Peak oxygen uptake and incident coronary heart disease in a healthy population – The HUNT Fitness Study.

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ABSTRACT

Aims: The majority of previous research on the association between cardiorespiratory fitness (CRF) and cardiovascular disease is based on indirect assessment of CRF in clinically referred predominantly male populations. Therefore, our aim was to examine the associations between VO_{2peak} measured by the gold-standard method of cardiopulmonary exercise testing and fatal and non-fatal coronary heart disease in a healthy and fit population.

Methods and results: Data on VO_{2peak} from 4,527 adults (51% women) with no previous history of cardiovascular or lung disease, cancer, and hypertension or use of antihypertensive medications participating in a large population-based health-study (The HUNT3 Study), were linked to hospital registries and the cause of death registry. Average VO_{2peak} was 36.0 ml/kg/min and 44.4 ml/kg/min among women and men, and 83.5% had low ten-year risk of cardiovascular disease at baseline. Average follow-up was 8.8 years, and 147 participants reached the primary end-point. Multi-adjusted Cox-regression showed 15% lower risk for the primary end-point per one-MET (metabolic equivalent task) higher VO_{2peak} (hazard ratio (HR) 0.85, 95% confidence interval (CI) 0.77-0.93), with similar results across sex. The highest quartile of VO_{2peak} had 48% lower risk of event compared to the lowest quartile (HR 0.52, 95% CI 0.33-0.82). Oxygen pulse and ventilatory equivalents of oxygen and carbon dioxide also showed significant predictive value for the primary end-point.

Conclusions: VO_{2peak} was strongly and inversely associated with coronary heart disease across the whole fitness continuum in a low-risk population sample. Implementation of CRF measurements in clinical practice may improve risk classification and optimize prevention.

Keywords. Cardiorespiratory fitness. Oxygen uptake. Coronary heart disease. Cardiopulmonary exercise testing. Primary prevention.

INTRODUCTION

Despite the decline in mortality from coronary heart disease (CHD),[1] CHD is still responsible for one third of all deaths in the adult population.[2] As much as fifty percent of the decline in mortality from myocardial infarction has been attributed to lower case-fatality, probably due to enhanced treatment options such as percutaneous coronary interventions (PCI) and optimized medical treatment.[3] The prevalence of CHD in the population therefore remains relatively unchanged.[1] Hence, there is great potential for further lowering the burden of CHD by early risk detection and preventive strategies.[4]

Cardiorespiratory fitness (CRF) is strongly associated with all-cause and cardiovascular mortality,[5-9] and might even be an important predictor of mortality beyond traditional risk factors such as hypertension, diabetes, cholesterol levels, and smoking.[10] A more limited number of studies also suggest that moderate to high CRF in apparently healthy people is associated with delayed CHD progression and reduced non-fatal events.[6, 8] Hence, a recent statement from the American Heart Association argued for routine implementation of CRF measurements in clinical practice in order to improve risk classification and optimize prevention.[10]

Most of the evidence, however, is based on studies from a limited number of cohorts including individuals referred to exercise testing for clinical reasons[11, 12], and the majority of the mortality risk burden has been associated with CRF levels below a threshold of 5-6 metabolic equivalents (METs).[13] Furthermore, women are lacking or underrepresented in most studies,[6, 7, 12, 14] and the generalizability of findings to apparently healthy, free-living populations is uncertain. Moreover, CRF levels in population studies are commonly predicted from submaximal or peak workload on a treadmill or cycle ergometer as opposed to the gold-standard method of cardiopulmonary exercise testing (CPET) by direct gas-analysis of peak oxygen uptake (VO_{2peak}).[5] To our knowledge only two relatively small cohorts,[14, 15] including middle-aged men, has examined the association between direct measurements of VO_{2peak} and risk of cardiovascular events, showing an inverse relationship. Studies investigating the association of VO_{2peak} and cardiovascular events in healthy population samples are therefore needed.

Thus, the aim of this study was to examine the prospective associations between $VO_{2peak,}$ measured by CPET, and fatal or non-fatal coronary heart disease events or revascularization, in a healthy

low-risk cohort of both men and women. Secondary, we aimed to assess the associations separately for myocardial infarction, chronic ischemic heart disease, coronary revascularization, and mortality, as well as the prognostic value of other CPET measures such as ventilatory equivalents and oxygen pulse.

MATERIAL AND METHODS

Study design and participants

The prospective cohort study involved participants from the HUNT3 Fitness Study, a sub-study of the third wave of the Nord-Trøndelag Health Study (HUNT3). In 2006-2008, all inhabitants in Nord-Trøndelag county in mid-Norway were invited to participate in HUNT3. Of 93,860 eligible adults, 50,807 inhabitants participated (54.1 %)[16] by answering self-reported questionnaires and undergoing clinical and biochemical measurements. Participants from four pre-selected municipalities with no previous history of cardiovascular and lung disease, cancer, sarcoidosis, and hypertension or use of antihypertensive medications were invited to CPET (n = 12,609). Of these, 5,633 showed up for exercise testing, and a total of 4,527 completed the exercise test as well as having no other missing variables for the main analyses. The study was approved by the Regional Committee for Medical Research Ethics, and the Norwegian Data Inspectorate approved the HUNT Study. All subjects gave informed consent to participation.

Self-reported and clinical measurements

Smoking status (current, former, occasional, and never smoker) and pack-years of cigarettes, alcohol consumption (frequency of alcohol intake per week over the last 12 months), family history of cardiovascular disease (CVD) (myocardial infarction or stroke in first degree relative), and leisure-time physical activity was gathered from self-reported questionnaires. Physical activity was dichotomized to adherence or non-adherence to physical activity guidelines (detailed information available in Supplementary data). Clinical examinations were performed by trained personnel measuring weight to nearest half kilogram, as well as height and waist circumference to nearest centimetre in standing position. An oscillometry-based Dinamap 845XT (Critikon) was used for measuring resting heart rate and blood pressure. Blood samples were analysed for non-fasting serum-levels of total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides, glucose, creatinine, and C-reactive protein (CRP). Dyslipidemia was defined as total-cholesterol over 7.0, HDL

under 1.3 and 1.0 for men and women respectively, or triglycerides over 1.7. Further information on measurement of clinical variables in HUNT3 has been described elsewhere.[16]

Cardiopulmonary exercise testing

Participants were given a ten-minute warm-up and acclimatization to treadmill walking and running while establishing initial treadmill inclination and speed at the same time. The participants were equipped with a heart rate monitor and a face-covering mask before initiating the test on the treadmill. An individualized ramp-protocol was used where speed (0.5-1 $km \cdot h^{-1}$), inclination (1-2 %), or a combination of both, was increased roughly every minute or when participants had stable oxygen uptake values over 30 seconds. Continuous measurement of exhaled gases was done by using a mixing chamber gas analyser (MetaMax II; Cortex Biophysik Gmbh, Leipzig, Germany). Routine gas and volume calibration was performed at standardized intervals several times per day. Further information regarding equipment and calibration is available in Supplementary data. Comprehensive data of the reliability of the measurements has been previously published.[17] Test-retest correlation of oxygen uptake was 0.99 ($p<0.001$) and the coefficient of variation was 1.8%. The test was defined as reaching true VO_{2max} if measurements showed a plateau in oxygen consumption as well as a respiratory exchange ratio (RER) above 1.05. In the present study both tests meeting criteria for VO_{2max} as well as tests classified as VO_{2peak} are labelled VO_{2peak}. Oxygen pulse was calculated as maximal oxygen consumption in millilitres divided by heart-rate at peak exercise. Ventilatory efficiency equivalents was calculated as minute ventilation divided by CO_2 ventilation or O_2 consumption in litres per minute at peak and steady state submaximal exercise.

Follow-up and information on end-points

Follow-up was ensured by linking baseline data from The HUNT3 Study to a local, validated hospital database (Nord-Trøndelag Hospital Trust myocardial infarction registry), the regional health trust database on diagnoses and procedures, and the Norwegian Cause of Death Registry (NCDR). We used ICD-10 (International Classification of Disease-10) codes I20, I21, I24, I25 to define end-points of CHD. Information on coronary revascularization including percutaneous coronary intervention (PCI)

and coronary artery bypass graft surgery (CABG) was based on relevant codes from Nomesco classification of surgical and radiological procedures (NCSP and NCRP). Information on cause and date of death was gathered from the NCDR. Death from CHD was based ICD-10 codes I20-I25. Primary end-point was defined as diagnosis of, or death from, CHD, or coronary revascularization (PCI or CABG), whichever came first.

Statistical analyses

Ten-year risk of CVD was calculated and classified as low, medium or high based on the recently published NORRISK2 risk prediction algorithm (detailed information available in Supplementary data).[18] The Cox proportional hazards model was employed with attained age as the time scale. Time under risk was calculated as time since participation in the HUNT3 Fitness Study (ranging from June 14th 2007 until June 19th 2008) until censoring (death of non-coronary cause), event, or end of follow-up (December 31st 2016). The proportional hazards assumption was investigated by testing Schoenfeld residuals. Analyses were performed with VO_{2peak} expressed as a continuous variable by METs (one MET equals ~3.5 ml/kg/min) and by comparing quartiles of VO_{2peak}. Quartiles of VO_{2peak} was age and sex-specific by generating percentiles of VO_{2peak} in categories of sex and age (in deciles) before merging these in quartiles of VO_{2peak} (Q1 to Q4). This ensured equal age and gender distribution across quartiles. Main analyses were adjusted for age and sex in model 1, and in addition, smoking status, alcohol intake, and family history of CVD in model 2. Adjustment for BMI was included in sub-analyses performed on exercise variables not being weight scaled. We examined evidence of interaction with VO_{2peak} across covariates, and test for linear trend across categories Q1 to Q4 was performed by tests for log-linearity of the hazard ratios. Results are presented as hazard ratios for effect estimates and 95% confidence intervals (95% CIs) for evaluation of precision. Analyses were performed using STATA15.1 (StataCorp, Texas, US).

RESULTS

Characteristics of the population

Baseline characteristics of the 4,527 participants are shown in Table 1. Briefly, mean age was 48.2 (standard deviation (SD) 13.5, range 19 to 89) years, and gender distribution was balanced (51% women). Average VO_{2peak} in the population was 36.0 ml/kg/min and 44.4 ml/kg/min among women and men, respectively. The pre-defined VO_{2max} criteria were reached in 80% of participants. Cut-offs of VO_{2peak} for Q1 to Q4 across sex and ten-year age groups is found in Supplementary table 1. Levels of cardiovascular risk-factors were consistently decreasing from Q1 through Q4 (except alcohol use being higher in higher quartiles of VO_{2peak}). Clustering of cardiovascular risk at baseline was generally low, as 83.5% had low ten-year risk of CVD and mortality as measured by the NORRISK2 risk prediction model.

Peak oxygen uptake and primary end-point

During a total follow-up time of 40,060 person-years (mean 8.8, SD 1.0) 147 participants (3.3%) reached the composite primary end-point of diagnosis of or death from CHD, or intervention by PCI or CABG. Incidence rate of primary end-point was 3.7 events per 1000 person-years. In the combined analyses of men and women, the risk for primary end-point was 16% (95% CI 8-23) lower per one MET higher VO_{2peak} after adjustment for age and sex (model 1), and 15% (95% CI 7-23) when additionally adjusted for smoking status, alcohol use and family history of CVD in the multi-adjusted model 2 (Table 2). The same direction and comparable magnitude of effects were seen in analyses stratified by sex. The Kaplan-Meier graph (Figure 1) illustrates the higher event-free survival from the primary composite end-point for the higher quartiles of VO_{2peak}. Analyses by quartiles of VO_{2peak} showed approximately 50% (95% CI 18-67) lower risk in Q4 compared to Q1 and testing for trend across quartiles of VO_{2peak} showed a significant linear trend ($p<0.005$ for model 1 and 2 in combined analyses). Further, when subdivided by baseline VO_{2peak} level, participants in the highest quartiles had

similar protective effect per one MET higher VO_{2peak}. There was no evidence of statistical interaction between covariates in model 1 and 2.

Sub-analyses

Analyses with myocardial infarction, chronic ischemic heart disease, coronary revascularization by PCI or CABG, and mortality as respective end-points is presented in Table 3. In general, the effect estimates were in line with findings in the main analyses. However, noticeably the association with all-cause mortality was weak, with a 6% (95% CI -5-16) lower mortality risk per one MET higher VO_{2peak} in the multi-adjusted model. Mortality incidence rate was 2.2 per 1000 person-years (91 deaths of any cause). Malignant disease was the leading cause of death (48%), while cardiovascular and respiratory disease accounted for only 20% and 3% of deaths during follow-up, respectively.

Hazard ratios for the primary end-point in the analyses stratified by presence of cardiovascular risk factors (blood pressure > 140/90, diabetes, obesity, smoking status, dyslipidemia, and physical activity adherence) showed results consistent to findings in the main analyses (data not shown), suggesting no effect modification by baseline levels of cardiovascular risk factors. Stratified analyses for primary end-point by groups of ten-year risk of CVD (n=4,149) gave similar results; low-medium risk group: 14% (95% CI 2-24) lower risk per one MET higher VO_{2peak}, and high-risk group: 16% (95% CI 0-29) lower risk per one MET higher VO_{2peak} (multi-adjusted model). One percent higher ten-year cardiovascular risk were associated with 12% (95% CI 8-16) higher risk of the primary end-point.

Cox regression analyses on oxygen pulse showed 8% (95% CI 2-13) lower risk for primary end-point per unit higher value adjusted as for in the multi-adjusted model plus adjustment for BMI. We also tested whether the oxygen equivalent ($EqVO_2$) at peak exercise was associated with primary end-point (adjusted as for the multi-adjusted model plus BMI). These analyses gave a 3% (95% CI 0-6) and 5% (95% CI -1-11) higher risk per unit higher ratio for peak (n=4,497) and submaximal (n=2,506) values, respectively. Only sixteen participants had $EqVO_2$ values above the upper normal limit of 50. Analyses per unit higher $EqVCO_2$ gave 4% increased risk for primary end-point for peak (95% CI 0-8, n=4,497) and submaximal values (95% CI -3-13, n=2,503), respectively. Having values of $EqVCO_2$ above the upper normal limit of 30 was associated with 39% (95% CI -6-106) higher risk

of the primary end-point. Additional results from these analyses are available in supplementary material (Supplementary table 2).

Sensitivity analyses

In analysis for the primary end-point after adjusting for BMI, systolic blood pressure, dyslipidemia, C-reactive protein, and diabetes in addition to the variables in model 1 and 2 the HR was slightly attenuated to 0.90 (95% CI 0.81-1.00, n=4,421). Separate analyses for the primary end-point excluding first two years of follow-up showed similar results as the main analyses, HR 0.89 (95% CI 0.81-0.98) in model 2. We also performed analyses for primary end-point on those subjects reaching the pre-defined VO_{2max} criteria, with similar results as in the main analyses (model 2: HR 0.83, 95% CI 0.74-0.93). Time on study instead of attained age was tested as time scale for the main analyses without notable effect on estimates.

DISCUSSION

Our results show that peak oxygen uptake (VO_{2peak}) is inversely related to the risk of coronary heart disease in healthy men and women. The influence of VO_{2peak} was similar for both acute diagnoses like myocardial infarction and chronic diagnoses such as angina pectoris, and the reduced risk with higher VO_{2peak} was similar both in fit and unfit sub-populations. Further, ventilatory equivalents and oxygen pulse were also associated with incident coronary heart disease.

VO_{2peak} and coronary heart disease

Our main finding of 17% (men) and 12% (women) lower adjusted risk of CHD per one MET higher VO_{2peak} is in line with the large meta-analysis from 2009 by Kodama et al.[9] estimating a 15% lower risk per one MET higher exercise capacity. In general, our findings support several earlier studies describing an inverse relationship between cardiorespiratory fitness and fatal[12, 19, 20] and non-fatal[6, 8, 21] CHD. Even though precision was lower in women, the consistent size and direction of estimates makes erroneous conclusions less likely.

The association between fitness and CHD was strong despite the fit population sample in this study. In fact, the average VO_{2peak} among women in this study was higher than the average among men in other studies.[6, 8, 14, 22] The average sixty-year-old male in our study had roughly the same fitness level as the group with highest estimated fitness in the meta-analysis by Kodama et al.[9] A 28% higher VO_{2peak} compared to the normal material from the US FRIEND cohort[23] further confirms the fit sample of our participants. Test for trend across the quartiles of VO_{2peak} showed a significant linear relationship, and when analysing within the highest-fit quartile of VO_{2peak} we found the same association per one MET higher VO_{2peak}, indicating that higher cardiorespiratory fitness is protective with no apparent upper threshold. Cut-offs of CRF for risk reduction have, however, been recommended earlier. Exercise capacity <5 METs has been proposed as a single threshold of increased risk,[13] as well as cut-offs of 8 and 6 METs for fifty year old men and women, respectively, in another study.[9] Our results suggest that there is still a large potential for risk-reduction even when having CRF beyond such thresholds, and implicitly, thresholds may vary between populations.

The risk reduction of CHD per one MET higher VO_{2peak} was consistent across stratified analyses by presence of the cardiovascular risk factors, which is in line with findings from several other studies.[19, 24]

The associations of VO_{2peak} to all-cause mortality was not statistically significant, and the effect-estimates of 6% lower relative risk of all-cause mortality per one MET is somewhat lower than findings from a large meta-analysis.[9] The fact that mortality from CVD and respiratory diseases contributed to only 23% of deaths in total may explain these weak associations.

Our results showed a clear predictive value of oxygen pulse, a less studied CPET variable, for future CHD. Similar findings was recently shown in a publication from the Kuopio Ischemic Heart Disease cohort.[25] The oxygen pulse trajectory is included for judgment of myocardial ischemia as a non-invasive measure of stroke volume,[26] and our findings support the prognostic value of this parameter, even without assessing the trajectory during test. Ventilatory equivalents for oxygen ($EqVO_2$) and carbon dioxide ($EqVCO_2$) at peak exercise also showed predictive value of CHD, while missing data of submaximal measures gave low precision of estimates especially for $EqVCO_2$.

Strengths and limitations

Our study has several methodological strengths. Firstly, cardiorespiratory fitness was measured by direct assessment of peak oxygen consumption during maximal exercise, and this study is the first to use this gold-standard method in a healthy sample of the general population to evaluate associations with cardiovascular events and mortality. The precise measurements of exposure may have compensated somewhat for the relatively few end-points accumulated over the follow-up period. A study from the FRIEND database showed significant differences between cardiorespiratory fitness reference data based on direct measurement of VO_{2peak}, and VO_{2peak} estimated from exercise test data.[23] The same study found that there were notable differences especially in the extremes of estimated fitness. This reflects the need for follow-up studies based on directly measured cardiorespiratory fitness. Definition of end-points by linkage to hospital databases and national registries ensured high-quality data with negligible loss to follow-up. We did not have specific information on emigration during follow-up, however emigration from the county is known to be very

low.[16] The healthy population also reduced the risk of reverse confounding from clinical or subclinical disease as an explanation for the association. Sensitivity analyses with exclusion of participants with events during the first two years of follow-up supported this assumption.

Participating in voluntary exercise testing introduces the possibility of self-selection towards more active participants, which may reduce external validity. However, an earlier study compared the HUNT3 Fitness population with the healthy part of the total HUNT3 population and found a similar cardiovascular risk profile in men and women, but the Fitness study participants were slightly more active, weighed less, and had lower waist circumference.[27] The same study showed that the prevalence of cardiovascular risk factors was slightly lower in the Fitness population compared to the general HUNT3 population (5.6% vs 6.4%).

Clinical implications

The update on clinical recommendations for CPET from 2016 highlighted the potential for risk-stratification and prevention of non-communicable disease prevention among apparently healthy individuals.[28] Our results confirm that the level of CRF predicts CHD, with a preventive influence of higher values, in a healthy free-living population. Higher VO_{2peak} was protective against both chronic and acute ischemic heart disease, respectively. Further, motivation for primary disease prevention by medical therapy in the population is known to be low,[29] partly due to low adherence, high costs and potential side-effects. Exercise on the other hand is associated with healthy living and reduced morbidity, and the potential for primary prevention is great, and not fully exploited.[4] Taking full advantage of exercise as (preventive) medicine is an important challenge to the society as the scientific evidence show great health-impact of as little as one MET higher CRF. Such a modest increase in CRF is easily achieved over a few months of regular exercise,[30] and thus, exercise may be an efficient way of reducing the cardiovascular risk.

Conclusion

In conclusion, peak oxygen uptake was strongly and inversely related to coronary heart disease in a large cohort of apparently healthy and low-risk men and women. These findings support the notion

that VO_{2peak} has predictive value along the whole fitness continuum and implementation of CRF measurements in clinical practice may improve risk classification and optimize prevention. Interventions aiming to increase VO_{2peak} may have substantial benefits in reducing the burden of coronary heart disease.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Bhatnagar P, Wickramasinghe K, Wilkins E and Townsend N. Trends in the epidemiology of cardiovascular disease in the UK. *Heart*. 2016;102:1945-1952.
2. Sanchis-Gomar F, Perez-Quilis C, Leischik R and Lucia A. Epidemiology of coronary heart disease and acute coronary syndrome. *Ann Transl Med*. 2016;4:256.
3. Smolina K, Wright FL, Rayner M and Goldacre MJ. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: linked national database study. *BMJ*. 2012;344:d8059.
4. Ford ES and Capewell S. Proportion of the decline in cardiovascular mortality disease due to prevention versus treatment: public health versus clinical care. *Annu Rev Public Health*. 2011;32:5-22.
5. Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J and Ross R. Impact of Cardiorespiratory Fitness on All-Cause and Disease-Specific Mortality: Advances Since 2009. *Prog Cardiovasc Dis*. 2017;60:11-20.
6. Khan H, Jaffar N, Rauramaa R, Kurl S, Savonen K and Laukkanen JA. Cardiorespiratory fitness and nonfatalcardiovascular events: A population-based follow-up study. *Am Heart J*. 2017;184:55-61.
7. Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH and Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA*. 1989;262:2395-401.
8. Tikkanen E, Gustafsson S and Ingelsson E. Associations of Fitness, Physical Activity, Strength, and Genetic Risk With Cardiovascular Disease: Longitudinal Analyses in the UK Biobank Study. *Circulation*. 2018.
9. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N and Sone H. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA*. 2009;301:2024-35.
10. Ross R, Blair SN, Arena R, Church TS, Despres JP, Franklin BA, Haskell WL, Kaminsky LA, Levine BD, Lavie CJ, Myers J, Niebauer J, Sallis R, Sawada SS, Sui X, Wisloff U, American Heart Association Physical Activity Committee of the Council on L, Cardiometabolic H, Council on Clinical C, Council on E, Prevention, Council on C, Stroke N, Council on Functional G, Translational B and Stroke C. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation*. 2016;134:e653-e699.
11. Myers J, Prakash M, Froelicher V, Do D, Partington S and Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med*. 2002;346:793-801.
12. Kokkinos PF, Faselis C, Myers J, Narayan P, Sui X, Zhang J, Lavie CJ, Moore H, Karasik P and Fletcher R. Cardiorespiratory Fitness and Incidence of Major Adverse Cardiovascular Events in US Veterans: A Cohort Study. *Mayo Clin Proc*. 2017;92:39-48.
13. Kokkinos P, Myers J, Faselis C, Panagiotakos DB, Doumas M, Pittaras A, Manolis A, Kokkinos JP, Karasik P, Greenberg M, Papademetriou V and Fletcher R. Exercise capacity and mortality in older men: a 20-year follow-up study. *Circulation*. 2010;122:790-7.
14. Laukkanen JA, Lakka TA, Rauramaa R, Kuhanen R, Venalainen JM, Salonen R and Salonen JT. Cardiovascular fitness as a predictor of mortality in men. *Arch Intern Med*. 2001;161:825-31.

15. Talbot LA, Morrell CH, Metter EJ and Fleg JL. Comparison of cardiorespiratory fitness versus leisure time physical activity as predictors of coronary events in men aged < or = 65 years and > 65 years. *Am J Cardiol*. 2002;89:1187-92.
16. Krokstad S, Langhammer A, Hveem K, Holmen TL, Midthjell K, Stene TR, Bratberg G, Heggland J and Holmen J. Cohort Profile: the HUNT Study, Norway. *Int J Epidemiol*. 2013;42:968-77.
17. Loe H, Rognmo O, Saltin B and Wisloff U. Aerobic capacity reference data in 3816 healthy men and women 20-90 years. *PLoS One*. 2013;8:e64319.
18. Selmer R, Igland J, Ariansen I, Tverdal A, Njolstad I, Furu K, Tell GS and Klemsdal TO. NORRISK 2: A Norwegian risk model for acute cerebral stroke and myocardial infarction. *Eur J Prev Cardiol*. 2017;24:773-782.
19. Farrell SW, Finley CE, Barlow CE, Willis BL, DeFina LF, Haskell WL and Vega GL. Moderate to High Levels of Cardiorespiratory Fitness Attenuate the Effects of Triglyceride to High-Density Lipoprotein Cholesterol Ratio on Coronary Heart Disease Mortality in Men. *Mayo Clin Proc*. 2017.
20. Gander JC, Sui X, Hebert JR, Hazlett LJ, Cai B, Lavie CJ and Blair SN. Association of Cardiorespiratory Fitness With Coronary Heart Disease in Asymptomatic Men. *Mayo Clin Proc*. 2015;90:1372-9.
21. Berry JD, Pandey A, Gao A, Leonard D, Farzaneh-Far R, Ayers C, DeFina L and Willis B. Physical fitness and risk for heart failure and coronary artery disease. *Circ Heart Fail*. 2013;6:627-34.
22. Al-Mallah MH, Juraschek SP, Whelton S, Dardari ZA, Ehrman JK, Michos ED, Blumenthal RS, Nasir K, Qureshi WT, Brawner CA, Keteyian SJ and Blaha MJ. Sex Differences in Cardiorespiratory Fitness and All-Cause Mortality: The Henry Ford ExercIse Testing (FIT) Project. *Mayo Clin Proc*. 2016;91:755-62.
23. Kaminsky LA, Arena R and Myers J. Reference Standards for Cardiorespiratory Fitness Measured With Cardiopulmonary Exercise Testing: Data From the Fitness Registry and the Importance of Exercise National Database. *Mayo Clin Proc*. 2015;90:1515-23.
24. Katzmarzyk PT, Church TS and Blair SN. Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. *Arch Intern Med*. 2004;164:1092-7.
25. Laukkanen JA, Araujo CGS, Kurl S, Khan H, Jae SY, Guazzi M and Kunutsor SK. Relative peak exercise oxygen pulse is related to sudden cardiac death, cardiovascular and all-cause mortality in middle-aged men. *Eur J Prev Cardiol*. 2018;25:772-782.
26. Guazzi M, Adams V, Conraads V, Halle M, Mezzani A, Vanhees L, Arena R, Fletcher GF, Forman DE, Kitzman DW, Lavie CJ, Myers J, European Association for Cardiovascular P, Rehabilitation and American Heart A. EACPR/AHA Scientific Statement. Clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *Circulation*. 2012;126:2261-74.
27. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen O, Vatten L and Wisloff U. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci Sports Exerc*. 2011;43:1465-73.
28. Guazzi M, Arena R, Halle M, Piepoli MF, Myers J and Lavie CJ. 2016 Focused Update: Clinical Recommendations for Cardiopulmonary Exercise Testing Data Assessment in Specific Patient Populations. *Circulation*. 2016;133:e694-711.
29. Biondi-Zoccai G, Wu Y, Serrano CV, Jr., Frati G, Agostoni P and Abbate A. Aspirin underuse, non-compliance or cessation: definition, extent, impact and potential solutions in

the primary and secondary prevention of cardiovascular disease. *Int J Cardiol*. 2015;182:148-54.
30. Weston KS, Wisloff U and Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med*. 2014;48:1227-34.

1 **TABLES**

2

Table 1. Baseline characteristics of the study population stratified by sex

	Women	Men
n	2,316	2,211
Age (years, mean SD)	47.65 (13.6)	48.76 (13.5)
Physical measurements		
Waist circumference (cm, mean SD)	85.8 (10.6)	94.6 (9.1)
Body mass index (kg/cm2, mean SD)	25.4 (3.9)	26.6 (3.2)
Systolic blood pressure (mmHg, mean SD)	123.5 (15.4)	132.0 (14.3)
Diastolic blood pressure (mmHg, mean SD)	69.9 (9.8)	76.4 (10.3)
Resting heart rate (beats/min, mean SD)	67.5 (10.0)	64.6 (10.9)
Biochemical measurements		
Total cholesterol (mmol/l, mean SD)	5.43 (1.11)	5.48 (1.02)
HDL cholesterol (mmol/l, mean SD)	1.53 (0.34)	1.25 (0.29)
Triglycerides (mmol/l, mean SD)	1.23 (0.67)	1.79 (1.12)
Glucose, non-fasting (mmol/l, mean SD)	5.21 (1.01)	5.53 (1.51)
Creatinine (umol/l, mean SD)	72.2 (10.7)	85.8 (11.4)
C-reactive protein (mg/l, median IQR)	0.80 (1.40)	0.90 (1.20)
Behavioural		
Regular alcohol intake* (%)	15.2	21.2
Pack years of cigarettes (n, median IQR)	6.4 (11.7)	8.4 (15.6)
Smoker (%)	19.3	18.0
Physically active (%) †	26.2	29.5
Disease in first-degree relative		
Cardiovascular disease (%)	19.4	17.7
Cardiovascular risk ‡		

Low	94.3	72.3
Moderate	2.9	9.9
High	2.8	17.9
Exercise testing variables		
VO_{2peak} (ml/min/kg, mean SD)	36.0 (7.8)	44.4 (9.2)
VO_{2max} criteria met (%)	76.9	83.3
RER_{peak} (mean SD)	1.12 (0.07)	1.12 (0.07)
Heart $rate_{peak}$ (beats/min, mean SD)	179.1 (14.8)	180.1 (15.5)
Oxygen $pulse_{peak}$ (ml/heart beat)	13.9 (2.6)	21.0 (3.9)
$EqVCO2_{peak}$	24.48 (2.83)	24.47 (2.94)
$EqVO2_{peak}$	33.33 (4.67)	33.25 (4.71)

SD, standard deviation; IQR, interquartile range; HDL, high-density lipoprotein; RER, respiratory exchange ratio; EqVCO2, ventilatory equivalent for carbon dioxide; EqVO2, ventilatory equivalent for oxygen

* Regular alcohol intake over once per week.

† Adherence to physical activity guidelines.

‡ Ten-year cardiovascular risk assessed by NORRISK2 risk prediction algorithm.

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Table 2. Hazard ratios for primary end-point* per one MET higher VO_{2peak}, and by quartiles of VO_{2peak}

			Model 1		Model 2	
		Events (n)	**HR**	**95% CI**	**HR**	**95% CI**
Total	Per 1-MET		0.84	0.77-0.92	0.85	0.77-0.93
	Q1	58	1	-	1	-
	Q2	34	0.58	0.38-0.88	0.58	0.38-0.90
	Q3	27	0.46	0.29-0.73	0.48	0.30-0.76
	Q4	28	0.49	0.31-0.78	0.52	0.33-0.82
Men	Per 1-MET		0.82	0.75-0.91	0.83	0.75-0.92
	Q1	44	1	-	1	-
	Q2	23	0.50	0.30-0.83	0.51	0.31-0.85
	Q3	20	0.43	0.26-0.74	0.46	0.27-0.79
	Q4	18	0.40	0.23-0.70	0.43	0.24-0.75
Women	Per 1-MET		0.89	0.74-1.08	0.88	0.73-1.08
	Q1	14	1	-	1	-
	Q2	11	0.79	0.36-1.74	0.76	0.35-1.69
	Q3	7	0.51	0.21-1.28	0.49	0.20-1.22
	Q4	10	0.79	0.35-1.78	0.77	0.34-1.77

*Coronary heart disease, revascularization by PCI or CABG, coronary heart disease mortality.; HR, hazard ratio; CI, confidence interval; Q1-Q4, quartile 1 to 4;

Model 1: adjusted for age and sex

Model 2: model 1 + smoking status, alcohol use, and family history of CVD.

Table 3. Hazard ratio per one MET higher VO_{2peak} for secondary end-points

		Model 1		Model 2	
	Events (n)	**HR**	**95% CI**	**HR**	**95% CI**
Myocardial infarction	74	0.80	0.70-0.90	0.80	0.70-0.91
Chronic ischemic heart disease	74	0.89	0.79-1.01	0.90	0.79-1.02
Coronary revascularization	85	0.83	0.74-0.93	0.83	0.74-0.94
All-cause mortality	91	0.90	0.80-1.01	0.94	0.84-1.05
Cardiovascular mortality	18	0.73	0.54-0.98	0.78	0.58-1.04

MET, metabolic equivalent task; HR, hazard ratio; CI, confidence interval.

Model 1: adjusted for age and sex

Model 2: model 1 + smoking status, alcohol use, and family history of CVD.

FIGURES

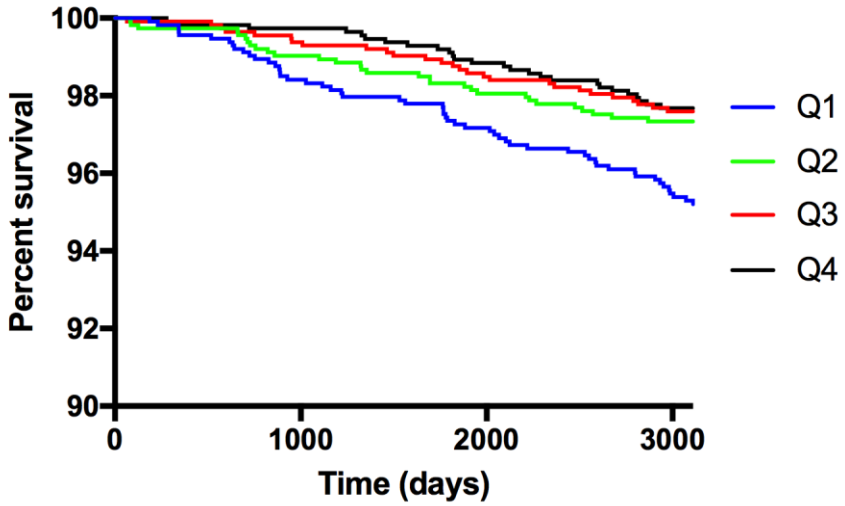

Figure 1: Kaplan-Meier curve for survival from primary end-point according to age and sex-specific quartiles of VO_{2peak} (Q1-Q4).

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SUPPLEMENTARY DATA

Supplementary methods

Cardiopulmonary exercise testing

The heart rate monitor used for the test was a Polar S610 or Polar RS300 (Polar, Kempele, Finland). The face-covering mask was a Hans-Rudolph V (Shawnee, US). The treadmill was a DK7830 (DK City, Taichung, Taiwan).

Two-point gas calibration towards carbon dioxide and oxygen of known concentrations was done before the first test every day, as well as after every fifth test throughout the day. Volume calibration was done before the first test every day as well as after every third test using a 3L standardized syringe (Calibration syringe D, Sensormedics, CareFusion, San Diego, CA, USA). Ambient air was checked before each test.

Self-reported information on physical activity

Physical activity was dichotomized to adherence or non-adherence to physical activity guidelines defined as minimum 75 minutes per week of high-intensity exercise or minimum 150 minutes per week of moderate intensity exercise. This was calculated by combining average frequency (never, less than once a week, once a week, two to three times a week, or almost every day), duration (less than 15 minutes, 15-29 minutes, 30 to 60 minutes, over 60 minutes) and average intensity (graded as 6-20 on the scale of rated perceived exertion) of weekly leisure-time physical activity. Minutes of leisure time physical activity was calculated as average time (minutes) spent exercising per session multiplied with average frequency of exercise per week. Moderate and high intensity was defined as rate of perceived exertion 13-14 and 15-20, respectively.

Utilization of the NORRISK2 risk-prediction algorithm

Ten-year risk of cardiovascular disease was calculated and classified as low, medium or high based on the recently published NORRISK2 risk prediction algorithm.[18] High risk: > 5% ten-year risk in age-

group < 54 years, >10% ten-year risk in age-group 55-64 years, and > 15% ten-year risk in the age-group ≥ 65 years. Low risk: < 4% ten-year risk in age-group < 54 years, <8% ten-year risk in age-group 55-64 years, and < 12% ten-year risk in the age-group ≥ 65 years. We adapted the mentioned risk calculator to our population by including ages under 45 years of age in the lowest age-group (originally 45-54 years), and ages over 74 years in the oldest age-group (originally 65-74 years).

Supplementary tables

Supplementary table 1. Cut-offs for VO_{2peak} in ml/kg/min by age, sex and quartiles of VO_{2peak}

			Q1		Q2		Q3		Q4	
	Age	**n**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**
Men	<30	219	27.2	48.5	48.8	54.6	54.7	59.3	59.4	75.1
	30-39	379	21.6	43.2	43.2	48.3	48.4	54.0	54.0	69.9
	40-49	569	26.8	41.5	41.5	46.9	46.9	51.3	51.3	71.3
	50-59	563	21.8	36.2	36.2	41.3	41.4	46.5	46.5	65.1
	60-69	365	20.6	33.7	33.7	38.5	38.6	42.7	42.9	59.6
	>70	116	17.4	28.3	28.5	33.2	33.2	37.2	37.4	58.3
Women	<30	252	22.4	37.4	37.5	42.3	42.4	48.5	48.5	66.7
	30-39	443	20.7	34.3	34.4	39.1	39.1	43.6	43.7	59.0
	40-49	595	20.4	32.5	32.5	37.3	37.3	41.9	42.0	67.1
	50-59	571	13.1	29.4	29.5	33.3	33.4	37.4	37.5	51.0
	60-69	350	17.3	27.0	27.0	30.3	30.4	33.6	33.7	46.6
	>70	105	14.9	22.9	23.0	25.7	25.9	29.2	29.3	66.3

Supplementary table 2. Hazard ratio for primary end-point by oxygen pulse and ventilatory equivalents

		(n)	HR*	95% CI
Peak oxygen pulse		4,320		
	Per unit		0.92	0.87-0.98
	Q1		1	
	Q2		0.97	0.64-1.47
	Q3		0.77	0.49-1.23
	Q4		0.32	0.16-0.64
Submaximal EqVO2		2,506		
	Per unit		1.05	0.99-1.11
	Q1		1	
	Q2		0.99	0.48-2.07
	Q3		1.06	0.52-2.16
	Q4		1.51	0.78-2.92
Peak EqVO2		4,497		
	Per unit		1.03	1.00-1.06
	Q1		1	
	Q2		1.23	0.76-1.98
	Q3		1.12	0.69-1.81
	Q4		1.50	0.95-2.38
Submaximal EqVCO2		2,503		
	Per unit		1.04	0.97-1.13
	Q1		1	
	Q2		1.27	0.60-2.70
	Q3		1.24	0.59-2.62
	Q4		1.58	0.77-3.24

Peak EqVCO2	4,495		
	Per unit	1.04	1.00-1.08
	Q1	1	
	Q2	1.18	0.70-1.99
	Q3	1.35	0.81-2.24
	Q4	1.43	0.87-2.35

HR, hazard ratio; CI, confidence interval; Q1 to Q4, quartiles of the respective parameters.

*Adjusted for age, sex, smoking status, alcohol use, family history of CVD and BMI.

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