mean	SD
LAESV index (A-L), mL/m^2	34.2	7.1	31.3	6.1
LAESV index (MOD), mL/m^2	31.2	6.5	28.2	5.6
Mitral E-wave, cm/s	67	16	72	16
Mitral A-wave, cm/s	50	16	56	18
Mitral E/A	1.5	0.5	1.4	0.6
Mitral E/e'	6.4	2.2	6.5	2.0
Lateral e', cm/s	12.5	3.5	13.2	3.6
Septal e', cm/s	9.6	2.6	10.1	2.9
Mitral deceleration time, ms	214	69	208	68
Pulmonary vein S, cm/s	55	12	61	12
Pulmonary vein D, cm/s	50	12	49	12
Pulmonary vein S/D ratio	1.2	0.4	1.3	0.3
TRV maximum, m/s	2.3	0.3	2.3	0.4

A indicates late diastolic (atrial) mitral inflow; A-L, area-length; D, peak diastolic velocity; E, early diastolic mitral inflow; e', peak early diastolic mitral annular velocity; LAESV, left atrial end-systolic volume; LV, left ventricular; MOD, summation of disc method; S, peak systolic velocity; TRV, tricuspid regurgitant velocity.

CRF and LA Volume

Figure 2 shows the positive correlation of LAVI with $VO_{2\%pred}$, and Table 3 shows the positive association with VO_{2peak} and PA volume as well. Participants adhering to PA guidelines had borderline significantly higher LAVI (age- and sex-adjusted mean difference, 1.7 [95% CI, −0.1 to 3.5]; P=0.06). In multiple regression analyses, introducing an interaction term between age and $VO_{2peak}/VO_{2\%pred}$ improved the predictions, but other or higher-order interaction terms did not. VO_{2peak}, age, and the interaction of age and VO_{2peak} predicted LAVI (model 1: LAVI~VO_{2peak}+Age+VO_{2peak}×Age, $P<0.0001$, R^2=0.09). The linear regression with $VO_{2\%pred}$, age, sex, and the interaction of age and $VO_{2\%pred}$ (model 2: LAVI~$VO_{2\%pred}$+Age+$VO_{2\%pred}$×Age+Sex, $P<0.0001$, R^2=0.10) performed similarly. Sex did not contribute significantly in the prediction equation with VO_{2peak}. The resulting regression equations were used to predict LAVI on the basis of age and $VO_{2peak}/VO_{2\%pred}$. In sex-specific models, the same model 2 (omitting the sex term) had the lowest Akaike information criterion for men ($P<0.002$; R^2=0.11), whereas none of the tested models for women significantly predicted LAVI (a model with splines for VO_{2peak} at 25, 35, and 45 mL/kg had the lowest Akaike information criterion; R^2=0.06; P=0.06). The higher predicted LAVI with advanced age and higher $VO_{2peak}/VO_{2\%pred}$ is presented in Figure 3A and 3B, as well as the male-only $VO_{2\%pred}$ model in Figure 3C. Predicted LAVI by $VO_{2\%pred}$ with associated 95% CIs and 95% prediction intervals by sex for age 40 and 70 years is shown in Figure S1.

Diastolic Function

There were no statistically significant associations between LAVI and e', E/e', and tricuspid regurgitation (Table S1) as well as other indexes of diastolic dysfunction (pulmonary vein systolic and diastolic velocities and their ratio, transmitral flow early and late velocities and their ratio, and mitral deceleration time; data not shown). Septal e' showed a borderline association to $VO_{2\%pred}$, but no other association was found in age- and sex-adjusted multiple regression

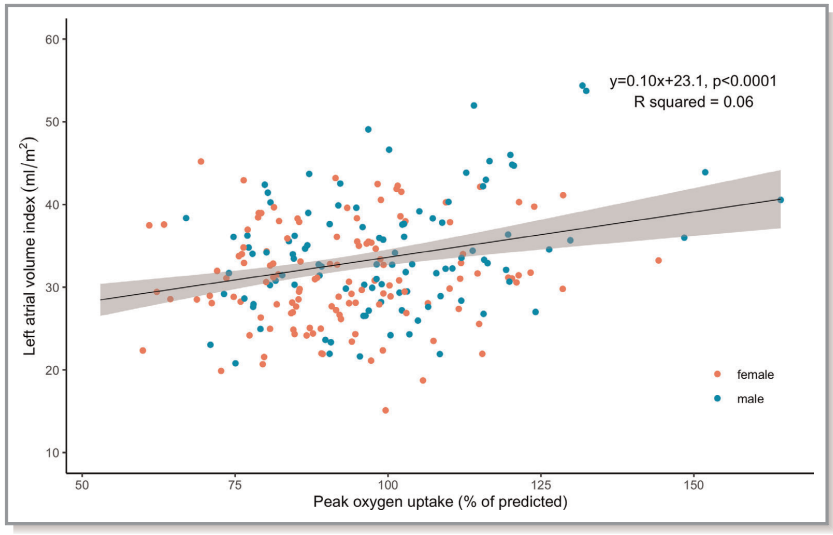

Figure 2. Scatterplot of the relationship between left atrial volume index (mL/m^2) and percentage of age- and sex-predicted peak oxygen uptake (n=229).

analyses with echocardiographic indexes of diastolic function as dependent variable and VO_{2peak} and $VO_{2\%pred}$ as independent variables (Table S2).

Sensitivity Analyses

Excluding those with only single-plane measurement of LAVI, those with >0.5-cm difference between 2- and 4-chamber measurements, and those with diastolic dysfunction (n=3), did not make notable changes to the main results. LAVI was systematically lower when measured by the biplane method of discs compared with the A-L method (LAVI [summation of disc method] mean, 29.5 mL/m^2; mean difference, 3.3 [95% CI, 3.1–3.5] mL/m^2; P<0.0001; n=209), and 22% had LAVI >34 mL/m^2 when measured by the summation of disc method. Predicted LAVI based on model 1 and model 2 was therefore also lower, and higher $VO_{2peak}/VO_{2\%pred}$ was needed to reach thresholds for enlarged LAVI (Figure S2). The same effect modification between $VO_{2peak}/VO_{2\%pred}$ and age was seen when using LAVI measured by the biplane method of discs.

Using a different approach fitting a restricted cubic spline model for the prediction of LAVI by $VO_{2\%pred}$ showed similar results as model 2 (Data S1, Figure S3). Allowing for different β coefficients at different levels of $VO_{2\%pred}$, this model showed an effect on LAVI when $VO_{2\%pred}$ was >100%.

We also fitted spline models allowing different β coefficients at different ages, with similar findings as in the main models (Data S1, Figure S4).

Discussion

Our main finding is that higher CRF is associated with higher LAVI in a healthy, free-living general population sample, and

Table 3. Linear Regression Analyses With LA Volume Index (A-L Method) as Dependent Variable

		Univariate Model			Age- and Sex-Adjusted Model		
Variable	n	β (95% CI)	R^2	P Value	β (95% CI)	R^2	P Value
VO_{2peak}	229	0.18 (0.09–0.28)	0.06	<0.001	0.20 (0.08–0.33)	0.07	0.002
$VO_{2\%pred}$	229	0.10 (0.05–0.15)	0.06	<0.001	0.08 (0.03–0.13)	0.08	0.001
PA volume	225	0.01 (0.003–0.015)	0.04	0.006	0.009 (0.003–0.015)	0.08	0.005
Age	229	0.03 (−0.03 to 0.01)	0.004	0.34	N/A	N/A	N/A

A-L indicates area-length; LA, left atrial; PA volume, minutes of physical activity per week with high-intensity activity weighted 2:1; $VO_{2\%pred}$, percentage of age- and sex-predicted VO_{2peak}; VO_{2peak}, peak oxygen consumption; N/A, not applicable.

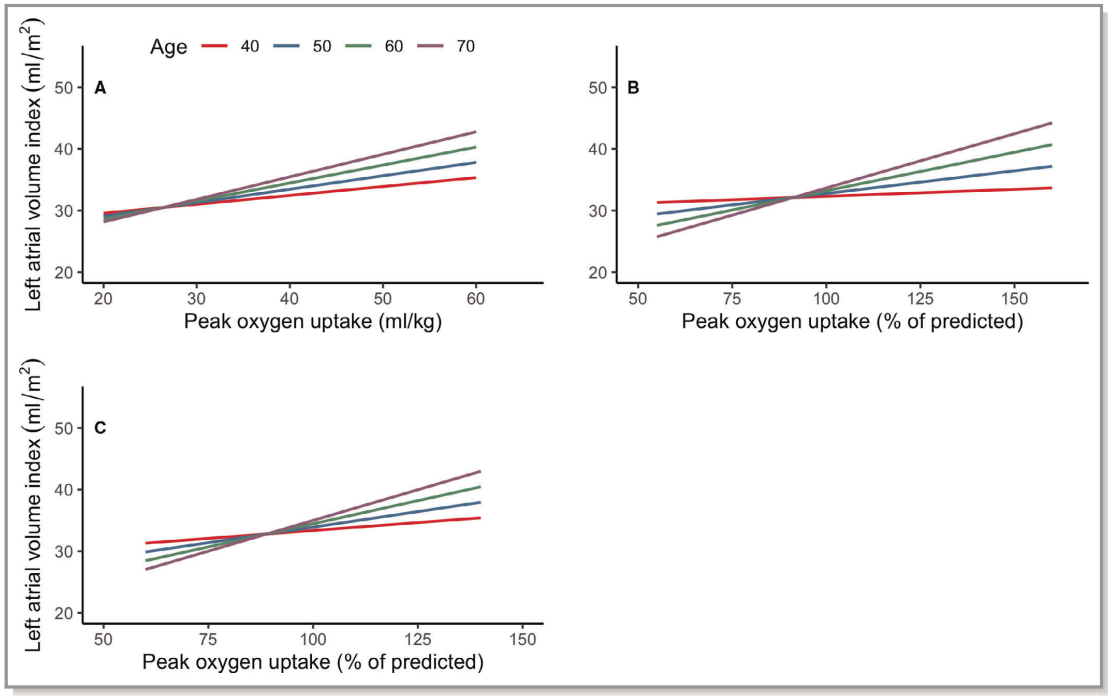

Figure 3. Prediction diagrams of left atrial volume index for ages 40, 50, 60, and 70 years by peak oxygen uptake (VO_{2peak}) (n=229) (**A**), percentage of age- and sex-predicted VO_{2peak} (n=229) (**B**), and percentage of age- and sex-predicted VO_{2peak} in men only (n=103) (**C**).

the effect is modified by age. In fact, participants aged ≥60 years with VO_{2peak} as expected by age and sex were predicted to have LAVI at the threshold for pathological enlargement of 34 mL/m^2 (A-L method). Higher LAVI was not associated with other indexes of diastolic dysfunction. The main findings are summarized in Figure 4.

CRF, Diastolic Function, and LA Size

Although evidence on the associations between LAVI and CRF in healthy populations is lacking, the main finding from this study is supported by studies showing enlarged LAVI in competitive endurance athletes[7,8] and recreational runners.[9] Authors from a study comparing athletes and normal subjects argued that LAVI appears to be determined by a complex interplay between LV end-diastolic volume, PA level, and LV mass.[20] Sex was not an independent predictor in the latter study, which is in line with our results showing that LAVI was not different between sexes after adjusting for VO_{2peak}, a proxy of LV size. An interventional study randomizing adults to high-intensity exercise or yoga control showed that LA volume increased significantly more in the high-intensity exercise group,[21] although another study based on the same exercise intervention showed that LA mechanical remodeling was more prominent than LV mechanical remodeling in the high-intensity exercise group.[22]

A modest proportion of the LAVI variance was explained by the $VO_{2peak}/VO_{2\%pred}$ models. However, the mean $VO_{2\%pred}$ of the study population was 5% lower than expected by age and sex, and few subjects had high $VO_{2\%pred}$ values, especially in women. In fact, only 26% of women had $VO_{2\%pred}$ higher than expected by age and sex, compared with 47% of the men. It is likely that this contributed largely to the lack of significant associations between LAVI and $VO_{2peak}/VO_{2\%pred}$ in women. The differences by sex for the association between CRF and LAVI should be investigated further.

Higher LAVI was not associated with diastolic dysfunction in our study, which is supported by a study from a US preventive health clinic showing that the association between LAVI and LV diastolic filling pressures measured by E/e' differs by CRF level.[11] Another study also showed that E/e' was associated with LAVI among normal subjects, but not among athletes.[20] In patients with LV diastolic dysfunction, low CRF is associated with enlarged LAVI,[23] and therefore it seems to be a U- (or at least J-) shaped relationship between LAVI and CRF, where enlarged LAVI and low CRF are

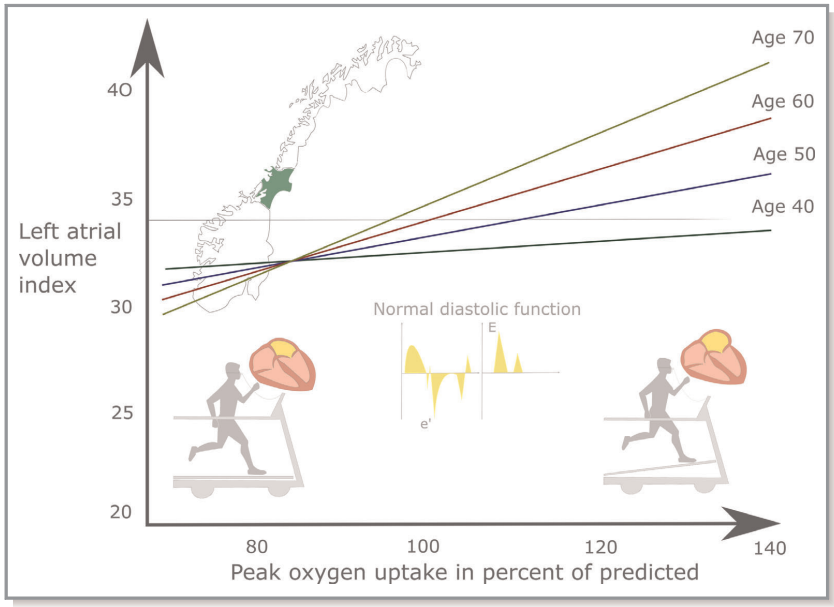

Figure 4. Take-home figure. Indexed left atrial volume was positively associated with cardiorespiratory fitness in healthy adults, and the effect was more pronounced with advanced age. Left atrial volume was not associated with other indexes of left ventricular diastolic dysfunction.

associated with diastolic dysfunction and a poor prognosis, whereas enlarged LAVI and high CRF probably are associated with a favorable prognosis. Higher prognostic value of diastolic dysfunction compared with enlarged LAVI has been shown in a study from the general population,[24] supporting that enlarged LAVI may convey other information than just as a marker of chronically elevated filling pressures.

The negative prognostic influence of LAVI is shown in high-risk populations, such as patients with type 2 diabetes mellitus and myocardial infarction.[1,2] However, longitudinal studies on the prognostic value of enlarged LAVI in fit populations are lacking, which should be evaluated in future studies. An interventional study did show LA mechanical remodeling in response to high-intensity exercise without significant electrical remodeling,[22] further supporting this notion.

A study investigating exercise capacity and LAVI in normal and diseased subjects showed that exercise capacity was negatively correlated to LAVI in systolic and diastolic dysfunction, but positively correlated to LAVI in normal subjects, supporting our findings,[25] as the population in our study was previously healthy.

It is likely that the physiological high blood flow obtained during PA may trigger LA remodeling in fit individuals in a similar way as the high-pressure states found in relation to LV pathological features. The number of years of endurance exercise training has been shown to be an independent predictor of enlarged LAVI in athletes,[10] which is supportive of our finding of an effect modification between age and CRF on LAVI showing higher predicted LAVI by CRF with advanced age. Cumulative PA volume over years may therefore be an important stimulus to enlarged LAVI in healthy subjects from the general population as well. Animal models showing increased LA fibrosis in response to exercise training further support the possibility of cumulative, and possibly irreversible, changes to the LA over time.[26] Although data on cumulative PA over many years were not available in our study, it may be argued that LA enlargement caused by this stimulus may be less harmful as PA behavior generally is associated with lower cardiovascular risk. Thus, it may be hypothesized that LAVI is not a good marker of risk in a healthy and fit population.

Clinical Implications

Our study indicates that CRF should be considered when assessing LAVI, and as previously discussed it may be argued that LAVI is not a marker of risk in healthy and fit subpopulations. As Nistri et al previously stated,[20] a dichotomous classification of normal/pathological LAVI without taking variables such as athletic status into account increases risk of misclassification, further suggesting that specific cutoffs based on sporting activity should be proposed. Our

study supports these findings and further implies that CRF or exercise training status should be considered when assessing LAVI also in nonathletes. Our findings indicate that making such precautions is especially important among fit individuals of advanced age. In fact, one metabolic equivalent task (metabolic equivalent task=3.5 mL/kg per minute) higher VO_{2peak} translated to ≈1.3 mL higher predicted LAVI in 70-year-old patients in our study. Our results also suggest that briefly assessing patients' leisure-time PA could help clinicians in interpreting findings of enlarged LAVI. Another alternative to the somewhat resource-demanding ergospirometry is estimating CRF through nonexercise equations, which could be a feasible alternative in clinical practice.[12,19]

Strengths and Limitations

The study has several methodological strengths. CRF was measured by ergospirometry during incremental symptom-limiting exercise, dedicated echocardiographic recordings were obtained to assess LA volume, and leisure-time PA was measured by a validated questionnaire.

Although mean VO_{2peak} values were lower in our study population than in the reference population, CRF reference values from Norwegian populations in general are somewhat higher than in other populations.[27] Given the relationship between LAVI and CRF found in this study, this may partly explain the high LAVI values found in this study compared with a recent meta-analysis.[28] Another potentially important cause may be the inclusion of LAVI from studies assessing LAVI on the basis of one view only, and the meta-analyses did not take difference in length between 4- and 2-chamber view into account. The discrepancy between the recommendations, the large meta-analyses, and the present results reflects the need for high-quality echocardiographic studies from different populations. The A-L method for LA volume evaluation is known to yield higher values for LAVI compared with the summation of disc method, and the discrepancy in our material was similar to what was expected.[29] However, sensitivity analyses showed the same associations to CRF regardless of method of LAVI quantification.

The cross-sectional design limits conclusions about causation, and the selection of healthy individuals made echocardiographic diastolic dysfunction practically absent. Furthermore, we did not follow specific guidelines for examination of diastolic dysfunction when ejection fraction was reduced,[6] as the number of participants with reduced ejection fraction was negligible. Data on fat-free body mass were not available, and it is reasonable that fit individuals have a lower proportion of body fat that may influence the indexed LA volume. Obesity may also affect LAVI[30]; and although it is strongly related to CRF, it is possible that the presented associations could be different in populations of different body composition. As all participants were in sinus rhythm, the results should not be generalized to individuals with arrhythmias. However, although the participants answered to be free of any cardiovascular disease, it is possible that some participants may have had undiagnosed asymptomatic paroxysmal atrial fibrillation. This is especially important as previous studies have shown increased atrial fibrillation prevalence in populations with a long history of endurance exercise training,[31] and atrial fibrillation is associated with enlarged atria. However, it is less likely that subjects with high CRF are asymptomatic from their atrial fibrillation compared with subjects with low CRF. Furthermore, self-reported diagnoses of atrial fibrillation in HUNT3 have been validated, showing a negative predictive value of 98.4% for the self-reported questionnaire.[32] Thus, it is unlikely that confounding from atrial fibrillation has significantly affected the presented results.

Conclusions

In a healthy population, LA volume was positively associated with CRF, but not with diastolic dysfunction. On the basis of this study from a healthy population and earlier studies from diseased populations, there seems to be a U- or J-shaped relationship between CRF and LA size. Age-relative CRF should be considered when LA volume is included in clinical decision making. Enlarged LA volume may not be a marker of risk in fit subpopulations, which should be further studied.

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Disclosures

None.

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LEFT ATRIAL VOLUME, CARDIORESPIRATORY FITNESS, AND DIASTOLIC FUNCTION IN HEALTHY INDIVIDUALS. THE HUNT STUDY, NORWAY.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS AND RESULTS

Self-reported questionnaires and clinical measurements

Information on smoking status (current, former, occasional, and never) and previous medical history was gathered from self-reported questionnaires. Trained nurses performed anthropometric measurements. Weight were measured with participants wearing light clothes to the nearest half kilogram, height without shoes to the first decimal, and waist and hip circumference to the nearest centimeter while standing with hands in a relaxed position. Blood pressure and resting heart rate were measured three times using an oscillometry-based Dinamap 845XT (Critikon) with the participants resting in sitting position with cuff on arm. Non-fasting blood samples analyzed for total-cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose were drawn.

Supplemental methods to echocardiographic measurements

For 21 participants left atrial volume index (LAVI) was estimated from one plane only. The endocardial border of the left atrium (LA) was traced in end-systole with start and stop at the mitral annulus, so a straight line closed the mitral annulus. The pulmonary veins and the LA appendage were not included in the trace.

Mitral inflow pulsed-wave Doppler indices were assessed from apical 4-chamber with sample volume at the tip of the mitral leaflets. Peak mitral annular longitudinal velocities were assessed from the septal and lateral wall by pulsed-wave tissue Doppler with sample volume localized to the basal part of the myocardium (near the insertion of the mitral leaflets). The peak velocities were measured at the outer contour of the Doppler spectrum at low gain settings. The average of the septal and lateral early diastolic velocity was used for calculation of the ratio of the early mitral inflow to the early diastolic mitral annular velocity (E/e'). It was up to the

discretion of the physician echocardiographer to assess if the image quality was appropriate for analyses of left ventricular diastolic function.

Supplemental methods to cardiorespiratory fitness measurements

Familiarization to treadmill use was given during a ten-minute warm-up while establishing initial treadmill inclination and speed for the test. An individualized protocol was used with increasing inclination (2%), speed (1 km/h), or both (1% and 0.5 km/h) every minute until voluntary exhaustion. Maximal oxygen uptake (VO_{2max}) was achieved when the respiratory exchange ratio was ≥1.05 and oxygen uptake did not increase >2 mL/kg/min despite increasing workload. For simplicity the term VO_{2peak} including those not meeting VO_{2max} criteria is used in the manuscript. Heart rate was measured by a heart rate monitor from Polar (S610i; Polar Electro Oy, Kempele, Finland).

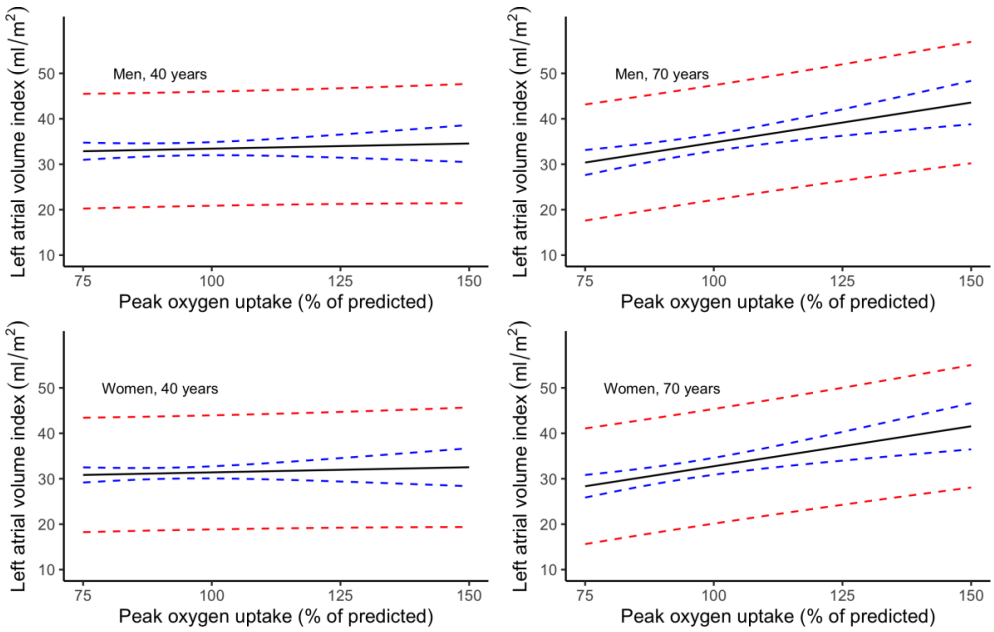
Restricted cubic spline models

A model was fitted using restricted cubic splines for LAVI by percent of predicted peak oxygen consumption from age and sex ($VO_{2\%pred}$) (based on model 2 from main results). Both a linear and a cubic spline model was fitted, but the cubic model had the lowest Akaike information criterion (AIC). Knots were set 80%, 100%, and 120% of $VO_{2\%pred.}$ The AIC for model 2 and the restricted cubic splines model was similar despite the loss of freedom from introducing splines (AIC 1502.2 vs AIC 1504.9, respectively).

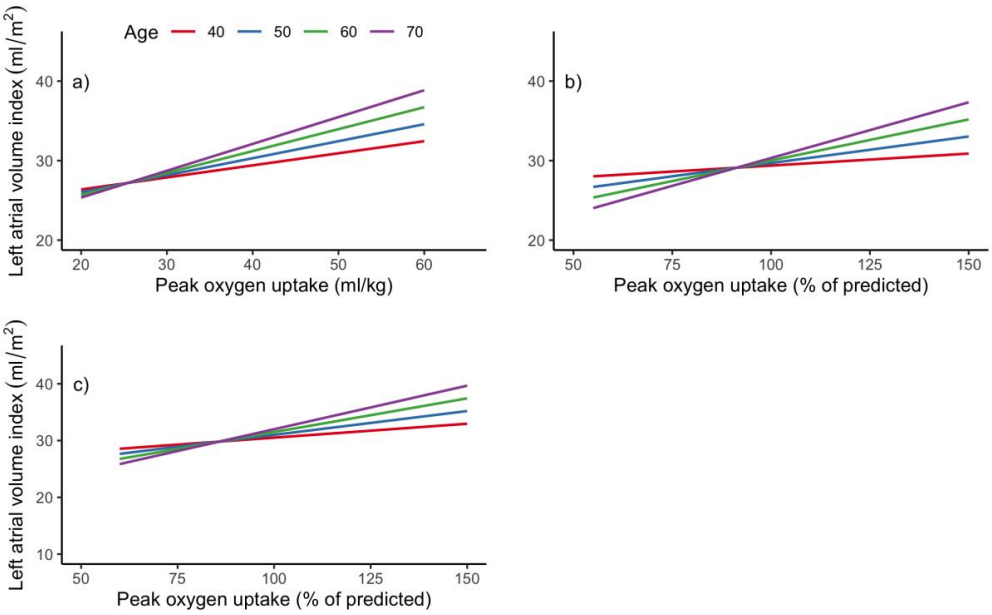
Since age is considered both in $VO_{2\%pred}$ and in age itself the observations could potentially be observed by non-additivity of the age-effect on LAVI. In addition to the previously tested models including higher-order interactions for age we therefore fitted models by a restricted cubic splines approach for the age covariate. Different number and cut-offs for knots were explored with knots at 45 and 65 years showing the lowest AIC. These models gave

similar results as the chosen models from main results, as shown in Supplemental Figure 4. Neither the overall or sex-specific models had AIC lower than the models presented in the main results. None of the models for women significantly predicted LAVI, as for the reported main models.

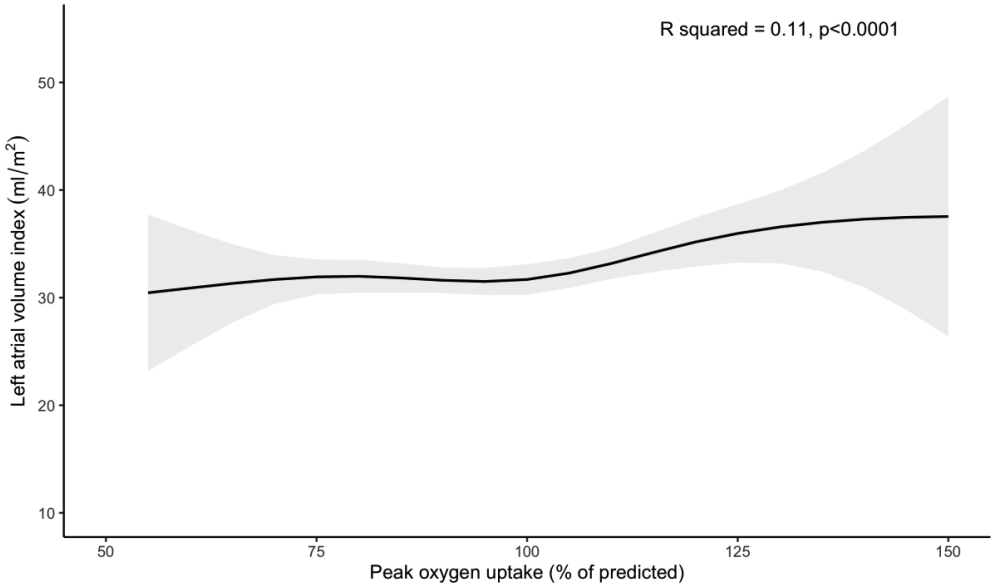
SUPPLEMENTAL FIGURES



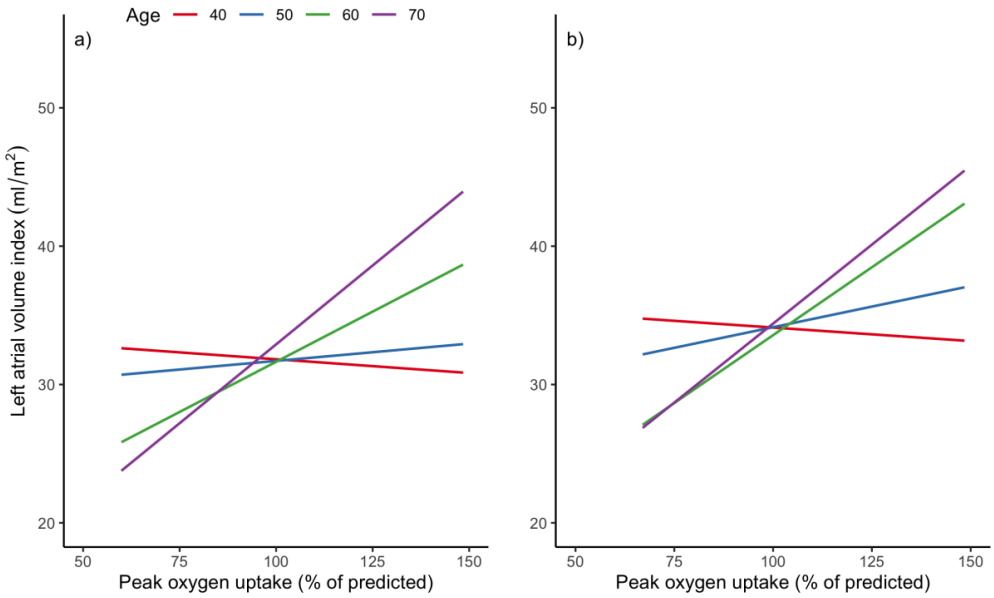
Supplemental Figure 1. Prediction diagrams of left atrial volume index (A-L method) for ages 40 and 70 years for men and women by percent of age and sex predicted VO_{2peak} with confidence (blue dotted line) and prediction (red dotted line) intervals based on main results model 2 (n=229).



Supplemental Figure 2. Prediction diagrams of left atrial volume index measured by biplane method of discs for different ages by a) VO_{2peak} (n=209), b) percent of age and sex predicted VO_{2peak} (n=209), and c) percent of age and sex predicted VO_{2peak} in men only (n=96).



Supplemental Figure 3. Prediction of left atrial volume index (A-L method) based on a restricted cubic spline model for percent of age and sex predicted VO_{2peak} (n=229).



Supplemental Figure 4: Prediction of left atrial volume index (A-L method) by percent of age and sex-predicted VO_{2peak} based on a restricted cubic spline model for age. a) Sexes combined (n=229), b) men only (n=103).

SUPPLEMENTAL TABLES

Supplemental Table 1. Linear regression analyses with left atrial volume index as the dependent variable and indices of diastolic dysfunction as independent variables

		Univariate model			Age and sex-adjusted model		
	n	***β (95% CI)***	**R_2**	**p**	***β (95% CI)***	**R_2**	**p**
E/e' ratio	228	0.17 (-0.28-0.61)	-0.002	0.46	0.14 (-0.36-0.63)	0.04	0.59
Septal e'	228	0.03 (-0.28-0.34)	-0.004	0.84	0.24 (-0.11-0.59)	0.04	0.19
Lateral e'	228	-0.04 (-0.29-0.20)	-0.004	0.72	0.12 (-0.20-0-43)	0.04	0.48
TRV max	35	6.03 (-1.1-13.1)	0.07	0.09	5.48 (-1.57-12.5)	0.06	0.12

95% CI = 95 % confidence interval, E = peak early diastolic mitral inflow velocity, e' = peak early diastolic mitral annular velocity, TRV = tricuspid regurgitant velocity.

Supplemental Table 2: Linear regression analyses with indices of diastolic dysfunction as dependent variable and VO_{2peak}/$VO_{2\%pred}$ as independent variable

		Univariate model			Age and sex-adjusted model		
	n	***β (95% CI)***	**R_2**	**p**	***β (95% CI)***	**R_2**	**p**
VO_{2peak} as independent variable							
E/e' ratio	240	-0.04 (-0.07- -0.007)	0.02	0.017	-0.01 (-0.05-0.04)	0.12	0.73
Septal e'	240	0.08 (0.04-0.12)	0.06	<0.0001	0.045 (-0.002-0.092)	0.36	0.06
Lateral e'	240	0.10 (0.04-0.15)	0.05	0.001	0.035 (-0.03-0.10)	0.43	0.27
TRV max	39	0.002 (-0.01-0.02)	-0.023	0.76	0.01 (-0.01-0.03)	-0.01	0.50
$VO_{2\%pred}$ as independent variable							
E/e' ratio	240	0.002 (-0.02-0.02)	-0.004	0.77	-0.003 (-0.02-0.014)	0.13	0.75
Septal e'	240	0.002 (-0.02-0.02)	0.0001	0.84	0.018 (-0.0001-0.04)	0.36	0.051
Lateral e'	240	-0.01 (-0.04-0.02)	-0.002	0.44	0.011 (-0.01-0.03)	0.44	0.33
TRV max	39	0.003 (-0.003-0.01)	0.04	0.33	0.003 (-0.004-0.009)	0.07	0.45

95% CI = 95% confidence interval, VO_{2peak} = peak oxygen consumption, $VO_{2\%pred}$ = percent of age and sex predicted VO_{2peak}, E = peak early diastolic mitral inflow velocity, e' = peak early diastolic mitral annular velocity, TRV = tricuspid regurgitant velocity.

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