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Oxygen uptake at aerobic threshold is inversely associated with fatal cardiovascular and all-cause mortality events

Running Title: Oxygen uptake at aerobic threshold and mortality

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ABSTRACT

Purpose: We aimed to assess the associations of oxygen uptake at aerobic threshold (VO_2 at AT) with cardiovascular and all-cause mortality.

Design: VO_2 at AT was assessed in 1,663 middle-aged men in a cohort study. Hazard ratios (HRs) were calculated for sudden cardiac death (SCD), fatal coronary heart disease (CHD) and cardiovascular disease (CVD), and all-cause mortality.

Results: During a median follow-up of 25.6 years, 138 SCDs, 209 fatal CHDs, 333 fatal CVDs, and 719 all-cause mortality events occurred. On adjustment for established risk factors, the HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.48 (0.28-0.82), 0.48 (0.31-0.74), 0.57 (0.41-0.79), and 0.66 (0.53-0.82) respectively comparing extreme quartiles of VO_2 at AT. On further adjustment for peak VO_2, the HRs were 0.87 (0.48-1.56), 0.83 (0.52-1.34), 0.91 (0.63-1.30), and 0.88 (0.69-1.12) respectively. Addition of VO_2 at AT to a standard CVD mortality risk prediction model was associated with a C-index change of 0.0085 (95% CI: -0.0002-0.0172; p=0.05) at 25 years.

Conclusion: VO_2 at AT is inversely associated with cardiovascular and all-cause mortality events, but the associations are partly dependent on peak VO_2. VO_2 at AT may improve the prediction of the long-term risk for CVD mortality.

Keywords: oxygen uptake at aerobic threshold; cardiopulmonary exercise testing; cardiovascular disease; mortality; risk prediction

KEY MESSAGES

- Oxygen uptake at aerobic threshold (VO_2 at AT), a cardiopulmonary exercise testing parameter, may be a useful prognostic tool for adverse clinical outcomes in the general population.
- In a population-based prospective cohort study of men, VO_2 at AT was inversely associated with cardiovascular and all-cause mortality events and improved the prediction of cardiovascular mortality.
- In populations who cannot achieve maximal VO_2, VO_2 at AT may serve as a useful prognostic tool; however, further studies are warranted.

Introduction

Maximal oxygen uptake (VO_{2max}), commonly used as a measure of cardiorespiratory fitness (CRF), is considered the gold standard for assessing aerobic capacity.(1) VO_{2max} is assessed using maximal-effort graded exercise testing and gas exchange analysis and has been commonly referred to as peak VO_2 (henceforth referred to as such) in clinical population studies, as some participants are not able to attain maximal aerobic capacity due to exercise limitations such as limb fatigue or pain.(2) The relationship existing between peak VO_2, cardiovascular disease (CVD) and all-cause mortality has been well established. Consistently, several well-designed large-scale epidemiological studies have reported robust inverse and independent associations of peak VO_2 with the risk of CVD,(3) all-cause mortality,(3) and mortality from vascular and non-vascular outcomes.(4, 5) Accumulating evidence also suggests that peak VO_2 may improve CVD risk prediction above that of traditional cardiovascular risk factors.(3, 6) In certain situations where participants are not able to attain maximal aerobic capacity, peak VO_2 may not accurately represent aerobic capacity(7) thereby limiting its prognostic value. Oxygen uptake at aerobic threshold (VO_2 at AT) and VO_2 at ventilatory threshold (VO_2 at VT) are two additional cardiopulmonary exercise testing (CPX) parameters, which are also measures of exercise capacity and indices of cardiac and respiratory functioning. These parameters can be assessed by submaximal level exercise testing and during maximal exercise testing.(8) Both VO_2 at AT and VO_2 at VT are mainly influenced by the onset of lactic acidosis occurring at submaximal exercise intensities.(8, 9) However, whiles VO_2 at AT corresponds to the first increase in blood lactate concentrations above resting levels during incremental exercise testing;(10) VO_2 at VT represents the point reached during incremental exercise testing (intensity limit of prolonged activity),(9) beyond which a transition to anaerobic metabolism begins or where lactate clearance is no longer able to keep up with lactate production.(11) As reported in the 2016 update of

clinical recommendations for CPX by the European Society of Cardiology/European Association for Cardiovascular Prevention & Rehabilitation and the American Heart Association (AHA), peak VO_2 and VO_2 at VT have been consistently demonstrated to have prognostic significance in some specific patient populations.(12) We have recently shown strong linear and inverse associations of VO2 at VT with fatal cardiovascular and all-cause mortality outcomes, and the ability of VO_2 at VT to significantly improve the prediction and classification of long-term CVD mortality risk beyond established cardiovascular risk factors in a general population setting.(13) Our overall data supported the conclusion that VO_2 at VT may be a suitable proxy for peak VO_2 in apparently healthy populations. Since aerobic exercise training improves CRF and aerobic capacity,(14) VO_2 at AT may also be used as a proxy for peak VO_2 to indicate the level of aerobic exercise capacity. However, there are no data available showing the relationships of VO_2 at AT with fatal CVD and all-cause mortality outcomes. The prognostic significance of VO_2 at AT for adverse outcomes is also unknown. VO_2 at AT is a potentially useful and safe parameter which can be assessed from respiratory gases, as it can be defined at submaximal exercise levels with respiratory gas analyzers; compared to the assessment of peak VO_2, which may difficult to determine sometimes due to exercise limitations. As previously evaluated for VO_2 at VT, there is therefore a need to investigate if VO_2 at AT will be a useful prognostic tool for adverse clinical outcomes in apparently healthy populations. This will ultimately be of importance in improving the diagnostic value of clinical CPX testing. In this context, we aimed to evaluate the associations of VO_2 at AT with the risk of sudden cardiac death (SCD), fatal coronary heart disease (CHD) and CVD events, and all-cause mortality in a population-based cohort of 1,663 apparently healthy middle-aged men from eastern Finland.

Materials and methods

Study design and population

Study participants included a representative sample of men living in the city of Kuopio and its surrounding rural communities in eastern Finland, who were recruited into the Kuopio Ischemic Heart Disease (KIHD) risk factor study. This prospective population-based cohort study was designed to investigate potential risk factors for atherosclerotic CVD and other related chronic disease outcomes. Participants were men aged 42-61 years during baseline examinations performed between March 1984 and December 1989. There was a total of 3433 randomly selected men who were potentially eligible and of these, 3235 were eligible for inclusion into the study. Of the 3235 men, 2682 (82.9 %) volunteered to participate and 553 declined to give informed consent or did not respond to the invitation. The current analysis included 1,663 men with complete information on VO_2 at AT, covariates, and specified outcomes. The study protocol and design was approved by Research Ethics Committee of the University of Eastern Finland, Kuopio, Finland. Each participant included in the study provided written informed consent.

Assessment of oxygen uptake at aerobic threshold

A submaximal symptom-limited exercise tolerance test was performed between 8:00 am and 10:00 am using an electronic braked cycle ergometer.(15) Oxygen uptake was assessed using a respiratory gas analyzer (Medical Graphics, MCG, St. Paul, Minnesota) during a submaximal symptom-limited cycle ergometer exercise tolerance test. The analyzer expressed VO_2 as an average value recorded over 8 seconds. All exercise tests were conducted under the supervision of an experienced physician and assisted by an experienced nurse to ensure safety.

The main exposure variable of the current study using CPX data was VO_2 at AT which refers to the aerobic gas exchange threshold, indicating the first increase in blood lactate concentration leading to an over-proportional increase in carbon dioxide (CO_2) output as related to O_2 uptake increase due to the bicarbonate buffering of the proton resulting from the dissociation of lactic acid.(10) The threshold (VO_2 at AT) was defined from essential respiratory gas exchange changes. There is a compensatory increased stimulus for pulmonary ventilation mediated via carotid bodies as a consequence of a rising CO_2 partial pressure. In addition, pulmonary minute ventilation was increasing over-proportionally first time during increased exercise intensity at the VO_2 at AT. After an initial linear relationship between increased exercise workload and respiratory gases exchange increase (VO_2 and VCO_2), there is sudden upward bend indicating that excess CO_2 has been exhaled during increasing exercise workload. The intersection between two linear lines of respiratory gases (VO_2 and CO_2) for the upper and the lower part of the function indicates VO_2 at AT which is, by definition, an oxygen uptake. In this population study, the definition of aerobic threshold was solely based on changes in respiratory gases as reported previously,(10) which is a non-invasive approach using a visual and graphical methodology of respiratory gases. Graphical determination was done by plotting VCO_2 on the y-axis and VO_2 on the x-axis. VO_2 at AT was expressed as an absolute amount i.e in ml/min.

Assessment of risk markers

Blood sample collection, physical measurements, assessment of lifestyle characteristics, and measurement of serum lipids, lipoproteins and other biochemical characteristics have been described in detail in previously published reports.(16, 17) In brief, the cholesterol content of lipoprotein fractions were measured from fresh samples after combined ultracentrifugation and precipitation and serum triglycerides were assessed enzymatically (Boehringer Mannheim, Mannheim, Germany).(16) The glucose

dehydrogenase method (Merck, Darmstadt, Germany) was used to measure fasting plasma glucose after protein precipitation by trichloroacetic acid.(18) Self-administered questionnaires were used to assess smoking, alcohol consumption, blood pressure, use of medication, and baseline diseases,(16) all of which have been described previously.(19-21) Adulthood socioeconomic status (SES) was assessed as a combined measure of income, education, occupation, occupational prestige, material standard of living, and housing conditions.(22) Peak VO_2 was used as a measure of CRF, which was assessed by using a respiratory gas exchange analyzer during the cycle ergometer exercise test. A detailed description of the measurement of peak VO_2 has been reported elsewhere.(15) Peak VO_2 was also expressed in ml/min.

Ascertainment of outcomes

All deaths that occurred from study enrollment through to 2013 were included. There were zero losses to follow-up recorded in the KIHD study. Participants are under continuous annual surveillance for the development of new cardiovascular events, which include incident cases and deaths.(23) The sources of information on all outcomes were based on a comprehensive review of all available hospital records, questionnaires administered to health workers, wards of healthcare centres or hospitals, interviews with informants, electrocardiograms of study participants, registers of deaths as well as death certificates, and medico-legal reports. Sudden cardiac deaths were diagnosed and classified based on presenting complaints, electrocardiographic findings, cardiac enzyme levels, findings of autopsies (80% of all cardiac deaths), and history of CHD as well as the clinical history and findings reported from hospital and by paramedics. Sudden cardiac death was diagnosed when the death occurred within an hour of the onset of an abrupt change in symptoms or within 24 hours after the onset of symptoms; including those cases not witnessed when clinical and autopsy findings did not reveal a non-cardiac cause of sudden death or after successful resuscitation from ventricular tachycardia and/or ventricular fibrillation.(5) Coronary

heart disease and CVD deaths were coded using the *International Classification of Diseases, Ninth Revision (ICD-9),* and *International Statistical Classification of Diseases, 10th Revision (ICD-10),* codes. Two physicians cross-checked the documents in great detail. The Independent Events Committee of the KIHD study, blinded to clinical data, performed classification of all outcomes.

Statistical analysis

Descriptive analyses were conducted to summarize the baseline characteristics of the participants. We calculated age-adjusted partial correlation coefficients to assess the cross-sectional associations of VO_2 at AT with several risk factors and markers. Hazard ratios (HRs) with 95% confidence intervals (CIs) for SCD, fatal CHD and CVD events, and all-cause mortality were estimated using Cox proportional hazard models. Schoenfeld residuals were used to confirm the assumptions of the proportionality of hazards.(24) To assess the shape of the relationship between VO_2 at AT and each outcome, HRs were calculated within quartiles of baseline VO_2 at AT and plotted against mean values of VO_2 at AT within each quartile. Floating variances were used to calculate 95% CIs for the log hazard ratio in each group (including the reference group), to allow for comparisons across the groups irrespective of the arbitrarily chosen reference category (bottom quartile).(25) VO_2 at AT was modelled as both continuous [per standard deviation (SD) increase] and categorical (quartiles) variables. Hazard ratios were progressively adjusted for age; other traditional cardiovascular risk factors [body mass index (BMI), systolic blood pressure (SBP), high-density lipoprotein cholesterol (HDL-C), alcohol consumption, history of diabetes mellitus, smoking status, prevalent CHD, resting heart rate, physical activity, and SES]; and finally peak VO_2. Collinearity diagnostics employing the variance inflation factor (VIF)(26) showed no evidence of collinearity between VO_2 at AT and peak VO_2 (VIF=1.58). We performed subgroup analyses using tests of interaction to assess statistical evidence of any differences in hazard ratios across levels/categories of

pre-specified individual level characteristics, including age at survey, BMI, SBP, HDL-C, history of diabetes mellitus, smoking status, and prevalent CHD. Sensitivity analysis involved excluding the first five years of follow-up.

To evaluate whether adding information on VO_2 at AT to conventional cardiovascular risk factors would be associated with an improvement in the prediction of CVD mortality risk, we calculated measures of discrimination for censored time-to-event data (Harrell's C-index (27)) and reclassification.(28, 29) To investigate the change in C-index on the addition of VO_2 at AT, 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_2 at AT. Because there were too few deaths at 10 years, the incremental predictive value of VO_2 at AT for the 25-year risk of CVD mortality was rather examined. Reclassification analysis was restricted to the first 25 years of follow-up and was assessed using the net-reclassification-improvement (NRI)(28, 29) and integrated-discrimination-improvement (IDI)(28) by comparing the model containing conventional risk factors to the predicted risk from the model containing conventional risk factors plus VO_2 at AT. Reclassification analysis was based on predicted 25-year CVD mortality risk categories of low (<8%), intermediate (8 to <30%), and high (≥30%) risk as previously reported.(30) All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

Results

Baseline characteristics and correlates of VO_2 at AT

The mean age and BMI at study entry were 52 [standard deviation (SD), 5] years and 27.0 (SD, 3.5) kg/m^2 respectively. The mean (SD) value of VO_2 at AT at baseline was 1,267 (321) ml/min (**Table 1**). Weak to moderate inverse correlations were observed for values of VO_2 at AT and lipids (total cholesterol

and triglycerides). Weak positive correlations were observed for physical measures (BMI and physical activity), HDL-C, and serum creatinine lipids. VO_2 at AT was strongly correlated with peak VO_2 (r = 0.64) in age-adjusted analysis. The strong association remained the same in correlational analysis further adjusted for BMI. Values of VO2 at AT were significantly lower in men with diabetes compared with men without diabetes, smokers compared to non-smokers, and men with prevalent CHD compared to men without prevalent CHD. During a median (IQR) follow-up of 25.6 (20.1-27.1) years (37,496 person-years at risk), a total of 138 SCDs, 209 fatal CHDs, 333 fatal CVDs, and 719 all-cause mortality events were recorded.

VO_2 at AT and outcome events

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_2 at AT was linearly and inversely associated with SCD, fatal CHD, fatal CVD, and all-cause mortality (**Figure 1**). **Table 2** shows the associations of VO_2 at AT with each outcome assessed. The age-adjusted HRs (95% CIs) 1 SD increase in VO_2 at AT for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.64 (0.53-0.77), 0.65 (0.56-0.76), 0.75 (0.66-0.84), and 0.82 (0.75-0.88) respectively. These were only minimally attenuated to 0.70 (0.57-0.85), 0.70 (0.59-0.81), 0.79 (0.70-0.89), and 0.85 (0.79-0.92) respectively after adjustment for established cardiovascular risk factors. The associations were less robust on further adjustment for peak VO_2. Alternatively, comparing the top versus bottom quartile of VO_2 at AT, the age-adjusted HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.37 (0.22-0.62), 0.39 (0.25-0.59), 0.48 (0.35-0.67), and 0.57 (0.46-0.71) respectively. After adjustment for established cardiovascular risk factors, the corresponding hazard ratios were 0.48 (0.28-0.82), 0.48 (0.31-0.74), 0.57 (0.41-0.79), and 0.66 (0.53-0.82) respectively. On further adjustment for peak VO_2, the

corresponding HRs were attenuated to 0.87 (0.48-1.56), 0.83 (0.52-1.34), 0.91 (0.63-1.30), and 0.88 (0.69-1.12) respectively (**Table 2**). Generally, the associations demonstrated did not vary significantly by levels or categories of several clinically relevant characteristics (**Figures 2 and 3**). However, for all outcomes except for SCD, there was some evidence of effect modification by SBP (*p* for interaction for all < 0.05). Stronger inverse associations were observed in men with lower SBP (< 133 mmHg) compared to weaker or modest associations in men with higher SBP (≥ 133 mmHg). There was also evidence of effect modification by history of CHD and smoking status for the association of VO_2 at AT with SCD (*p* for interaction for all < 0.05). While statistically significant inverse associations were observed in men with a history of CHD and current smokers, there were weak or modest associations for men without a history of CHD and non-smokers (**Figure 3**). To put the strength of the associations of VO_2 at AT with the risk of all outcomes assessed into context, direct comparisons were made to associations of peak VO_2 with these outcomes. Peak VO_2 was inversely and independently associated with each outcome. The associations were also independent of VO_2 at AT (**Table 3**).

VO_2 at AT and CVD mortality risk prediction

A CVD mortality risk prediction model containing conventional risk factors yielded a C-index of 0.7070 (95% CI: 0.6795-0.7345; $p<0.001$). After addition of VO_2 at AT measurements to this prognostic model, the C-index increased by 0.0085 (95% CI: -0.0002-0.0172; $p=0.05$) and yielded an overall NRI of 2.78% (-0.65-6.22%; $p=0.11$) for the predicted 25-year CVD mortality risk categories. The IDI was 0.0071 (0.0028-0.0115; $p=0.001$).

To compare the predictive ability of VO_2 at AT with peak VO_2 in the same sample, information on peak VO_2 was added to the model containing conventional risk factors. There was a C-index change of 0.0191 (95% CI: 0.0046-0.0336; $p=0.01$). 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: 5.51%, 0.56-10.47%; p=0.03). The IDI was 0.0257 (0.0168-0.0345; p<0.001).

Discussion

Although previous population studies including the KIHD cohort have consistently shown higher CRF (as measured by peak VO_2) to be protective of fatal cardiovascular and all-cause mortality events, the associations of VO_2 at AT with these outcomes have not been previously evaluated. In this population of middle-aged Finnish men, we have shown for the first time that this submaximal exercise test parameter is strongly, linearly, and inversely associated with the risk of SCD, fatal CHD and CVD, and all-cause mortality in analyses adjusted for established cardiovascular risk factors. The associations were however attenuated after further adjustment for peak VO_2. There were modest inverse correlations of VO_2 at AT with several established cardiovascular risk factors. As expected, VO_2 at AT was strongly correlated with peak VO_2. In contrast, the inverse association between peak VO_2 and each outcome was independent of VO_2 at AT in analyses conducted in the same participants, which suggests peak VO_2 as a stronger risk indicator than VO_2 at AT. Except for evidence of effect modification by SBP, smoking status, and history of CHD, the associations of VO_2 at AT with outcomes remained generally consistent across several clinically relevant subgroups. The association was stronger in men with a history of CHD compared to men without a history of CHD, reflecting existing evidence which suggests that aerobic exercise capacity has more beneficial effects on mortality risk in individuals with pre-existing cardiometabolic disease compared to individuals without pre-existing.(31, 32) Our results suggest that VO_2 at AT may be an important determinant of fatal cardiovascular and all-cause mortality events. Furthermore, addition of VO_2 at AT was associated with a marginally statistically significant improvement in CVD mortality prediction beyond traditional risk factors; however, the incremental benefit was small. Addition of

information on VO_2 at AT was not associated with significant improvement in reclassification of participants across clinically meaningful risk categories. Further analyses in the same set of participants showed the improvement provided by peak VO_2 assessment in prediction of CVD mortality risk was better than that of VO_2 at AT.

Among these three CPX variables – peak VO_2, VO_2 at VT, and VO_2 at AT – the prognostic values of peak VO_2 and VO_2 at VT have been assessed extensively in the literature, whereas there is very limited data on VO_2 at AT. In a recent study, we have investigated the association of VO_2 at VT with fatal cardiovascular and all-cause mortality outcomes as well as its prognostic value in a general male Caucasian population.(13) In the current study, using VO_2 at AT as our exposure, we have replicated the same objectives as investigated previously. Our main exposure was VO_2 at AT which represents aerobic gas exchange at submaximal exercise levels as assessed by the first increase in blood lactate concentration; which is indicated by an over-proportional increase in CO_2 output as related to O_2 uptake increase due to the bicarbonate buffering of the proton resulting from the dissociation of lactic acid. Both VO_2 at AT and VO_2 at VT can be assessed at submaximal levels reflecting endurance exercise capacity which can be improved by regular exercise training.(10)

Several mechanisms have been postulated to underpin the protective effects of CRF on cardiovascular and all-cause mortality outcomes; and these include both physiological and metabolic processes.(33) Given that VO_2 at AT is a measure of aerobic exercise capacity and therefore an index of the level of physical activity, we hypothesize that these same mechanistic processes may underlie the associations demonstrated. Physical activity improves aerobic exercise capacity, which ultimately confers long-term benefits on the cardiovascular system. Indeed, it has been shown that moderate intensity regular exercise for at least 6 months can improve aerobic exercise capacity by 1 metabolic equivalent (MET).(34) Physical activity exhibits several suggested beneficial effects on cardiovascular function and these

include: (i) beneficial modulation of cardiovascular risk factors including markers of subclinical heart disease such as natriuretic peptides and cardiac troponin T(35, 36); (ii) increase in cardiac output, left ventricular function, oxygen utilization, and the formation of collateral vessels;(37, 38) (iii) regulation of cardiac autonomic function and vagal control of heart rate;(39) and (iv) anti-inflammatory effects, by reduction in levels of risk markers such as C-reactive protein,(40, 41) which is associated with an increased risk of cardiovascular and all-cause mortality outcomes.(42) Physical activity may also improve endothelial function, blood pressure and lipid levels, thus preventing or retarding the atherosclerotic process.(37, 43) The findings of graded and independent associations between VO_2 at AT and the outcomes assessed are consistent with indications of causal relationships, but these will require robust evidence from intervention studies. Cardiorespiratory fitness is known to be influenced by both genetic as well as environmental factors, with physical activity accounting for a greater part of the variation.(44) Though physical activity improves CRF, this depends on several factors such as baseline health and fitness status, type, duration, and intensity of physical activity, as well as familial factors. Given that these same reasons may relate to our exposure (VO_2 at AT), exercise trials may not enable causal inferences, as interventions that improve VO_2 at AT will interact with initial physical fitness levels as well as cardiovascular risk factors in modulating the risk of outcomes to be ascertained. In the absence of clinical trials however, Mendelian randomisation studies of genetic variants related to VO_2 at AT may provide another route to assess causality.(45) It has been reported that genetic factors account for 25-40% of the variation in CRF,(44) although the genetic contribution to the level of VO_2 at AT has not been well studied.

As demonstrated in our previous study which reported strong inverse and independent associations of VO2 at VT with fatal cardiovascular and all-cause mortality outcomes;(13) the current findings also highlight a protective effect of VO_2 at AT on fatal cardiovascular and all-cause mortality outcomes. The

continuous nature of the association between VO_2 at AT and CVD mortality and the findings from risk prediction analyses suggest that VO_2 at AT may be potentially suitable for population-level risk assessment. However, the improvements provided by VO_2 at AT in risk prediction and reclassification were modest and may not be clinically meaningful. In a head-to-head comparison, we compared incremental improvements afforded by VO_2 at AT assessment with those afforded by peak VO_2. In addition to being a stronger risk indicator, peak VO_2 also provides better improvement in the prediction of CVD mortality risk than VO_2 at AT. The overall results show that information on VO_2 at AT cannot be used to replace peak VO_2, but may be used as an alternative or proxy for peak VO_2 in subjects unable to achieve peak VO_2. Given that CPX parameters (such as VO_2 at AT and VO_2 at VT) are still being underutilized, there have been recommendations for continued research into their clinical utility across all patient populations.(12) Given that this is the first study on the topic, data from future studies are needed to establish any role of VO_2 at AT in CVD risk assessment and prevention in general and specific patient populations.

Strengths and limitations

Several notable strengths of the current study deserve consideration. This is the first prospective evaluation of the associations between VO_2 at AT and the risk of cardiovascular and all-cause mortality outcomes. Our analyses employed a well characterized large cohort, which involved a high response rate, a long follow-up period, and there were no losses during follow-up. Our sample was selected to be a nationally representative population-based cohort of middle-aged Finnish men, making it possible to generalize the results in male populations. Participants in this study have undergone annual monitoring and outcomes have been checked using well-linked established databases for outcomes.(5, 6) We recorded information on various CVD outcomes using validated endpoint definitions. We had access to a

comprehensive panel of lifestyle and biological markers, allowing adequate adjustment for potential confounders. Our analyses were comprehensive and the observed associations are robust. Our results showed consistent associations across several clinically relevant subgroups. There were a number of limitations which also deserve consideration. The KIHD study included middle-aged men based on a genetically and an ethnically homogeneous population from eastern Finland and therefore our findings cannot be extrapolated widely to women and other ethnicities. Given the population-based nature of the study, VO_2 at AT assessment was based on respiratory gas changes methodology, as blood samples with the assessment of lactic acid was not available during CPX. A one-time assessment of VO_2 at AT was used and therefore we could not correct for regression dilution. The assessment based on baseline measurements could have under-estimated our associations as reproducibility substudies of peak VO_2 in the KIHD study have reported high within-person variability in peak VO_2 levels.(46)

Conclusions

This prospective study shows strong linear and inverse associations of VO_2 at AT with cardiovascular and all-cause mortality outcomes, which are partly dependent on peak VO_2. In addition, VO_2 at AT significantly improves the prediction of the long-term risk for CVD mortality when added to established risk factors. However, peak VO_2 remained the best indicator and predictor of risk and VO2 at AT could be used as a proxy should peak VO_2 not be achieved.

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Disclosure of interest

The authors report no conflicts of interest

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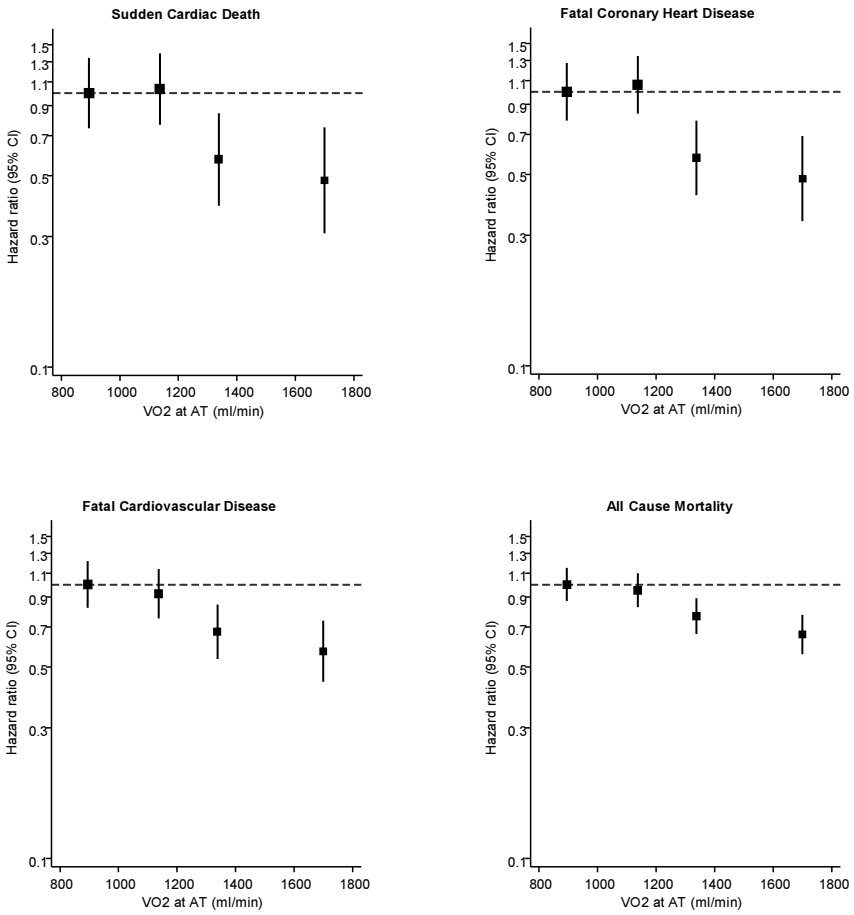
Figure Legends

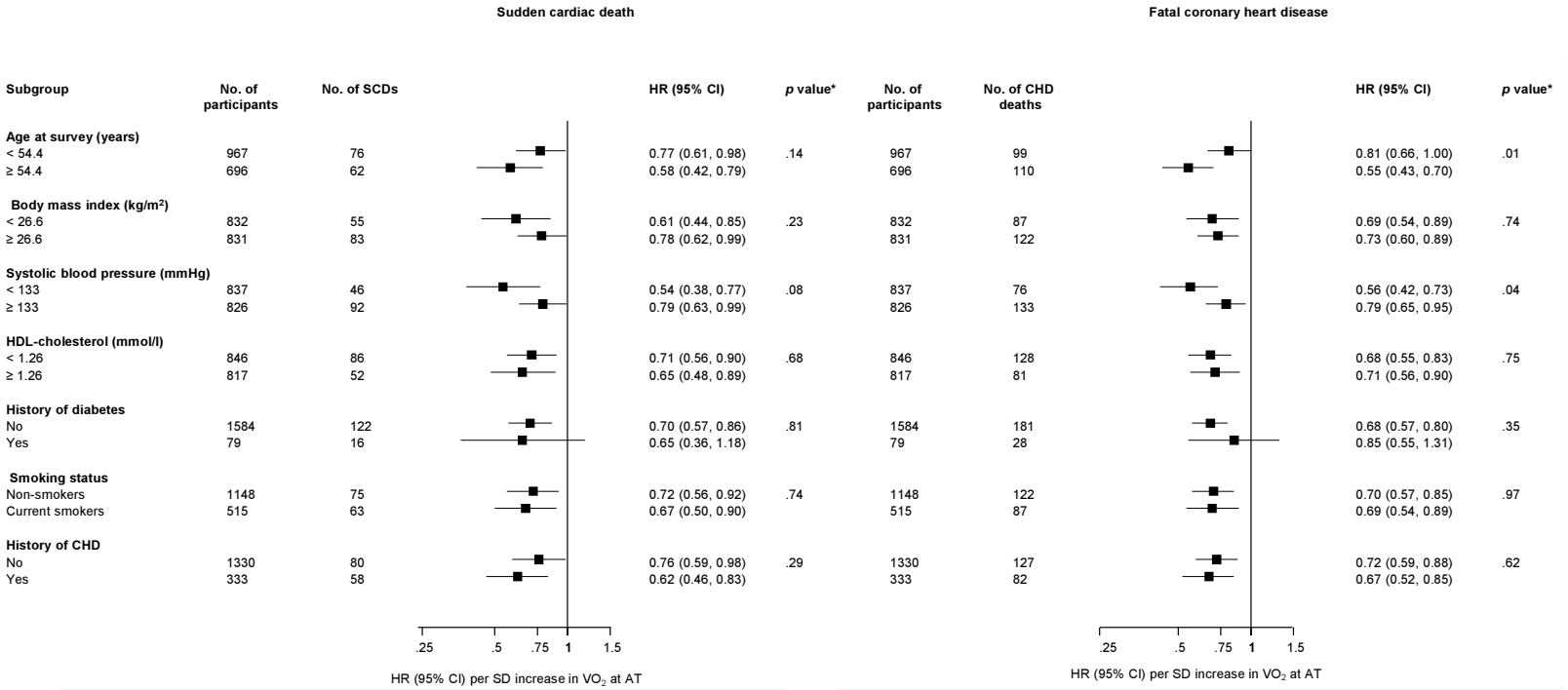
Figure 1. Hazard ratios for sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality by quartiles of VO_2 at AT



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

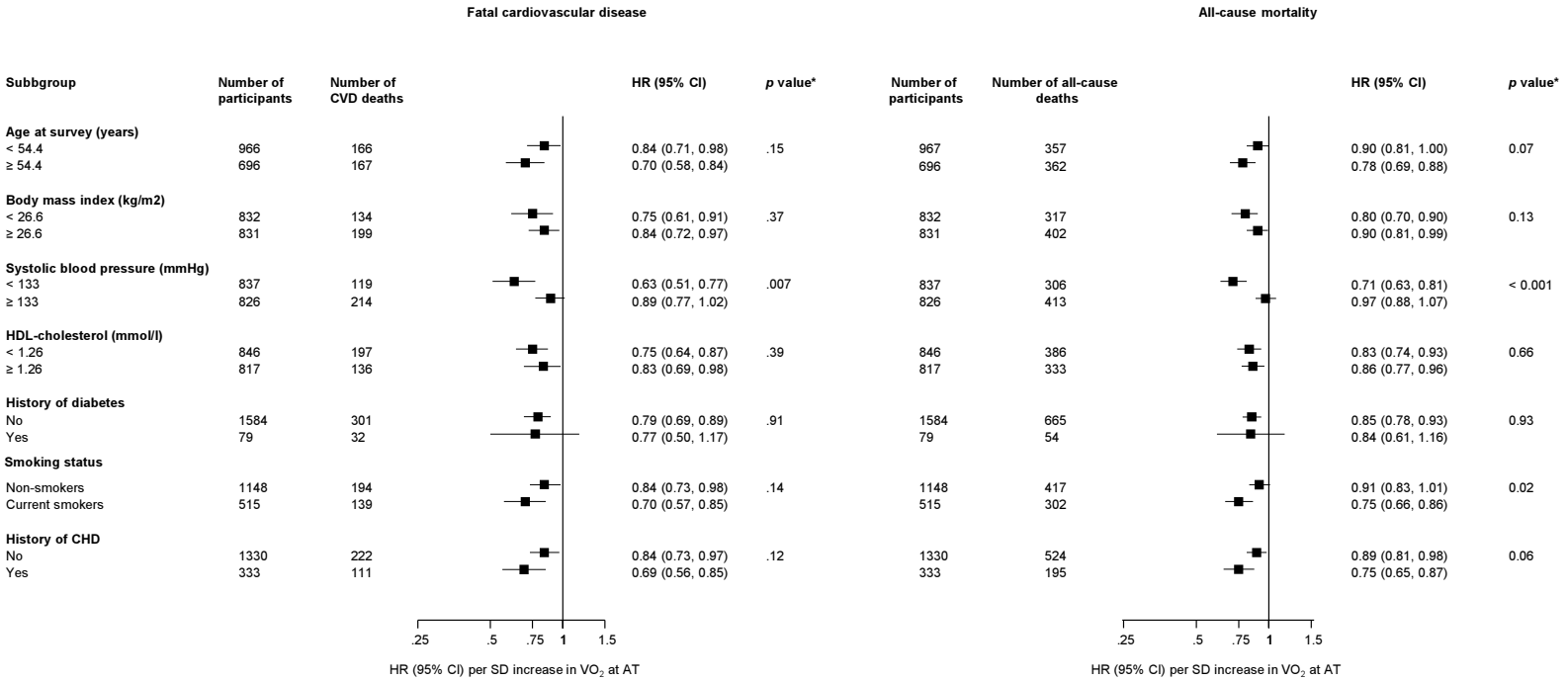
VO_2 at AT, oxygen uptake at aerobic threshold

Figure 2. Hazard ratios for sudden cardiac death and fatal coronary heart disease by several participant level characteristics



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; CHD, coronary heart disease; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; SCD, sudden cardiac death; SD, standard deviation; VO_2 at AT, oxygen uptake at aerobic threshold; *, *p*-value for interaction; cut-offs for age, body mass index, systolic blood pressure, and HDL cholesterol are based on median values.

Figure 3. Hazard ratios for fatal cardiovascular disease and all-cause mortality by several participant level characteristics



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; CVD, cardiovascular disease; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; sudden cardiac death; SD, standard deviation; VO_2 at AT, oxygen uptake at aerobic threshold; *, *p*-value for interaction; cut-offs for age, body mass index, systolic blood pressure, and HDL cholesterol are based on median values.

Table 1. Baseline Participant Characteristics and Correlates of VO_2 at AT

	Mean (SD), median (IQR), or %	Partial correlation r (95% CI)[a]	Absolute difference (95% CI) in values of VO_2 at aerobic threshold per 1 SD higher or compared to reference category of correlate[b]
VO_2 at aerobic threshold (ml/min)	1,267 (321)	-	-
Questionnaire/Prevalent conditions			
Age at survey (years)	52.2 (5.4)	-0.05 (-0.10, -0.00)*	-15.90% (-31.30, -0.50)*
Alcohol consumption (g/week)	76.0 (128.9)	-0.02 (-0.07, 0.03)	-6.47% (-21.91, 8.97)
History of diabetes			
No	96.8	-	Ref
Yes	3.2	-	-39.21% (-111.63, 33.21)**
Smoking status			
Other	69.0	-	Ref
Current	31.0	-	-109.01% (-141.94, -76.09)**
History of hypertension			
No	70.8	-	Ref
Yes	29.2	-	-21.70% (-55.74, 12.34)
History of CHD			
No	80.0	-	Ref
Yes	20.0	-	-123.49% (-162.23, -94.75)***
Use of anti-hypertensives			
No	81.8	-	Ref
Yes	18.2	-	-127.46% (-167.54, -87.38)***
Medication for dyslipidemia			
No	99.6	-	Ref
Yes	0.4	-	-114.92% (-352.75, 122.91)
Physical measurements			
BMI (kg/m^2)	27.0 (3.5)	0.15 (0.10, 0.19)***	46.57% (31.31, 61.83)***
SBP (mmHg)	134.1 (16.4)	0.02 (-0.02, 0.07)	7.59% (-7.92, 23.09)
DBP (mmHg)	89.4 (10.4)	0.03 (-0.02, 0.08)	8.87% (-6.54, 24.28)
Physical activity (kj/day)	1,548 (1,436)	0.13 (0.09, 0.18)***	42.75% (27.47, 58.03)***
Peak VO_2 (ml/min)	2,496 (608)	0.64 (0.61, 0.67)***	225.46% (212.53, 238.40)***
Lipid markers			
Total cholesterol (mmol/l)	5.87 (1.05)	-0.07 (-0.12, -0.03)*	-23.63% (-39.02, -8.23)*
HDL-C (mmol/l)	1.28 (0.30)	0.12 (0.07, 0.17)***	37.89% (22.59, 53.19)***
Triglycerides (mmol/l)	1.09 (0.78-1.54)	-0.08 (-0.13, -0.03)**	-26.28% (-41.76, -10.80)**
Metabolic and renal markers			
Fasting plasma glucose (mmol/l)	5.33 (1.22)	-0.04 (-0.09, 0.01)	-12.82% (-28.25, 2.60)
Serum creatinine (μmol/1)	89.3 (13.2)	0.07 (0.02, 0.12)*	22.34% (6.28, 38.39)*
Estimated GFR ($ml/min/1.73\ m^2$)	87.4 (17.5)	-0.06 (-0.11, -0.01)*	-20.19% (-36.58, -3.81)*

BMI, body mass index; CHD, coronary heart disease; CRF, cardiorespiratory fitness; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; SD, standard deviation; SBP, systolic blood pressure, VO_2 at AT, oxygen uptake at aerobic threshold; VO_{2max} , maximal oxygen uptake; [a] , Partial correlation coefficients between VO_2 at aerobic thresholds and the row variables;. [b] , Absolute change in values of VO_2 at aerobic threshold per 1-SD increase in the row variable (or for categorical variables, the absolute difference in mean values of VO_2 at aerobic thresholds for the category versus the reference) adjusted for age; asterisks indicate the level of statistical significance: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Table 2. Associations of VO_2 at AT With Sudden Cardiac Death, Fatal Coronary Heart Disease, Fatal Cardiovascular Disease, and All-Cause Mortality

Models **Oxygen uptake at aerobic threshold (ml/min)**	**Sudden cardiac death**		**Fatal coronary heart disease**		**Fatal cardiovascular disease**		**All-cause mortality**	
	138 cases		**209 cases**		**333 cases**		**719 cases**	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p value*
Age-adjusted								
Per 1 SD increase	0.64 (0.53-0.77)	< 0.001	0.65 (0.56-0.76)	< 0.001	0.75 (0.66-0.84)	< 0.001	0.82 (0.75-0.88)	< 0.001
Quartile 1 (323-1053)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (1054-1228)	0.92 (0.61-1.39)	0.69	0.98 (0.70-1.38)	0.92	0.87 (0.65-1.15)	0.32	0.91 (0.74-1.10)	0.32
Quartile 3 (1229-1470)	0.49 (0.30-0.79)	0.003	0.51 (0.35-0.75)	0.001	0.61 (0.45-0.82)	0.001	0.70 (0.57-0.85)	< 0.001
Quartile 4 (1471-2542)	0.37 (0.22-0.62)	< .001	0.39 (0.25-0.59)	< 0.001	0.48 (0.35-0.67)	< 0.001	0.57 (0.46-0.71)	< 0.001
Multivariate-adjusted*								
Per 1 SD increase	0.70 (0.57-0.85)	< .001	0.70 (0.59-0.81)	< 0.001	0.79 (0.70-0.89)	< 0.001	0.85 (0.79-0.92)	< 0.001
Quartile 1 (323-1053)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (1054-1228)	1.03 (0.68-1.58)	0.88	1.06 (0.75-1.49)	0.74	0.92 (0.70-1.23)	0.59	0.95 (0.78-1.16)	0.64
Quartile 3 (1229-1470)	0.57 (0.35-0.93)	0.03	0.57 (0.39-0.85)	0.006	0.67 (0.50-0.91)	0.01	0.77 (0.62-0.94)	0.01
Quartile 4 (1471-2542)	0.48 (0.28-0.82)	0.007	0.48 (0.31-0.74)	< 0.001	0.57 (0.41-0.79)	00.001	0.66 (0.53-0.82)	< 0.001
Multivariate-adjusted* plus peak VO_2								
Per 1 SD increase	0.90 (0.72-1.12)	0.34	0.87 (0.73-1.05)	0.14	0.96 (0.84-1.10)	0.54	0.97 (0.88-1.06)	0.49
Quartile 1 (323-1053)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (1054-1228)	1.22 (0.79-1.87)	0.36	1.22 (0.86-1.73)	0.26	1.04 (0.78-1.39)	0.78	1.02 (0.84-1.25)	0.83
Quartile 3 (1229-1470)	0.84 (0.50-1.41)	0.51	0.80 (0.53-1.22)	0.31	0.89 (0.65-1.23)	0.49	0.91 (0.73-1.13)	0.38
Quartile 4 (1471-2542)	0.87 (0.48-1.56)	0.64	0.83 (0.52-1.34)	0.45	0.91 (0.63-1.30)	0.60	0.88 (0.69-1.12)	0.30

*, 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_2 at AT, oxygen uptake at aerobic threshold; VO_{2max}, maximal oxygen uptake

Table 3. Associations of peak VO_2 With Sudden Cardiac Death, Fatal Coronary Heart Disease, Fatal Cardiovascular Disease, and All-Cause Mortality

Models VO_{2max} (ml/min)	**Sudden cardiac death**		**Fatal coronary heart disease**		**Fatal cardiovascular disease**		**All-cause mortality**	
	138 cases		**209 cases**		**333 cases**		**719 cases**	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p value*
Age-adjusted								
Per 1 SD increase	0.44 (0.36-0.54)	< 0.001	0.47 (0.40-0.55)	< 0.001	0.55 (0.48-0.63)	< 0.001	0.67 (0.61-0.73)	< 0.001
Quartile 1 (713-2085)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (2089-2483)	0.40 (0.27-0.61)	< 0.001	0.44 (0.31-0.62)	< 0.001	0.53 (0.40-0.69)	< 0.001	0.65 (0.54-0.78)	< 0.001
Quartile 3 (2487-2882)	0.25 (0.15-0.42)	< 0.001	0.28 (0.19-0.42)	< 0.001	0.35 (0.25-0.47)	< 0.001	0.52 (0.42-0.64)	< 0.001
Quartile 4 (2883-4841)	0.15 (0.08-0.28)	< 0.001	0.20 (0.12-0.33)	< 0.001	0.28 (0.20-0.41)	< 0.001	0.42 (0.33-0.53)	< 0.001
Multivariate-adjusted*								
Per 1 SD increase	0.54 (0.43-0.67)	< 0.001	0.56 (0.47-0.67)	< 0.001	0.62 (0.54-0.72)	< 0.001	0.73 (0.67-0.81)	< 0.001
Quartile 1 (713-2085)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (2089-2483)	0.46 (0.30-0.70)	< 0.001	0.49 (0.35-0.69)	< 0.001	0.56 (0.42-0.73)	< 0.001	0.67 (0.55-0.81)	< 0.001
Quartile 3 (2487-2882)	0.36 (0.21-0.60)	< 0.001	0.38 (0.25-0.58)	< 0.001	0.43 (0.31-0.59)	< 0.001	0.61 (0.49-0.76)	< 0.001
Quartile 4 (2883-4841)	0.25 (0.13-0.49)	< 0.001	0.32 (0.19-0.53)	< 0.001	0.39 (0.27-0.57)	< 0.001	0.54 (0.42-0.69)	< 0.001
Multivariate-adjusted* plus VO_2 at AT								
Per 1 SD increase	0.57 (0.44-0.74)	< 0.001	0.60 (0.49-0.75)	< 0.001	0.64 (0.54-0.75)	< 0.001	0.75 (0.67-0.84)	< 0.001
Quartile 1 (713-2085)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (2089-2483)	0.49 (0.32-0.75)	0.001	0.53 (0.37-0.75)	< 0.001	0.58 (0.44-0.76)	< 0.001	0.69 (0.57-0.84)	< 0.001
Quartile 3 (2487-2882)	0.41 (0.24-0.71)	0.001	0.46 (0.29-0.71)	0.001	0.46 (0.33-0.65)	< 0.001	0.65 (0.52-0.82)	< 0.001
Quartile 4 (2883-4841)	0.32 (0.15-0.66)	0.002	0.43 (0.24-0.75)	0.003	0.45 (0.29-0.69)	< 0.001	0.61 (0.46-0.80)	< 0.001

*, 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_2 at AT, oxygen uptake at aerobic threshold