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## **Associations of cardiovascular and all-cause mortality events with oxygen uptake at ventilatory threshold**

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### **Abbreviations**

**BMI** = body mass index  
**CHD** = coronary heart disease  
**CRF** = cardiorespiratory fitness  
**CVD** = cardiovascular disease  
**HDL-C** = high-density lipoprotein cholesterol  
**HR** = hazard ratio  
**IDI** = integrated-discrimination-improvement  
**KIHD** = Kuopio Ischaemic Heart Disease  
**NRI** = net reclassification improvement  
**SCD** = sudden cardiac death  
**SD** = standard deviation  
**SBP** = systolic blood pressure  
**VT** = ventilatory threshold  
**VO<sub>2</sub>** = oxygen uptake

## **ABSTRACT**

*Background:* Oxygen uptake ( $\text{VO}_2$ ) at ventilatory threshold (VT), is a cardiopulmonary exercise testing parameter which may be a proxy for peak  $\text{VO}_2$ . We aimed to assess the associations of  $\text{VO}_2$  at VT with sudden cardiac death (SCD), fatal coronary heart disease (CHD) and cardiovascular disease (CVD), and all-cause mortality.

*Methods and Results:*  $\text{VO}_2$  at VT was assessed during a submaximal exercise test using respiratory gas analyzers in the Kuopio Ischemic Heart Disease cohort of 1,639 middle-aged men. Hazard ratios (HRs) (95% CIs) were assessed. During a median follow-up of 25.6 years, 121 SCDs, 202 fatal CHDs, 312 fatal CVDs, and 703 all-cause mortality events occurred.  $\text{VO}_2$  at VT was correlated with peak  $\text{VO}_2$  ( $r = 0.90$ ) and linearly associated with each outcome. Comparing extreme quartiles of  $\text{VO}_2$  at VT, the HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality on adjustment for established risk factors were 0.37 (0.18-0.78), 0.32 (0.18-0.57), 0.45 (0.30-0.69), and 0.50 (0.38-0.64) respectively. The HRs were 1.02 (0.36-2.91), 1.43 (0.63-3.25), 1.46 (0.79-2.71), and 1.02 (0.69-1.51) respectively on further adjustment for peak  $\text{VO}_2$ . Addition of  $\text{VO}_2$  at VT to a CVD mortality risk prediction model containing established risk factors significantly improved risk discrimination and reclassification at 25 years.

*Conclusions:* There are linear and inverse associations of  $\text{VO}_2$  at VT with fatal cardiovascular and all-cause mortality events, which are dependent on peak  $\text{VO}_2$ . Inclusion of  $\text{VO}_2$  at VT in the standard established risk factors panel significantly improves the prediction and classification of long-term CVD mortality risk.

*Keywords:* Oxygen uptake at ventilatory threshold; peak oxygen uptake; cardiopulmonary exercise testing; risk prediction; fatal cardiovascular disease; all-cause mortality

## 1. Introduction

Peak oxygen uptake ( $\text{VO}_2$ ) which is used as a measure of cardiorespiratory fitness (CRF), is a cardiopulmonary exercise testing (CPX) parameter which is considered to be one of the best measures for assessing cardiovascular fitness and aerobic capacity.[1] Peak  $\text{VO}_2$  has been consistently shown to be inversely and independently associated with incident cardiovascular disease (CVD) events, cardiovascular mortality, and total mortality in large-scale epidemiological cohorts.[2-4] Evidence also suggests that Peak  $\text{VO}_2$  adds additional prognostic value beyond established risk factors in predicting vascular disease and mortality risk.[2, 5, 6] There is a growing appreciation of the value of exercise testing at the anaerobic threshold, a concept which was introduced by Wasserman and colleagues.[7] Oxygen uptake at ventilatory threshold (VT), often referred to as the anaerobic threshold, is a parameter assessed at submaximal level of CPX.[8] For majority of healthy individuals, the anaerobic threshold lies at exercise intensities between 50% and 75% of  $\text{VO}_2$  max; while in trained endurance athletes, it can reach intensities as high as 80% of  $\text{VO}_2$  max.[9] Beyond the anaerobic threshold of exercise testing, there is accumulation of lactic acid which eventually results in metabolic acidosis.[7] Unlike peak  $\text{VO}_2$  which is restricted by the capability of the cardiorespiratory system to deliver oxygen to exercising muscles and thus may not be the best measure of endurance,  $\text{VO}_2$  at VT is highly correlated with endurance performance.[10, 11] It has been suggested that the anaerobic threshold could be a determinant of physiological fitness and since it assesses the capacity to perform sustained aerobic activity,  $\text{VO}_2$  at VT is considered a reliable index of aerobic capacity. Though there is emerging evidence on the potential clinical application of  $\text{VO}_2$  at VT as a CPX variable, there are still persisting uncertainties. In 2016, the European Association for Cardiovascular Prevention & Rehabilitation and the American Heart Association (AHA) updated their clinical recommendations for CPX data assessment in specific patient populations; and recommended the inclusion of  $\text{VO}_2$  at VT in the algorithm for presurgical assessment.[12] However, the clinical value of  $\text{VO}_2$  at VT in general populations has not been established and there is no prospective data (to our knowledge) on the nature of the relationships of  $\text{VO}_2$  at VT with

adverse outcomes. Given the relative ease at which this submaximal exercise testing measure can be assessed noninvasively using respiratory gases, it will be clinically useful to know if  $\text{VO}_2$  at VT can be used as a suitable proxy for peak  $\text{VO}_2$ .

Our first objective to assess the nature and magnitude of the associations of  $\text{VO}_2$  at VT with the risk of sudden cardiac death (SCD), fatal coronary heart disease (CHD) and CVD events, and all-cause mortality using the established Kuopio Ischemic Heart Disease (KIHD) population-based cohort comprising healthy middle-aged men from eastern Finland. A second objective was to investigate the extent to which  $\text{VO}_2$  at VT measurements could improve the prediction of CVD mortality when added to conventional risk factors.

## **2. Materials and Methods**

### *2.1. Study population*

The KIHD risk factor study is a prospective epidemiologic study of the incidence of atherosclerotic CVD and other related chronic disease outcomes among Finnish adults ( $N=3,433$ ). Participants of the KIHD study were selected to be a representative sample of men aged 42-61 years living in the city of Kuopio and its surrounding rural communities in eastern Finland. A complete description of the study design, objectives, and sampling strategy has been previously described.[13] Briefly, of the 3,433 randomly selected to be potentially eligible, 3,235 were found to be eligible for inclusion into the study and of this number, 2,682 (82.9%) volunteered to participate in the study. Baseline examinations were performed between March 1984 and December 1989. Of the 2,682 men, 1,027 had missing measurements on CPX parameters, relevant covariates, and our specified outcomes. Of these, 694 men did not have data on  $\text{VO}_2$  at VT measurements because they could not participate in exercise testing measurements as a result of prevalent disease including musculoskeletal problems or could not exercise to a sufficient intensity level to reach  $\text{VO}_2$  at VT. The final cohort comprised of 1,639 men with complete information with  $\text{VO}_2$  at VT and peak  $\text{VO}_2$  measurements, relevant risk markers, and the main outcomes. Less than

1% of data was missing for most covariates; whereas missing data was 25.9% for VO<sub>2</sub> at VT and 11.7% for peak VO<sub>2</sub> as a result of those participants unable to undergo exercise protocol because of musculoskeletal and orthopedic problems. The research protocol and study design were approved the institutional review board of the University of Eastern Finland, Kuopio, Finland and all study participants provided written informed consent.

## *2.2. Assessment of VO<sub>2</sub> at VT*

Oxygen uptake was assessed using a respiratory gas exchange analyzer (Medical Graphics, MCG, St. Paul, Minnesota) during a submaximal symptom-limited cycle ergometer exercise tolerance test performed between 8:00 am and 10:00 am.[14] The standardized testing protocol comprised a graded increase in the workload of 20 W/min until exhaustion. The gas analyzer expressed VO<sub>2</sub> as the average values recorded over 8 seconds. VO<sub>2</sub> at VT was defined based on changes in respiratory gases (VO<sub>2</sub> and VCO<sub>2</sub>) during exercise test. In this study, VO<sub>2</sub> at VT was defined at the level of exercise tests at which an increase in the ventilatory equivalent for oxygen (VE/VO<sub>2</sub>) occurs without an increment in the ventilatory equivalent for carbon dioxide production (VE/VCO<sub>2</sub>). Peak VO<sub>2</sub> which was assessed using a respiratory gas exchange analyzer during the cycle ergometer exercise test was used as a measure of CRF, as described in previous reports.[4, 14] To ensure safety, all tests were supervised by an experienced physician with the assistance of an experienced nurse.

## *2.3. Assessment of covariates*

Information on covariates was obtained by history, examinations, and measurements during the baseline visit. Detailed description of blood sample collection, physical measurements, assessment of lifestyle characteristics, and assays for lipids, lipoproteins and biochemical factors have been provided in previous reports.[15, 16] Serum levels of triglycerides were assessed enzymatically (Boehringer Mannheim, Mannheim, Germany). Fasting plasma glucose was measured by the glucose dehydrogenase

method (Merck, Darmstadt, Germany) after protein precipitation by trichloroacetic acid. Smoking, use of antihypertensive and lipid-lowering medications, and medical history were assessed by standardized self-administered questionnaires.[15] Alcohol consumption was assessed using the Nordic Alcohol Consumption Inventory. Hypertension was defined as systolic blood pressure (SBP)  $\geq$  140 mm Hg, diastolic blood pressure (DBP)  $\geq$  90 mm Hg, prior physician diagnosis of hypertension, or use of antihypertensive medications. History of CHD was based on a previous myocardial infarction, angina pectoris, the use of nitroglycerin for chest pain once a week or more frequently or chest pain. Physical activity was assessed from a 12-month physical activity history modified from the Minnesota Leisure-Time Physical Activity Questionnaire,[17] described in detail previously.[14] Adulthood socioeconomic status (SES) was assessed as a combined measure of income, education, occupation, occupational prestige, material standard of living, and housing conditions.[18] Heart rate was recorded at rest.

#### 2.4. Ascertainment of outcomes

Our outcomes of interest were SCD, fatal CHD and CVD, and all-cause mortality events. The ascertainment procedures have been described previously. Deaths that occurred from study enrollment through to 2013 were included. In the KIHD study, participants (using personal identification codes) are under continuous annual surveillance for mortality events as well as incident vascular events.[19] No losses to follow-up have been recorded in the KIHD study. Both deaths and hospitalization events were ascertained from discharge lists, death certificates, hospital records, local hospitals, informant interviews, health practitioner questionnaires, study electrocardiograms, medico-legal reports, and vital statistics offices. The diagnostic classification of SCDs was based on symptoms, electrocardiographic findings, cardiac enzyme elevations, autopsy findings, and history of CHD together with the clinical history and findings from hospital and paramedic staff, details of which have been previously described.[4, 20, 21] CHD and CVD deaths were coded using the *International Classification of Diseases, Ninth Revision (ICD-9)*, and *International Statistical Classification of Diseases, 10<sup>th</sup> Revision (ICD-10)*, codes.

Documents were cross-checked in detail by two physicians. The Independent Events Committee, masked to clinical data, performed classification of outcomes.

### *2.5. Statistical analysis*

We summarized baseline characteristics of study participants using descriptive statistics (i.e., means, medians, and percentages). Age-adjusted partial correlation coefficients were calculated to assess the cross-sectional associations of VO<sub>2</sub> at VT with risk markers. Cox proportional hazard models were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for SCD, fatal CHD and CVD events, and all-cause mortality. The proportionality-hazards assumption was confirmed using Schoenfeld residuals.[22] We assessed the shape of the association between VO<sub>2</sub> at VT and each outcome, by calculating the HRs within quartiles of baseline VO<sub>2</sub> at VT and plotting these against the mean values of VO<sub>2</sub> at VT within each quartile. Floating variances were used as described previously.[23, 24] We modelled our exposure as both continuous [per unit (ml/kg/min) and 1 MET increase] and categorical (quartiles) variables. We calculated age-, multivariable-, and multivariable plus peak VO<sub>2</sub>-adjusted HRs for each outcome. The multivariable-adjusted models controlled for age, body mass index (BMI), SBP, high-density lipoprotein cholesterol (HDL-C), alcohol consumption, history of diabetes mellitus, smoking status, prevalent CHD, resting heart rate, physical activity, and SES. Subgroup analyses were performed using interaction tests to assess statistical evidence of effect modification by levels/categories of pre-specified individual level characteristics.

To assess whether adding information on VO<sub>2</sub> at VT to documented established risk factors is associated with improvement in prediction of CVD mortality risk, we calculated measures of discrimination for censored time-to-event data (Harrell's C-index [25]) and reclassification.[26, 27] To investigate the change in C-index on the addition of VO<sub>2</sub> at VT, two CVD mortality risk prediction models were fitted: one model based on traditional risk factors (i.e., age, SBP, history of diabetes, total cholesterol, HDL-C, and smoking) and the second model with these risk factors plus VO<sub>2</sub> at AT.



Reclassification analyses was restricted to the first 25 years and was assessed using the net-reclassification-improvement (NRI)[26, 27] and integrated-discrimination-improvement (IDI)[26] by comparing the model containing conventional risk factors to the predicted risk from the model containing conventional risk factors plus VO<sub>2</sub> at VT and secondly, the conventional risk factor model combined with peak VO<sub>2</sub>. Reclassification analyses was based on predicted 25-year CVD mortality risk categories of low (<8%), intermediate (8 to <30%), and high (≥30%) risk as previously reported.[6] All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

### **3. Results**

#### *3.1. Baseline characteristics and correlates of VO<sub>2</sub> at VT*

The mean age at baseline was 52 [standard deviation (SD), 5] years. The baseline mean (SD) level of VO<sub>2</sub> at VT was 23.72 (6.10) ml/kg/min and the corresponding metabolic equivalent was 6.78 (1.74) (**Table 1**). There were weak to moderate inverse correlations of VO<sub>2</sub> at VT with age, physical measures (BMI, blood pressure, and resting heart rate), lipids (total cholesterol and triglycerides), and fasting plasma glucose. Positive correlations were observed for peak VO<sub>2</sub> (r = 0.90), HDL-C (r = 0.30) and physical activity (r = 0.11). Values of VO<sub>2</sub> at VT were lower by 96% in men with diabetes compared with men without diabetes, by 82% in current smokers compared with non-smokers, by 94% in men with a history of hypertension compared with those without a history, and by 98% in men with a prevalent history of CHD compared with men without a history.

#### *3.2. VO<sub>2</sub> at VT and outcome events*

During a median (IQR) follow-up of 25.6 (20.1-27.1) years, a total of 121 SCDs, 202 fatal CHDs, 312 fatal CVDs, and 703 all-cause mortality events occurred. In analyses adjusted for several established risk factors (age, BMI, SBP, HDL-C, alcohol consumption, history of diabetes mellitus, smoking status, prevalent CHD, resting heart rate, physical activity, and SES), VO<sub>2</sub> at VT showed graded and inverse

associations with SCD, fatal CHD, fatal CVD, and all-cause mortality (**Figure 1**). **Table 2** summarizes the associations of VO<sub>2</sub> at VT with each outcome assessed. The age-adjusted HRs (95% CIs) per unit increase in VO<sub>2</sub> at VT for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.90 (0.87-0.93), 0.89 (0.87-0.92), 0.92 (0.90-0.94), and 0.94 (0.93-0.95) respectively. The HRs (95% CIs) were only minimally attenuated to 0.94 (0.90-0.98), 0.93 (0.90-0.96), 0.94 (0.92-0.97), and 0.95 (0.94-0.97) respectively after multivariable adjustment. Further adjustment for peak VO<sub>2</sub> attenuated the associations. Alternatively, comparing the top versus bottom quartile of VO<sub>2</sub> at VT, the age-adjusted HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.18 (0.09-0.37), 0.18 (0.10-0.31), 0.27 (0.19-0.40), and 0.39 (0.31-0.49) respectively. After multivariable adjustment, the corresponding hazard ratios were 0.37 (0.18-0.78), 0.32 (0.18-0.57), 0.45 (0.30-0.69), and 0.50 (0.38-0.64) respectively. The associations were less robust on further adjustment for peak VO<sub>2</sub> (**Table 2**). The associations did not generally vary significantly by levels or categories of several clinically relevant characteristics, except for some evidence of effect modification by SBP for all outcomes. There was some evidence of effect modification by history of CHD for fatal CVD and all-cause mortality outcomes (*P* for interaction < 0.05). Though the associations were in the same direction, stronger protective associations were observed in men with a prevalent history of CHD compared with men without a prevalent history of CHD (**Appendix Supplements 1 and 2**). In a sensitivity analysis which was limited to a sample of 1,620 men after 19 men with atrial fibrillation were excluded, the associations remained consistent. Direct comparisons were made to the associations of VO<sub>2</sub> at AT with outcomes using peak VO<sub>2</sub> in the same set of participants. Peak VO<sub>2</sub> was inversely and independently associated with each outcome. The associations were also independent of VO<sub>2</sub> at VT (**Appendix Supplement 3**).

### 3.3. VO<sub>2</sub> at VT and CVD mortality risk prediction

A CVD mortality risk prediction model containing established risk factors yielded a C-index of 0.7023 (95% CI: 0.6741-0.7305). After addition of information on VO<sub>2</sub> at VT, the C-index was 0.7249 (0.6973-

0.7525), representing a significant increase of 0.0226 (0.0070 to 0.03822;  $P=0.004$ ). After taking into account inappropriate reclassification, there was a significant improvement in the classification of participants into predicted 25-year CVD mortality risk categories (NRI: 8.02%, 2.66-13.38%;  $P=0.003$ ). The IDI was 0.0280 (0.0191-0.0369;  $P<0.001$ ).

To compare the predictive ability of  $\text{VO}_2$  at VT with peak  $\text{VO}_2$  in the same participants, information on peak  $\text{VO}_2$  was added to the model containing conventional risk factors. There was a C-index change of 0.0324 (95% CI: 0.0140-0.0508;  $P = 0.001$ ), after adding  $\text{VO}_2$  peak in the model. There was a significant improvement in the classification of participants into CVD mortality risk categories (NRI: 10.44%, 4.46-16.43%;  $P=0.001$ ). The IDI was 0.0406 (0.0292-0.0520;  $P<0.001$ ).

#### **4. Discussion**

In this large-scale population-based prospective cohort study of apparently healthy Finnish men aged at 42-61 at inclusion and with more than 25 years of follow-up, we found strong and linear associations of  $\text{VO}_2$  at VT with cardiovascular and all-cause mortality events in analyses adjusted for several established cardiovascular risk factors. The associations were however partly dependent on peak  $\text{VO}_2$ . Oxygen uptake at VT was observed to be modestly and inversely correlated with several established cardiovascular risk factors; of note was the strong positive correlation of  $\text{VO}_2$  at VT with  $\text{VO}_2$ . In further analysis that assessed the association of peak  $\text{VO}_2$  with each outcome in the same set of participants, stronger inverse associations were demonstrated and which were independent of  $\text{VO}_2$  at VT; which implies that peak  $\text{VO}_2$  is a stronger risk indicator than  $\text{VO}_2$  at VT for these outcomes. Though there was suggestion of evidence of effect modification by SBP and history of CHD, the associations remained generally consistent across several clinically relevant subgroups. The stronger protective effect of  $\text{VO}_2$  at VT on fatal CVD and all-cause mortality events in men with a prevalent history of CHD may reflect previous findings which suggests that exercise training has more beneficial effects on adverse outcomes in individuals with pre-existing cardiometabolic disease compared with individuals without pre-existing

disease.[28, 29] Finally, addition of  $\text{VO}_2$  at VT significantly improved measures of discrimination and reclassification for CVD mortality risk when added to established risk factors. Additional analyses in the same set of participants showed that the improvement provided by peak  $\text{VO}_2$  assessment in the prediction of CVD mortality risk was somewhat better than that of  $\text{VO}_2$  at AT.

#### *4.1. Comparison with other studies*

We are unable to compare the current findings with previous work, as no prospective study to date has evaluated the associations of  $\text{VO}_2$  at VT with the risk of fatal outcomes. The results are however in line with findings from a recent study by our group using the KIHD cohort (unpublished data). We have shown that  $\text{VO}_2$  at aerobic thresholds (a measure of exercise capacity and also assessed by submaximal level exercise testing) is protective of fatal cardiovascular and all-cause mortality events, which were consistent with graded relationships. In addition, our novel findings are not surprising, given that  $\text{VO}_2$  at VT is a reliable measure of aerobic capacity.[8, 12] Peak  $\text{VO}_2$  which is considered the gold standard measure of aerobic capacity [1] has been demonstrated to be inversely and independently associated with vascular risk and mortality outcomes in several large epidemiological prospective cohort studies.[2-4] Indeed, our findings also show that  $\text{VO}_2$  at VT is strongly correlated with peak  $\text{VO}_2$ . Our findings demonstrating  $\text{VO}_2$  at VT to provide independent prognostic information for CVD mortality extend prior observations on the predictive ability of fitness on the risk of CVD mortality.[2, 5, 6]

#### *4.2. Potential biological mechanisms*

Compared with peak  $\text{VO}_2$ ,  $\text{VO}_2$  at VT is more highly correlated with endurance[10, 11] and is a determinant of physiological fitness and therefore provides an index of sustained physical or aerobic activity. This therefore suggests that the biological mechanisms by which exercise training provides benefits on the cardiovascular system, may underlie the protective associations demonstrated in the current study. Physical activity exerts cardioprotective effects via (i) improvement in cardiovascular risk

factors such as blood pressure, biomarkers of insulin resistance, lipid and glucose levels, natriuretic peptides, and cardiac troponin T;[30-32] (ii) its anti-inflammatory actions, by reduction in levels of inflammatory markers such as IL-18 and C-reactive protein;[33, 34] (iii) regulation of white adipose tissue mass and adipokine expression;[35] (iv) improvement in endothelial function which ultimately slows the atherosclerotic cascade;[36, 37] (v) vagal control of heart rate and regulation of cardiac autonomic function;[38] and (vi) increase in cardiac output, left ventricular function, oxygen utilization, and the formation of collateral vessels.[36, 39] In a meta-analysis of 160 randomized controlled trials, Lin and colleagues demonstrated that exercise significantly improved CRF with beneficial modulation of several cardiometabolic markers.[40]

#### *4.3. Implications of findings*

The results of our study support a protective effect of  $VO_2$  at VT on fatal cardiovascular and all-cause mortality outcomes, shows an incremental predictive value to CVD mortality of assessing  $VO_2$  at VT, and also shows the ability of  $VO_2$  at VT to reclassify subjects across clinically relevant thresholds of risk. The last decade has witnessed a growing volume of evidence in support of CPX, which has triggered the release of recommendations by several guideline bodies and associations.[8, 41] However, despite the numerous advances in the evidence base, the clinical application of CPX has not been well established, especially in general population settings. The European Association for Cardiovascular Prevention & Rehabilitation and the AHA have recently recommended  $VO_2$  at VT to be used in presurgical risk assessment algorithms.[12] Because common physical activities do not require maximal effort,  $VO_2$  at VT has also been used as an individualized exercise training prescription in both normal individuals and patients with cardiac conditions.[42, 43] Though the current findings suggest peak  $VO_2$  as a stronger risk indicator and provides better improvement in the prediction of CVD mortality risk than  $VO_2$  at VT, the overall data supports the consideration that  $VO_2$  at VT as a CPX variable may be a suitable proxy for peak  $VO_2$  or CRF in apparently healthy populations. Until recently,  $VO_2$  at VT has not been utilized

widely because of technical difficulties in its measurement. However, with the introduction of respiratory gas analyzers and automated data processes, it is relatively easy to analyze and compute this index.[7] Unlike peak  $\text{VO}_2$  which is determined during maximal or peak exercise testing and therefore may be difficult to assess during exercise limitations, during periods of low motivation, and in some patient populations;[44]  $\text{VO}_2$  at VT can be safely assessed at submaximal exercise levels. For example, Boyne and colleagues observed in 59 patients with stroke that  $\text{VO}_2$  at VT may be a more reliable measure of aerobic capacity than peak  $\text{VO}_2$ . [45] It is acknowledged that it may not always be possible to determine  $\text{VO}_2$  at VT reliably, especially in some patient populations, such as those with significant respiratory impairment or patients with heart failure.[7, 46] In addition, unlike  $\text{VO}_2$  at VT, the assessment of peak  $\text{VO}_2$  is limited by the ability of oxygen to be delivered to the exercising muscles and therefore peak  $\text{VO}_2$  cannot be sustained for a long period.[47] Our findings are timely and shows  $\text{VO}_2$  at VT to have potential applications in clinical settings. However, before any guideline recommendations can be made, further research is needed to replicate these findings in other populations and unequivocally establish the clinical utility of  $\text{VO}_2$  at VT as a risk assessment tool for major adverse cardiovascular outcomes in the general population.

#### *4.4. Strengths and limitations*

We have reported the first prospective evaluation of the associations between  $\text{VO}_2$  at VT and the risk of cardiovascular and all-cause mortality outcomes using a well characterised and established large population-based prospective cohort with a follow-up of over 25 years. The KIHD cohort had a high participation rate and there were no losses during follow-up which minimised the risk of selection bias. Our sample comprised a nationally representative ethnically and genetically homogeneous cohort which may reduce confounding by factors such as medical care. Participants in the KIHD cohort have been annually monitored and checked using established well-linked databases for outcome events, which were confirmed by physicians' review.[4, 5] Our analyses were comprehensive and employed adequate

statistical techniques, thereby ensuring the robustness of our results. We had access to and adjusted for several lifestyle and biological markers, assessed the shape of the relationships, conducted subgroup analyses which showed consistent direction of effect across several clinically relevant subgroups, and we have conducted risk prediction analyses. Some limitations should be considered when interpreting the results. The KIHD study included middle-aged Caucasian men from eastern Finland, so generalizability to other populations might be limited. However, we have no reason to believe that the pathophysiological pathways by which physical activity might have a protective effect on vascular and total mortality would be different in other age groups and populations of men. Our assessment of  $\text{VO}_2$  at VT was based on ventilatory equivalents method which is an estimation compared to the gold standard V-slope method.[48] Though the V-slope method has significant advantages over the ventilatory equivalents method, a number of studies have reported that no significant differences in mean  $\text{VO}_2$  at VT values between the two methods.[45, 48] We had no repeat measurements of  $\text{VO}_2$  at VT, which could have underestimated the associations, especially given that reproducibility sub-studies of peak  $\text{VO}_2$  in the KIHD study have reported high within-person variability (regression dilution ratio of 0.58) in peak  $\text{VO}_2$  levels.[49] There was some missing data for our exposure variables which could have potentially led to loss of information. However, the missing data were for participants who could not undergo exercise testing because of musculoskeletal or orthopedic issues; and given that the exposures were assessed in relation to cardiovascular outcomes, our associations are unlikely to be affected by the missing data. Moreover, the magnitude of the associations and narrow confidence intervals of our estimates suggest there was adequate power to demonstrate these associations. Though we adjusted for a comprehensive panel of covariates, residual confounding remains a potential alternative explanation for our findings, due to the observational design of the study.

## **Conclusions**

The results of this large prospective cohort study in middle-aged Caucasian men with long-term follow-up indicate strong linear and inverse associations of  $\text{VO}_2$  at VT with fatal cardiovascular and all-cause mortality events, which are partly dependent on peak  $\text{VO}_2$ . Additionally,  $\text{VO}_2$  at VT significantly improves the prediction and reclassification of the long-term risk for CVD mortality beyond established risk factors.



### Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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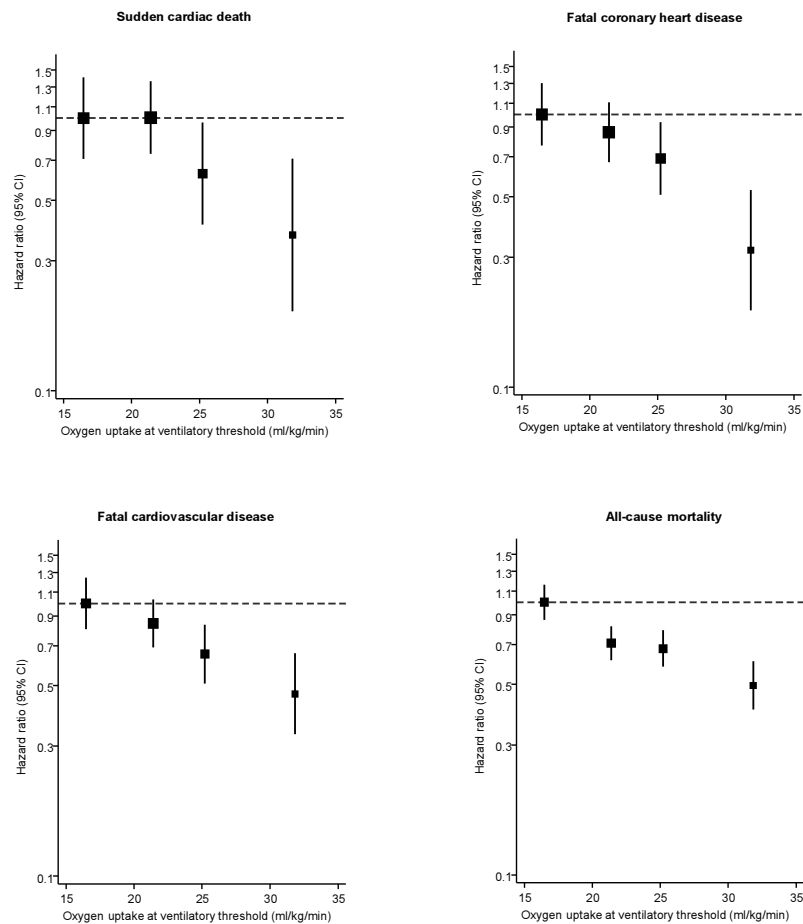
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## Figure Title and Legend

**Figure 1** Hazard ratios for sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality by quartiles of  $VO_2$  at VT



Hazard ratios were adjusted for age, body mass index, systolic blood pressure, high-density lipoprotein cholesterol, smoking status, alcohol consumption, prevalent coronary heart disease, history of diabetes mellitus, resting heart rate, physical activity, and socioeconomic status;  $VO_2$ , oxygen uptake; VT, ventilatory threshold

**Table 1.** Baseline participant characteristics and correlates of VO<sub>2</sub> at VT

	Mean (SD), median (IQR), or %	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in values of VO <sub>2</sub> at VT per 1 SD higher or compared to reference category of correlate‡
VO <sub>2</sub> at VT (ml/kg/min)	23.72 (6.10)	-	-
Metabolic equivalent	6.78 (1.74)	-	-
<b>Questionnaire/Prevalent conditions</b>			
Age at survey (years)	52.2 (5.4)	-0.32 (-0.36, -0.27)***	-85% (-89, -81)***
Alcohol consumption (g/week)	74.2 (113.7)	-0.09 (-0.13, -0.04)*	-40% (-54, -20)**
History of diabetes			
No	97.0	-	Ref
Yes	3.0	-	-96% (-99, -85)***
Smoking status			
Other	68.9	-	Ref
Current	31.1	-	-82% (-90, -67)***
History of hypertension			
No	70.8	-	Ref
Yes	29.2	-	-94% (-97, -88)***
History of CHD			
No	80.2	-	Ref
Yes	19.8	-	-98% (-99, -96)***
Use of anti-hypertensives			
No	81.9	-	Ref
Yes	18.1	-	-99% (-100, -98)***
Medication for dyslipidemia			
No	99.6	-	Ref
Yes	0.4	-	-100% (-100, -93)*
<b>Physical measurements</b>			
BMI (kg/m <sup>2</sup> )	26.9 (3.4)	-0.36 (-0.40, -0.31)***	-87% (-90, -84)***
SBP (mmHg)	134 (16)	-0.09 (-0.14, -0.04)***	-42% (-56, -23)**
DBP (mmHg)	89 (10)	-0.13 (-0.18, -0.08)***	-53% (-64, -38)***
Physical activity (kj/day)	1,515 (1,322)	0.11 (0.06, 0.16)**	87% (41, 147)***
Peak VO <sub>2</sub> (ml/kg/min)	31.4 (7.5)	0.90 (0.89, 0.91)***	26,408% (23,107, 30,178)***
RHR (beats/min)	62.5 (10.7)	-0.20 (-0.25, -0.16)***	-69% (-77, -59)***
<b>Lipid markers</b>			
Total cholesterol (mmol/l)	5.87 (1.06)	-0.06 (-0.10, -0.01)*	-28% (-45, -4)*
HDL-C (mmol/l)	1.28 (0.29)	0.30 (0.25, 0.34)***	458% (327, 629)***
Triglycerides (mmol/l)	1.10 (0.79-1.55)	-0.27 (-0.32, -0.23)***	-79% (-84, -73)***
<b>Metabolic and renal markers</b>			
Fasting plasma glucose (mmol/l)	5.32 (1.20)	-0.17 (-0.22, -0.13)***	-63% (-72, -51)***
Serum creatinine (μmol/l)	89.3 (13.7)	0.04 (-0.01, 0.09)	27% (-5, 71)
Estimated GFR (ml/min/1.73 m <sup>2</sup> )	87.6 (18.0)	-0.05 (-0.10, 0.00)	-26% (-45, 0)

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; RHR, resting heart rate; SD, standard deviation; SBP, systolic blood pressure; VO<sub>2</sub> at VT, oxygen uptake at ventilatory threshold; asterisks indicate the level of statistical significance: \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †Pearson correlation coefficients between VO<sub>2</sub> at VT and the row variables; ‡Percentage change in values of VO<sub>2</sub> at VT per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean values of VO<sub>2</sub> at VT for the category versus the reference) adjusted for age

1 metabolic equivalent corresponds to an oxygen uptake of 3.5 ml/kg/min at ventilatory thresholds

**Table 2.** Associations of VO<sub>2</sub> at VT with sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality

Models Oxygen uptake at ventilatory threshold	Sudden cardiac death		Fatal coronary heart disease		Fatal cardiovascular disease		All-cause mortality	
	121 cases		202 cases		312 cases		703 cases	
	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value
<b>Age-adjusted</b>								
Per unit (ml/kg/min)	0.90 (0.87-0.93)	< 0.001	0.89 (0.87-0.92)	< 0.001	0.92 (0.90-0.94)	< 0.001	0.94 (0.93-0.95)	< 0.001
Per 1 MET	0.69 (0.60-0.78)	< 0.001	0.67 (0.61-0.75)	< 0.001	0.73 (0.68-0.79)	< 0.001	0.80 (0.76-0.84)	< 0.001
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	0.72 (0.47-1.10)	0.124	0.63 (0.45-0.88)	0.007	0.67 (0.51-0.88)	0.004	0.64 (0.53-0.78)	< 0.001
Quartile 3 (23.22-27.69)	0.40 (0.24-0.67)	< 0.001	0.45 (0.31-0.66)	< 0.001	0.48 (0.35-0.65)	< 0.001	0.58 (0.47-0.70)	< 0.001
Quartile 4 (27.70-52.21)	0.18 (0.09-0.37)	< 0.001	0.18 (0.10-0.31)	< 0.001	0.27 (0.19-0.40)	< 0.001	0.39 (0.31-0.49)	< 0.001
<b>Multivariate-adjusted*</b>								
Per unit (ml/kg/min)	0.94 (0.90-0.98)	0.005	0.93 (0.90-0.96)	< 0.001	0.94 (0.92-0.97)	< 0.001	0.95 (0.94-0.97)	< 0.001
Per 1 MET	0.81 (0.70-0.94)	0.004	0.77 (0.68-0.86)	< 0.001	0.82 (0.75-0.90)	< 0.001	0.84 (0.79-0.89)	< 0.001
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	1.01 (0.65-1.58)	0.965	0.87 (0.61-1.23)	0.429	0.86 (0.65-1.15)	0.311	0.72 (0.59-0.88)	0.001
Quartile 3 (23.22-27.69)	0.62 (0.36-1.08)	0.094	0.69 (0.46-1.03)	0.073	0.67 (0.48-0.94)	0.019	0.68 (0.54-0.84)	0.001
Quartile 4 (27.70-52.21)	0.37 (0.18-0.78)	0.009	0.32 (0.18-0.57)	< 0.001	0.45 (0.30-0.69)	< 0.001	0.50 (0.38-0.64)	< 0.001
<b>Multivariate-adjusted* plus peak VO<sub>2</sub></b>								
Per unit (ml/kg/min)	1.03 (0.96-1.12)	0.415	1.04 (0.98-1.11)	0.183	1.04 (0.99-1.10)	0.083	1.00 (0.97-1.03)	0.929
Per 1 MET	1.12 (0.85-1.47)	0.415	1.16 (0.93-1.43)	0.183	1.16 (0.98-1.38)	0.083	1.00 (0.90-1.12)	0.929
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	1.41 (0.84-2.37)	0.191	1.44 (0.95-2.17)	0.085	1.26 (0.91-1.76)	0.164	0.90 (0.72-1.13)	0.363
Quartile 3 (23.22-27.69)	1.12 (0.55-2.29)	0.753	1.67 (0.97-2.85)	0.064	1.33 (0.86-2.04)	0.199	1.02 (0.77-1.35)	0.880
Quartile 4 (27.70-52.21)	1.02 (0.36-2.91)	0.971	1.43 (0.63-3.25)	0.391	1.46 (0.79-2.71)	0.227	1.02 (0.69-1.51)	0.932

\*, Hazard ratios are adjusted for age, body mass index, systolic blood pressure, high-density lipoprotein cholesterol, smoking status, alcohol consumption, prevalent coronary heart disease, history of diabetes mellitus, resting heart rate, physical activity, and socioeconomic status

VO<sub>2</sub> at VT, oxygen uptake at ventilatory threshold

1 MET is equivalent to 3.5 ml/kg/min of oxygen uptake at ventilatory thresholds