Aengevaeren, VL, Mosterd, A, Braber, TL, Prakken, NHJ, Doevendans, PA, Grobbee, DE, Thompson, PD, Eijsvogels, TMH and Velthuis, BK

The Relationship Between Lifelong Exercise Volume and Coronary Atherosclerosis in Athletes.

The Relationship Between Lifelong Exercise Volume and Coronary Atherosclerosis in Athletes

Aengevaeren, Lifelong exercise and coronary atherosclerosis

Vincent L. Aengevaeren, MD[1,2]

Arend Mosterd, MD PhD[3]

Thijs L. Braber, MD[3,4]

Niek H.J. Prakken, MD PhD[5]

Pieter A. Doevendans, MD PhD[6,7]

Diederick E. Grobbee, MD PhD [8]

Paul D. Thompson, MD[9]

Thijs M.H. Eijsvogels, PhD[1,10*]

Birgitta K. Velthuis, MD PhD[4*]

Radboud Institute for Health Sciences, Departments of Physiology[1] and Cardiology[2], Radboud University Medical Center, Nijmegen, The Netherlands

[3]Department of Cardiology, Meander Medical Center, Amersfoort, The Netherlands

[4]Department of Radiology, University Medical Center Utrecht, Utrecht, The Netherlands

[5]Department of Radiology, University Medical Center Groningen, Groningen, The Netherlands

[6]Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands

[7]Netherlands Heart Institute, Utrecht, The Netherlands.

[8]Julius Center for Health Sciences and Primary Care, University Medical

Center Utrecht, Utrecht, The Netherlands

[9]Division of Cardiology, Hartford Hospital, Hartford, CT, USA

[10]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

* Equal author contributions

Word Count: 3926

Word Count Abstract: 325

Total Number of Tables: 3

Total Number of Figures: 3

Author for correspondence:

Dr. Thijs Eijsvogels, Department of Physiology (392), Radboud university medical center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, Tel +31 24 36 13 674, Fax +31 24 36 16413, E-mail: Thijs.Eijsvogels@Radboudumc.nl; Twitter: @ThijsEijsvogels

ABSTRACT

Background. Higher levels of physical activity are associated with a lower risk of cardiovascular events. Nevertheless, there is debate on the dose-response relationship of exercise and CVD outcomes and whether high volumes of exercise may accelerate coronary atherosclerosis. We aimed to determine the relationship between lifelong exercise volumes and coronary atherosclerosis.

Methods. Middle aged men engaged in competitive or recreational leisure sports underwent a non-contrast and contrast-enhanced computed tomography scan to assess coronary artery calcification (CAC) and plaque characteristics. Participants reported lifelong exercise history patterns. Exercise volumes were multiplied by Metabolic Equivalent of Task (MET) scores to calculate MET-min/week. Participants were categorized as <1000 MET-min/week, 1000-2000 MET-min/week or >2000 MET-min/week.

Results. 284 men (55±7 years) were included. CAC was present in 150/284 (53%) participants with a median CAC score of 35.8 [9.3-145.8). Athletes with a lifelong exercise volume >2000 MET-min/week (n=75) had a significantly higher CAC score (9.4 [0-60.9] versus 0 [0-43.5], p=.02) and prevalence of CAC (68%,$OR_{adjusted}$=3.2 (95%CI: 1.6-6.6)) and plaque (77%, $OR_{adjusted}$=3.3 (95%CI: 1.6-7.1)) compared to <1000 MET-min/week (n=88, 43% and 56% respectively). Very vigorous intensity exercise (≥9 METs) was associated with CAC ($OR_{adjusted}$=1.47 (95%CI: 1.14-1.91)) and plaque ($OR_{adjusted}$=1.56 (95%CI: 1.17-2.08)). Among participants with CAC>0, there was no difference in CAC score (p=.20), area (p=.21), density (p=.25) and regions of interest (p=.20) across exercise volume groups. Among participants with plaque, the most active group (>2000 MET-min/week) had a lower prevalence of mixed plaques (48% versus 69%, $OR_{adjusted}$=0.35 (95%CI:

0.15-0.85) and more often had only calcified plaques (38% versus 16%, $OR_{adjusted}$=3.57 (95%CI: 1.28-9.97)) compared to the least active group (<1000 MET-min/week).

Conclusions. Participants in the >2000 MET-min/week group had a higher prevalence of CAC and atherosclerotic plaques. The most active group did however have a more benign composition of plaques, with fewer mixed plaques and more often only calcified plaques. These observations may explain the increased longevity typical of endurance athletes despite the presence of more coronary atherosclerotic plaque in the most active participants.

Keywords: coronary atherosclerosis; coronary artery calcium; exercise; coronary computed tomography angiography

CLINICAL PERSPECTIVE

What is new:

- This study improves understanding of coronary atherosclerosis in middle-aged athletes by analyzing CAC and atherosclerotic plaque characteristics with contrast-enhanced CT in relation to lifelong exercise.
- Athletes with a high lifelong exercise volume are more likely to have coronary atherosclerosis, but the most active athletes have a more benign composition of atherosclerotic plaques, i.e. less mixed and more often only calcified plaques.

What are the clinical implications:

- Physically active persons may have substantial, asymptomatic, coronary atherosclerosis.
- We showed substantial CAC and plaque in very active athletes, which is associated with an increased risk of cardiac events.
- As the atherosclerotic plaque types had a more benign composition, long-term follow-up of athletes needs to show whether atherosclerotic burden in athletes confers a similar risk as in the general population.
- Future studies unravelling the mechanisms leading to higher CAC and plaque prevalence in very active athletes are warranted.

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death worldwide, accounting for >17 million deaths per year[1]. Atherosclerotic coronary artery disease is the main cause of CVD morbidity and mortality. Computed tomography (CT) imaging allows assessment of coronary risk since the extent of coronary artery calcification (CAC) is an indicator of the coronaries' atherosclerotic plaque burden and the risk of future cardiovascular events[2, 3]. Furthermore, coronary angiography (CTCA) allows assessment and characterization of atherosclerotic plaques, which significantly determines risk estimation[3].

Higher levels of physical activity are associated with a lower risk of cardiovascular events[4, 5] and elite athletes live longer than the general population[6]. Nevertheless, there is debate on the dose-response relationship of exercise and CVD outcomes[7, 8] and whether high volumes of exercise may accelerate coronary atherosclerosis[9-11]. The relationship between physical activity and coronary atherosclerosis has been studied since 1960, when a post mortem study found a similar degree of coronary atherosclerosis in sedentary and active men[12]. Although a recent German study found no difference in CAC scores between marathon runners (n=108) and age-matched controls (n=864), these athletes had significantly higher CAC scores when compared to controls (n=216) who were matched for both age and CVD risk factors[13]. This contrasts with other observational studies that found either no association[14-16] or an inverse relationship[17, 18] between physical activity or fitness and CAC. Prior studies did not examine participants exposed to high volumes of exercise training for a prolonged period of time. Therefore, the question remains whether extreme exercise exposure accelerates the development of coronary artery atherosclerosis and calcification.

This study sought to determine the relationship between high volumes of exercise and CT guided assessment of CAC and atherosclerotic plaque characteristics. Others have shown that a high CAC area is directly associated with CVD risk, but that increased density of CAC is inversely associated with CVD risk[19]. Moreover, the type of plaque is important for the risk of cardiac events[3], with a lower risk attributed to calcified plaques compared to non-calcified and mixed plaques. We hypothesized that athletes that performed more lifelong exercise would demonstrate similar or higher CAC scores, but with a greater CAC density compared to athletes performing lower lifelong exercise volumes. We also expected athletes with the highest exercise volume to have more low-risk calcified plaques instead of non-calcified and mixed plaques. The enhanced plaque calcification may offset the increased CAC score and contribute to the superior life expectancy of athletes versus less active peers.

METHODS

Study population

This is an analysis of the Measuring Athlete's Risk of Cardiovascular Events (MARC) study, whose rationale and design have been published previously[20]. Men aged 45 years or older were eligible if they were asymptomatic, engaged in competitive or recreational leisure sports, were free of known CVD and had undergone a sports medical examination with bicycle exercise ECG that revealed no abnormalities, according to the responsible physician. We included only men because of their higher probability of coronary atherosclerosis and risk of exercise related cardiac arrest than women[21, 22]. Regional sports physicians assisted with recruiting potential participants, as they provided a flyer detailing the MARC study to athletes that underwent a sports medical

examination for comprehensive assessment of exercise tolerance. In the Netherlands, athletes often visit a sports physician either to improve their training patterns by determining their fitness (VO2 max), (an)aerobic threshold, peak heart rate and peak load (Watt) or to gain reassurance that they can sport safely. There was therefore no referral or medical condition underlying the examination in MARC participants. Exclusion criteria were 1) an abnormal sports medical examination according to the responsible physician, 2) known coronary artery disease, 3) contrast allergy and 4) renal impairment. The medical ethics committee approved the study and all participants provided written informed consent before participation. The study was conducted according to the Declaration of Helsinki. Baseline characteristics were obtained during the sports medical examination.

Lifelong exercise volume

Participants reported their lifelong exercise history including type of sport, year started and stopped, numbers of days a week, months per year, duration of the sessions and the level at which they performed for every sport. We assigned a metabolic equivalent of task (MET) for all reported sports[23]. We calculated the exercise volume per sport by multiplying the MET score for the specific sport with the reported exercise volume (session duration * frequency/week), months of practice per year and total years of practice. The lifelong exercise volume represents the sum of all sports activities between age 12 and the age at study participation and was expressed in MET-hours/week. We also calculated the average lifetime exercise exposure in MET-hours/week and MET-min/week by dividing the total lifetime exercise volume by age at participation minus 12 for average exercise volume per year and then divided this number by 52

for average exercise volume per week (MET-hours/week). MET-min/week was calculated from MET-hours/week multiplied by 60. Based on the international physical activity recommendation that individuals perform 500 to 1000 MET-min/week of exercise[24], we assigned study participants to a lifelong exercise volume group of <1000, 1000-2000 or >2000 MET-min/week. Moreover, we classified per individual the sport with the most lifelong hours as the dominant sport. Finally, we classified exercise as light (<3 MET), moderate (3-6 MET), vigorous (6-9 MET) or very vigorous (≥ 9 MET) intensity and calculated the average lifetime hours/week of exercise in the specific intensity ranges.

Cardiac Computed Tomography

Participants underwent a low dose cardiac CT using a 256-slice CT scanner (Philips Healthcare, Best, The Netherlands) with electrocardiographic gating according to guidelines[25]. A non-contrast CT was acquired to calculate the CAC score (scan parameters 120 kV, 60mAs), followed by CTCA. The total average radiation dose was 3.9±0.9 mSv (1.0±0.4 mSv for CAC score and 3.0±1.2 mSv for CTCA). CT scans were processed on a workstation (IntelliSpace Portal, Philips Healthcare) by experienced technicians, and assessed by two experienced cardiac radiologists who were blinded to the sports medical examination findings and exercise levels. The American Heart Association modified 16-segment coronary artery model was used to analyze plaque and CAC characteristics per segment[26, 27].

Coronary Artery Calcification and plaque characteristics

The Agatston CAC score was constructed by multiplying the calcified area (mm^2) of each plaque by 1,2,3 or 4 depending on the density of the plaque based on Hounsfield Units (HU), and summing up all CT slices[28]. Calcified areas are included in the score when the plaque density was above 130 Hounsfield Units (HU). Calcified areas received a density score of 1 when density was between 130 – 200 HU, 2 = 200 – 300 HU, 3 = 300 – 400 and 4 = >400 HU. The number of calcified areas are indicated by the regions of interest. CAC scores were dichotomized (CAC=0 and CAC>0) and categorized (0, >0 – 100 and >100). CTCA was used to segment CAC, assess plaque characteristics of plaques identified by the non-contrast CT scan and for the identification of plaques with calcification levels below the Hounsfield (<130 HU) threshold. We divided plaques into 1) calcified, 2) non-calcified, 3) mixed <130HU (detected with CTCA but not with CAC scoring) and 4) mixed >130 HU (detected with CTCA and CAC scoring) plaques.

Data analysis

All parameters were visually inspected for normality and checked for kurtosis and skewness. Continuous variables were reported as mean±SD when normally distributed or as median [interquartile range] when not normally distributed and categorical variables were presented as proportions. T-tests were used to compare continuous variables between individuals with CAC=0 *versus* CAC>0 when data were normally distributed. Mann-Whitney U tests were used to compare the characteristics of the CAC=0 *versus* CAC>0 groups when data were not normally distributed. Pearson Chi-Square tests were used to compare categorical variables. One-way ANOVA with Bonferroni post-hoc tests were used to compare participant characteristics between the lifelong exercise volume groups (<1000 / 1000-2000 / >2000 MET-min/week) when data was normally

distributed, and Kruskall-Wallis 1-way ANOVA tests were used when data were not normally distributed. Two-way repeated measures ANOVA was performed to describe the distribution of lifelong exercise patterns per group across age per decade. Binary logistic regression was used to calculate unadjusted and adjusted odds ratio's (ORs) for the association between exercise characteristics (volume / intensity / sport type) and CAC, coronary atherosclerosis and plaque type presence. Furthermore, we decided a priori to adjust for the following known cardiovascular risk factors: body mass index, systolic blood pressure, smoking, use of antihypertensive, cholesterol and family history of coronary heart disease. Additionally, we made a model in which we also adjusted for use of statins and diabetes, because these factors are known to influence coronary atherosclerosis[29, 30]. Moreover, to explore a potential non-linear relationship between lifetime exercise volume (MET-hours/week) and CAC or plaque, we performed restricted cubic spline regression analyses. The knots were placed at the 5th, 50th and 95th percentile[31, 32]. We performed a test for non-linearity, which compares models with the cubic spline terms and models with only the linear terms using the likelihood ratio test. Finally, we explored the association between lifetime exercise volume and CAC characteristics for only those participants with CAC>0, and the association between exercise characteristics (volume / intensity / sport type) and plaque characteristics for participants with any coronary atherosclerosis only. Statistical significance was assumed at $p<0.05$. Statistical analyses were performed using SPSS Statistics 21 (IBM Corp, Armonk, NY, USA). The cubic spline regression analysis was conducted using SAS software, version 9.3 (SAS, Cary, NC, USA).

RESULTS

A total of 284 participants from the original study population of n=318 (100% Caucasian) were included because 27 athletes did not return the lifelong exercise questionnaire and 7 athletes returned an incomplete questionnaire. CAC characteristics were not different between included and excluded athletes (data not shown). Frequency of the sports activities and dominant sports are summarized in **Supplemental Table 1.** Mean age (±SD) of the study population was 55.0±6.5 years, 150 of the 284 participants (53%) had CAC with a median CAC score of 35.8 [9.3-145.8]. Average lifetime exercise volume was 2.9 [1.9-4.4] hours/week, resulting in 1356 [851-2030] MET-min/week (**Supplemental Table 2**).

Athletes with CAC were older, had higher systolic and diastolic blood pressures, higher total cholesterol concentrations and more frequently used statins, were former smokers and had a positive family history for coronary heart disease compared to athletes without CAC (**Supplemental Table 2**). Athletes with CAC were also more physically active during their lifetime compared to athletes without CAC, as evidenced by more years of exercise, exercise sessions/week, hours/week, MET-min/week and subsequently more lifetime MET-hours. Logistic regression analyses confirmed the association between lifetime exercise volume (MET-hours/week) and CAC presence, with $OR_{adjusted}$=1.02 for CAC>0 per MET-hour/week (**Table 2**). Specifically, only very vigorous intensity exercise (hours/week) was associated with CAC presence with $OR_{adjusted}$=1.47 (95%CI: 1.14 – 1.91).

Figure 1 provides an overview of lifelong exercise patterns for each exercise volume group. CAC was more common in athletes with higher lifelong exercise volumes (**Table 1**). Athletes performing >2000 MET-min/week more frequently had CAC>0 (68%) as compared to the <1000 MET-min/week group (43%, **Table 1, Figure 2 Panel A**). CAC scores (9.4 [0-60.9] vs. 0

[0-43.5], p=.019), CAC area (4.3 [0-20.3] vs. 0 [0-16.8], p=.025) and number of regions of interest (2 [2-5] vs. 0 [0-3], p=.014) were all significantly higher in the >2000 MET-min/week *versus* <1000 MET-min/week group. We also found an increase in CAC score categories (p=.006) across the exercise volume groups (**Figure 2 Panel A**). Unadjusted (OR=2.80 (95%CI: 1.47 – 5.32)) and multivariable adjusted logistic regression analyses (OR=3.20 (95%CI: 1.56 – 6.57)) demonstrated a significantly higher CAC prevalence in >2000 MET-min/week *versus* <1000 MET-min/week (**Table 2**). However, there were no significant differences in CAC score (p=.20), area (p=.21), density (p=.25), and regions of interest (p=.20) across exercise volume groups when analyses were repeated only in participants with CAC>0 (**Supplemental Table 3**). Also, analysis of CAC location revealed no differences in the presence of CAC within each coronary vessel and in proximal *versus* distal segments (**Supplemental Table 3**). Analysis of coronary atherosclerosis characteristics (**Supplemental Table 4**) showed significant higher plaque prevalence (either calcified, non-calcified, mixed <130 HU or mixed >130HU) in the most active group (77%) *versus* the least active group (56%, **Figure 2 Panel B**). Unadjusted (OR=2.72 (95%CI: 1.37 – 5.39)) and multivariable adjusted logistic regression analyses (OR=3.35 (95%CI: 1.57 – 7.14)) confirmed these observations and demonstrated a significantly higher coronary atherosclerosis prevalence in >2000 MET-min/week *versus* <1000 MET-min/week (**Table 3**). Also prevalence of plaque appears to be specifically associated with hours of very vigorous intensity exercise ($OR_{adjusted}$=1.56 (95%CI: 1.17 – 2.08)), whereas hours of moderate and vigorous intensity exercise did not impact plaque prevalence. In participants with coronary atherosclerosis, a lower prevalence of mixed plaques was observed in the most active (48%) *versus* least active group (69%, **Figure 3 Panel A**) with $OR_{adjusted}$=0.35 (95%CI: 0.15 – 0.85). A difference in the prevalence of mixed plaques <130 HU was

responsible for this finding (43% in <1000 MET-min/week, 33% in 1000-2000 MET-min/week and 21% in >2000 MET-min/week group, p=.046) as no differences were observed in the prevalence of mixed plaques >130 HU across exercise volume groups (41%, 49% and 40% respectively, p=.47). The lower prevalence of mixed plaques in the highest exercise volume group appears to be largely mediated by hours of vigorous intensity exercise ($OR_{adjusted}$=0.83, 95%CI: 0.71 – 0.98), whereas moderate and very vigorous intensity did not impact plaque morphology. When considering dominant plaque types (either only calcified, only non-calcified or only mixed plaques), we observed that the most active group had significantly more often only calcified plaques compared to the least active group ($OR_{adjusted}$=3.57 (95%CI: 1.28 – 9.97), **Figure 3 Panel B**). Other types of plaque dominance (including mixed plaques <130 HU and >130 HU) did not significantly differ across exercise volume groups (p>.05). Exercise intensity was also not related to plaque dominance. Analysis of coronary atherosclerosis location revealed no differences in the presence of plaques within each coronary vessel and in proximal *versus* distal segments (**Supplemental Table 4**).

Finally, the test for non-linearity for cubic spline regression was non-significant for presence of CAC (p=.48) and presence of plaque (p=.29), indicating that there was no non-linear relationship with lifelong exercise volumes.

DISCUSSION

This study provides new insights in the association between lifelong exercise volumes and coronary atherosclerosis. Based on the non-contrast CT-scan, we found that participants with CAC>0 had a higher lifelong exercise volume compared to participants with CAC=0. Logistic

regression showed an $OR_{adjusted}$ of 1.02 per MET-hour/week and $OR_{adjusted}$=3.20 for >2000 MET-min/week *versus* <1000 MET-min/week for prevalence of CAC>0. CTCA confirmed our CAC data as we found that the most active group had a significantly higher prevalence of any type of plaque. However, among individuals with coronary atherosclerosis, a lower prevalence of mixed plaques and a higher prevalence of only calcified plaques was observed in the most *versus* least active athletes. Interestingly, very vigorous intensity exercise was associated with CAC and plaque presence, and vigorous intensity exercise was associated with reduced prevalence of mixed plaques. These findings suggest that athletes with the highest exercise volumes more often have CAC and atherosclerotic plaques, but their plaques are of a more benign composition.

Accelerated Coronary Artery Calcification

Athletes in the most active group show a higher prevalence of CAC and higher CAC scores. This is in agreement with a previous study, which showed higher CAC scores in German marathon runners when they were matched for age and risk factors with controls[13]. A major limitation of that study was that the history of the subjects' cardiovascular risk factors was unknown. Participants could have recently become runners and reduced their risk factors, however that would not undo the lifelong process of atherosclerosis. Support for this hypothesis is that 52% of the runners were former smokers. We quantified lifelong exercise patterns to account for changes in exercise volume throughout the lifetime (**Figure 1**) and therefore can determine the dose-response relationships between exercise exposure and coronary atherosclerosis more accurately. Athletes in the least active group performed an equivalent of ~1 hour/week of running throughout their entire lives (669 [405-802] MET-min/week), whereas athletes in the most active

group performed an equivalent of ~4 hours/week of running (2724 [2295-3526] MET-min/week). Our findings support a consistent pattern of an increased prevalence of CAC and CAC scores in athletes with high exercise volumes.

CAC and plaque characteristics

Atherosclerotic plaque characteristics can differ, which has an important effect on the risk of cardiac events. The CAC score is a multiplication of area and density, whereby an increase in area increases the risk of cardiovascular events and an increase in density lowers the risk of cardiovascular events [19]. We hypothesized that athletes would have similar or higher CAC scores because of a higher density of their plaques. Analysis in participants with CAC>0 showed that there was no difference in density across exercise volume groups. These findings emphasize that CAC characteristics (i.e., area, density, regions of interest and location) were comparable between exercise volume groups, despite a higher CAC prevalence in the most active athletes.

Our CTCA data revealed additional information on plaque composition. Among participants with plaques, we found a lower prevalence of mixed plaques and a higher prevalence of individuals with only calcified plaques in the >2000 MET-min/week group. A previous study estimated the 3-year probability of major adverse cardiac events at 6% for calcified plaques, 23% for non-calcified plaques and 38% for mixed plaques in a cohort of patients suspected of having coronary artery disease[3]. Therefore, plaque composition (fewer mixed, more only calcified) seems to be more benign in the most active athletes, which is supported by the lower prevalence of CVD in athletes[4, 5] and the superior life expectancy of elite athletes[6].

Influence of Exercise Intensity

We found a significant association between hours of very vigorous intensity exercise and presence of CAC and plaque, and an inverse association between vigorous exercise intensity and presence of mixed plaque. These observations are in line with findings from previous studies as extreme exercise appears to be related to cardiac troponin release[33], myocardial fibrosis[34], and atrial fibrillation[35]. It is therefore possible that not the duration of exercise is most important in the development of coronary atherosclerosis, but specifically the intensity of exercise. In contrast, epidemiological studies have shown that vigorous intensity exercise is associated with greater risk reductions in all-cause and cardiovascular mortality compared to moderate intensity exercise[36, 37]. Alternatively, exercise intensity may be a proxy for overall lifelong exercise volume as the most active exercisers (>2000 MET-min/week) reported the highest volume of very vigorous intensity exercise. Future (animal) studies exploring the mechanisms of CAC and plaque development following exposure to different exercise intensities are therefore needed.

Potential Underlying Mechanisms

The underlying mechanisms for the higher prevalence of CAC/plaque and its increased calcification in athletes with the highest exercise volume and intensity are unknown. Hypotheses for the potential underlying mechanisms include increased exposure to: 1) flexing of the coronary arteries at high heart rates with disruption of laminar blood flow, 2) high blood pressures during exercise, 3) increased levels of parathyroid hormone (PTH) due to their exercise training or 4) hypomagnesemia. Flexing of the coronary arteries during exercise may increase mechanical stress on the vessel wall and disturb flow patterns[38], potentially accelerating atherosclerosis[39]. High

blood pressure accelerates coronary artery calcification [30] and high blood pressures during exercise may have an influence on atherosclerosis when individuals are exposed for a substantial amount of time. Exercise is known to acutely increase PTH after exercise[40] and this might promote coronary calcification. Higher levels of PTH correlate with increased risk of atherosclerotic disease as assessed by whole body magnetic resonance imaging [41]. Alternatively, magnesium levels could also contribute to the increased CAC scores in athletes since magnesium levels are inversely related to CAC[42] and athletes may[43] have low magnesium levels. In conclusion, future studies are warranted to confirm which mechanisms are responsible for the higher CAC / plaque prevalence in the most active athletes.

Clinical relevance

Although active athletes have more CAC and plaque, they have fewer mixed plaques and more often have only calcified plaques. The combination of these plaque types results in a lower risk profile for future CVD. However, the difference between CAC=0 and CAC>0 is significant, with estimated 3-year probabilities of major adverse cardiac events of 2.1% for CAC score=0; 13% for a CAC score between 1 to 100; 16% for CAC score between 101 and 400; and 34% for a CAC score above 400[3]. Higher CAC categories were also associated with a higher event rate (CAC <100: 1/69 (1%); CAC 100 to <400: 3/25 (12%); and CAC >400: 3/14 (21%), p=.002) in German marathoners after 6.2 years of follow-up[44]. It is therefore prudent to aggressively manage atherosclerotic risk factors in athletes with high CAC scores, e.g. start with statins. Higher CAC scores may indicate higher risk in athletes, however it is likely that the athlete's risk is not similar to that of the general population. Exercise training increases coronary blood flow by increasing arteriolar diameters

and/or density and improves vasomotor reactivity of the coronary resistance arteries[45]. Therefore, beneficial vascular adaptations such as an improved coronary flow reserve[46, 47] may also allow athletes to better deal with coronary stenoses and experience fewer symptoms and events than the general population with a similar plaque burden. Follow-up studies focussed on clinical outcomes are warranted, to adequately advice athletes and minimalize their risk for future cardiovascular events.

Limitations

Limitations of this study include a potential recall bias as we requested the participants' lifelong historical exercise pattern. However, these athletes were dedicated exercisers who could remember their lifelong exercise activity very well and only 7 (2%) of the exercise questionnaires were incomplete. In addition, recall bias should affect all athletes in our cohort in the same way. This was an observational study and therefore we cannot exclude the possibility of residual confounding (from e.g. diet or alcohol intake). Furthermore, we only included recreational and competitive athletes and did not include a control group from the general population. Therefore, we cannot make any comparisons with non-athletes. Moreover, we only included men, so our results cannot be translated to women and follow-up research in female athletes is needed to allow sex-specific risk-estimation and counselling. Finally, we included only Caucasian men in the MARC study. As race is known to impact CAC distribution[22], findings from our study cannot be directly extrapolated to athletes of other races.

Recent studies demonstrated that the use of statins can promote calcification of atherosclerotic plaques[29, 48]. Therefore, we also analyzed the data excluding participants using

statins. This did not materially alter our results so we did not exclude these participants. Diabetes can accelerate atherosclerosis[30] so we also analyzed the data excluding participants with diabetes. This also did not alter our results so we chose not to exclude these participants.

A strength of our study is how we measured exercise volume. We chose to record lifelong exercise patterns as atherosclerosis is also a lifelong process[49]. We only included sports activities, so physical activity in other domains were not included (work, commuting, gardening, household activities). Unfortunately, this reduces the comparability of our exercise volumes with other studies. Another strength of this study is the combined use of both non-contrast CT and CTCA, to compare both CAC and otherwise non-detected atherosclerotic plaques.

Conclusion

In this study of middle aged men engaged in competitive or recreational leisure sports, participants in the >2000 MET-min/week group had a higher prevalence of CAC and atherosclerotic plaques. The most active group did however have a more benign composition of plaques, with fewer mixed plaques and more often only calcified plaques. These observations may explain the increased longevity typical of endurance athletes despite the presence of more coronary atherosclerosis in the most active participants.

Sources of funding: This work was supported by a grant from Sportcor, a Dutch national registry of sudden cardiac arrest in athletes, the Foundations 'Wetenschappelijk Onderzoek Hart- en Vaatziekten' and 'Bijstand Meander Medical Center' Amersfoort, the Röntgen Foundation Utrecht and Philips Healthcare. The funders had no role in study design, data collection and

analysis, decision to publish, or preparation of the manuscript. V.L.A is financially supported by a grant from the Radboud Institute for Health Sciences. T.M.H.E is supported by a Horizon 2020 grant from the European Commission (Marie Sklodowska-Curie Fellowship 655502).

Disclosures: V.L.A., T.L.B., N.H.J.P., P.A.D. and T.M.H.E. have no relationships or conflicts to disclose. A.M. reports serving as a consultant for Bayer, Merck, Novartis, and Pfizer; receiving speaker honoraria from Novartis. D.E.G. reports receiving research grants from Roche, Pfizer, Novartis. P.D.T. reports receiving research grants from the Amarin, Esperion, Regeneron, Sanolfi, and Pfizer; serving as a consultant for Aventis, Regeneron, Merck, Genomas, Abbvie, Sanolfi, and Pfizer; receiving speaker honoraria from Regeneron, Sanolfi, Amgen, Amarin, and Merck: owning stock in General Electric, JA Wiley Publishing, J&J, Abbvie, Abbott, Medtronic, Serapta and Cryolife. B.K.V. reports serving as a regular speaker for Philips Healthcare. All relationships are modest.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER, 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB, American Heart Association Statistics C and Stroke Statistics S. Heart disease and stroke statistics-2015 update: a report from the american heart association. *Circulation*. 2015;131:e29-e322. doi: 10.1161/CIR.0000000000000152.

2. Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA and Schwartz RS. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol*. 1998;31:126-133.

3. Hou ZH, Lu B, Gao Y, Jiang SL, Wang Y, Li W and Budoff MJ. Prognostic value of coronary CT angiography and calcium score for major adverse cardiac events in outpatients. *JACC Cardiovasc Imaging*. 2012;5:990-999. doi: 10.1016/j.jcmg.2012.06.006.

4. Maessen MF, Verbeek AL, Bakker EA, Thompson PD, Hopman MT and Eijsvogels TM. Lifelong Exercise Patterns and Cardiovascular Health. *Mayo Clin Proc*. 2016;91:745-754. doi: 10.1016/j.mayocp.2016.02.028.

5. Eijsvogels TM and Thompson PD. Exercise Is Medicine: At Any Dose? *JAMA*. 2015;314:1915-1916. doi: 10.1001/jama.2015.10858.

6. Garatachea N, Santos-Lozano A, Sanchis-Gomar F, Fiuza-Luces C, Pareja-Galeano H, Emanuele E and Lucia A. Elite athletes live longer than the general population: a meta-analysis. *Mayo Clin Proc*. 2014;89:1195-1200. doi: 10.1016/j.mayocp.2014.06.004.

7. Eijsvogels TM, Molossi S, Lee DC, Emery MS and Thompson PD. Exercise at the Extremes: The Amount of Exercise to Reduce Cardiovascular Events. *J Am Coll Cardiol*. 2016;67:316-329. doi: 10.1016/j.jacc.2015.11.034.

8. Lee DC, Lavie CJ, Sui X and Blair SN. Running and Mortality: Is More Actually Worse? *Mayo Clin Proc*. 2016;91:534-536. doi: 10.1016/j.mayocp.2016.01.013.

9. Eijsvogels TM, Fernandez AB and Thompson PD. Are There Deleterious Cardiac Effects of Acute and Chronic Endurance Exercise? *Physiol Rev*. 2016;96:99-125. doi: 10.1152/physrev.00029.2014.

10. Sharma S, Merghani A and Mont L. Exercise and the heart: the good, the bad, and the ugly. *Eur Heart J*. 2015;36:1445-1453. doi: 10.1093/eurheartj/ehv090.

11. Aengevaeren VL, Hopman MT and Eijsvogels TM. Fitness and Coronary Artery Calcification. *JAMA Intern Med*. 2016;176:716. doi: 10.1001/jamainternmed.2016.0898.

12. Spain DM and Bradess VA. Occupational physical activity and the degree of coronary atherosclerosis in "normal" men. A postmortem study. *Circulation*. 1960;22:239-242. doi:

13. Mohlenkamp S, Lehmann N, Breuckmann F, Brocker-Preuss M, Nassenstein K, Halle M, Budde T, Mann K, Barkhausen J, Heusch G, Jockel KH, Erbel R, Marathon Study I and Heinz Nixdorf Recall Study I. Running: the risk of coronary events : Prevalence and prognostic relevance of coronary atherosclerosis in marathon runners. *Eur Heart J*. 2008;29:1903-1910. doi: 10.1093/eurheartj/ehn163.

14. Shah RV, Murthy VL, Colangelo LA, Reis J, Venkatesh BA, Sharma R, Abbasi SA, Goff DC, Jr., Carr JJ, Rana JS, Terry JG, Bouchard C, Sarzynski MA, Eisman A, Neilan T, Das S, Jerosch-Herold M, Lewis CE, Carnethon M, Lewis GD and Lima JA. Association of Fitness in Young Adulthood With Survival and Cardiovascular Risk: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA Intern Med*. 2016;176:87-95. doi: 10.1001/jamainternmed.2015.6309.

15. Taylor AJ, Watkins T, Bell D, Carrow J, Bindeman J, Scherr D, Feuerstein I, Wong H, Bhattarai S, Vaitkus M and O'Malley PG. Physical activity and the presence and extent of calcified coronary atherosclerosis. *Med Sci Sports Exerc*. 2002;34:228-233. doi: Doi 10.1097/00005768-200202000-00008.

16. Hamer M, Venuraju SM, Lahiri A, Rossi A and Steptoe A. Objectively assessed physical activity, sedentary time, and coronary artery calcification in healthy older adults. *Arterioscler Thromb Vasc Biol*. 2012;32:500-505. doi: 10.1161/ATVBAHA.111.236877.

17. Delaney JAC, Jensky NE, Criqui MH, Whitt-Glover MC, Lima JAC and Allison MA. The association between physical activity and both incident coronary artery calcification and ankle brachial index progression: The Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2013;230:278-283. doi: DOI 10.1016/j.atherosclerosis.2013.07.045.

18. Sung J, Cho SJ, Choe YH, Choi YH and Hong KP. Prevalence of coronary atherosclerosis in asymptomatic middle-age men with high aerobic fitness. *Am J Cardiol*. 2012;109:839-843. doi: 10.1016/j.amjcard.2011.11.009.

19. Criqui MH, Denenberg JO, Ix JH, McClelland RL, Wassel CL, Rifkin DE, Carr JJ, Budoff MJ and Allison MA. Calcium density of coronary artery plaque and risk of incident cardiovascular events. *JAMA*. 2014;311:271-278. doi: 10.1001/jama.2013.282535.

20. Braber TL, Mosterd A, Prakken NH, Doevendans PA, Mali WP, Backx FJ, Grobbee DE, Rienks R, Nathoe HM, Bots ML and Velthuis BK. Rationale and design of the Measuring Athlete's Risk of Cardiovascular events (MARC) study : The role of coronary CT in the cardiovascular evaluation of middle-aged sportsmen. *Neth Heart J*. 2015;23:133-138. doi: 10.1007/s12471-014-0630-0.

21. Berdowski J, de Beus MF, Blom M, Bardai A, Bots ML, Doevendans PA, Grobbee DE, Tan HL, Tijssen JG, Koster RW and Mosterd A. Exercise-related out-of-hospital cardiac arrest in the general population: incidence and prognosis. *Eur Heart J*. 2013;34:3616-3623. doi: 10.1093/eurheartj/eht401.

22. McClelland RL, Chung H, Detrano R, Post W and Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*. 2006;113:30-37. doi: 10.1161/CIRCULATIONAHA.105.580696.

23. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, Greer JL, Vezina J, Whitt-Glover MC and Leon AS. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43:1575-1581. doi: 10.1249/MSS.0b013e31821ece12.

24. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A, American College of Sports M and American Heart A. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*. 2007;116:1081-1093. doi: 10.1161/CIRCULATIONAHA.107.185649.

25. Abbara S, Arbab-Zadeh A, Callister TQ, Desai MY, Mamuya W, Thomson L and Weigold WG. SCCT guidelines for performance of coronary computed tomographic angiography: a report of the Society of Cardiovascular Computed Tomography Guidelines Committee. *J Cardiovasc Comput Tomogr*. 2009;3:190-204. doi: 10.1016/j.jcct.2009.03.004.

26. Maffei E, Martini C, Arcadi T, Clemente A, Seitun S, Zuccarelli A, Torri T, Mollet NR, Rossi A, Catalano O, Messalli G and Cademartiri F. Plaque imaging with CT coronary angiography: Effect of intra-vascular attenuation on plaque type classification. *World J Radiol*. 2012;4:265-272. doi: 10.4329/wjr.v4.i6.265.

27. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, McGoon DC, Murphy ML and Roe BB. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation*. 1975;51:5-40.

28. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr. and Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827-832.

29. Puri R, Nicholls SJ, Shao M, Kataoka Y, Uno K, Kapadia SR, Tuzcu EM and Nissen SE. Impact of Statins on Serial Coronary Calcification During Atheroma Progression and Regression. *J Am Coll Cardiol*. 2015;65:1273-1282. doi: 10.1016/j.jacc.2015.01.036.

30. Kronmal RA, McClelland RL, Detrano R, Shea S, Lima JA, Cushman M, Bild DE and Burke GL. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*. 2007;115:2722-2730. doi: 10.1161/CIRCULATIONAHA.106.674143.

31. Durrleman S and Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8:551-561.

32. Desquilbet L and Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med*. 2010;29:1037-1057. doi: 10.1002/sim.3841.

33. Eijsvogels TM, Hoogerwerf MD, Oudegeest-Sander MH, Hopman MT and Thijssen DH. The impact of exercise intensity on cardiac troponin I release. *Int J Cardiol*. 2014;171:e3-4. doi: 10.1016/j.ijcard.2013.11.050.

34. van de Schoor FR, Aengevaeren VL, Hopman MT, Oxborough DL, George KP, Thompson PD and Eijsvogels TM. Myocardial Fibrosis in Athletes. *Mayo Clin Proc*. 2016;91:1617-1631. doi: 10.1016/j.mayocp.2016.07.012.

35. Myrstad M, Nystad W, Graff-Iversen S, Thelle DS, Stigum H, Aaronaes M and Ranhoff AH. Effect of years of endurance exercise on risk of atrial fibrillation and atrial flutter. *Am J Cardiol*. 2014;114:1229-1233. doi: 10.1016/j.amjcard.2014.07.047.

36. Lee DC, Pate RR, Lavie CJ, Sui X, Church TS and Blair SN. Leisure-time running reduces all-cause and cardiovascular mortality risk. *J Am Coll Cardiol*. 2014;64:472-481. doi: 10.1016/j.jacc.2014.04.058.

37. Gebel K, Ding D, Chey T, Stamatakis E, Brown WJ and Bauman AE. Effect of Moderate to Vigorous Physical Activity on All-Cause Mortality in Middle-aged and Older Australians. *JAMA Intern Med*. 2015;175:970-977. doi: 10.1001/jamainternmed.2015.0541.

38. Ding Z, Zhu H and Friedman MH. Coronary artery dynamics in vivo. *Ann Biomed Eng*. 2002;30:419-429.

39. Chiu JJ and Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev*. 2011;91:327-387. doi: 10.1152/physrev.00047.2009.

40. Bouassida A, Latiri I, Bouassida S, Zalleg D, Zaouali M, Feki Y, Gharbi N, Zbidi A and Tabka Z. Parathyroid hormone and physical exercise: a brief review. *J Sports Sci Med*. 2006;5:367-374.

41. Hagstrom E, Michaelsson K, Melhus H, Hansen T, Ahlstrom H, Johansson L, Ingelsson E, Sundstrom J, Lind L and Arnlov J. Plasma-parathyroid hormone is associated with subclinical and clinical atherosclerotic disease in 2 community-based cohorts. *Arterioscler Thromb Vasc Biol*. 2014;34:1567-1573. doi: 10.1161/ATVBAHA.113.303062.

42. Hruby A, O'Donnell CJ, Jacques PF, Meigs JB, Hoffmann U and McKeown NM. Magnesium intake is inversely associated with coronary artery calcification: the Framingham Heart Study. *JACC Cardiovasc Imaging*. 2014;7:59-69. doi: 10.1016/j.jcmg.2013.10.006.

43. Casoni I, Guglielmini C, Graziano L, Reali MG, Mazzotta D and Abbasciano V. Changes of magnesium concentrations in endurance athletes. *Int J Sports Med*. 1990;11:234-237. doi: 10.1055/s-2007-1024798.

44. Mohlenkamp S, Leineweber K, Lehmann N, Braun S, Roggenbuck U, Perrey M, Broecker-Preuss M, Budde T, Halle M, Mann K, Jockel KH, Erbel R and Heusch G. Coronary atherosclerosis burden, but not transient troponin elevation, predicts long-term outcome in recreational marathon runners. *Basic Res Cardiol*. 2014;109:391. doi: 10.1007/s00395-013-0391-8.

45. Laughlin MH, Bowles DK and Duncker DJ. The coronary circulation in exercise training. *Am J Physiol Heart Circ Physiol*. 2012;302:H10-23. doi: 10.1152/ajpheart.00574.2011.

46. Hildick-Smith DJ, Johnson PJ, Wisbey CR, Winter EM and Shapiro LM. Coronary flow reserve is supranormal in endurance athletes: an adenosine transthoracic echocardiographic study. *Heart*. 2000;84:383-389.

47. Haskell WL, Sims C, Myll J, Bortz WM, St Goar FG and Alderman EL. Coronary artery size and dilating capacity in ultradistance runners. *Circulation*. 1993;87:1076-1082.

48. Henein M, Granasen G, Wiklund U, Schmermund A, Guerci A, Erbel R and Raggi P. High dose and long-term statin therapy accelerate coronary artery calcification. *Int J Cardiol*. 2015;184:581-586. doi: 10.1016/j.ijcard.2015.02.072.

49. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med*. 2013;368:2004-2013. doi: 10.1056/NEJMra1216063.

FIGURE LEGENDS

Figure 1. Patterns of exercise volumes per decade. A gradual age-related increase in exercise volume was found in each exercise volume group (<1000 / 1000-2000 / >2000 MET-min/week). Data was averaged per decade and available for all participants (n=284) for decades between age 12 and 50. For decade 50-60 (n=192) and 60-70 (n=64), data was available in a subgroup only.

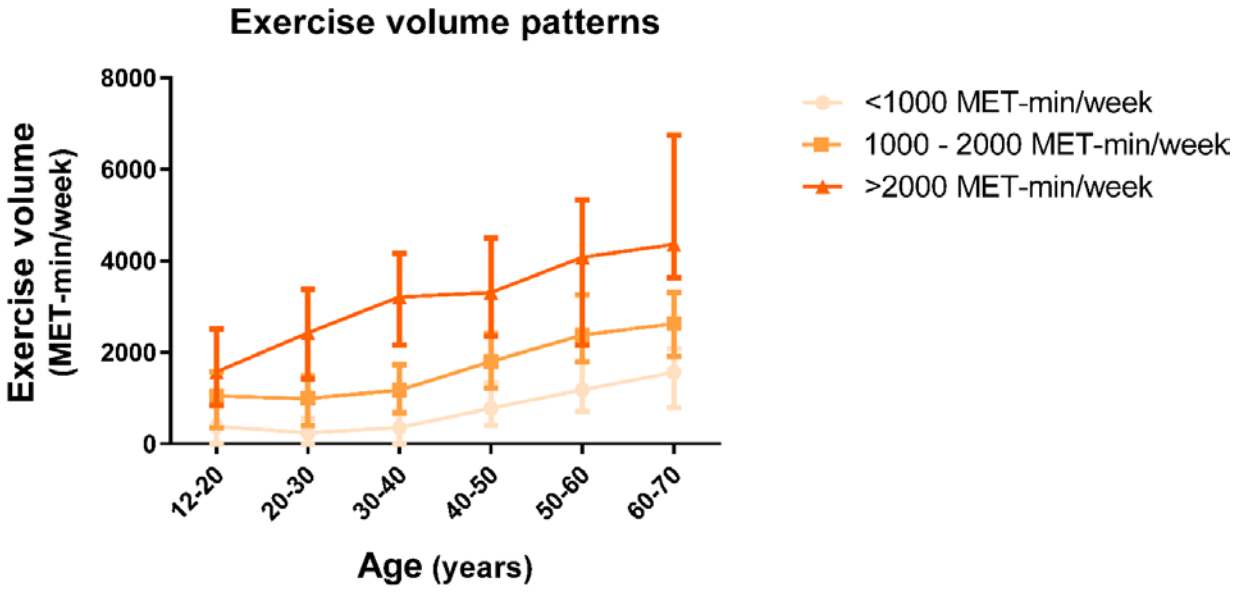
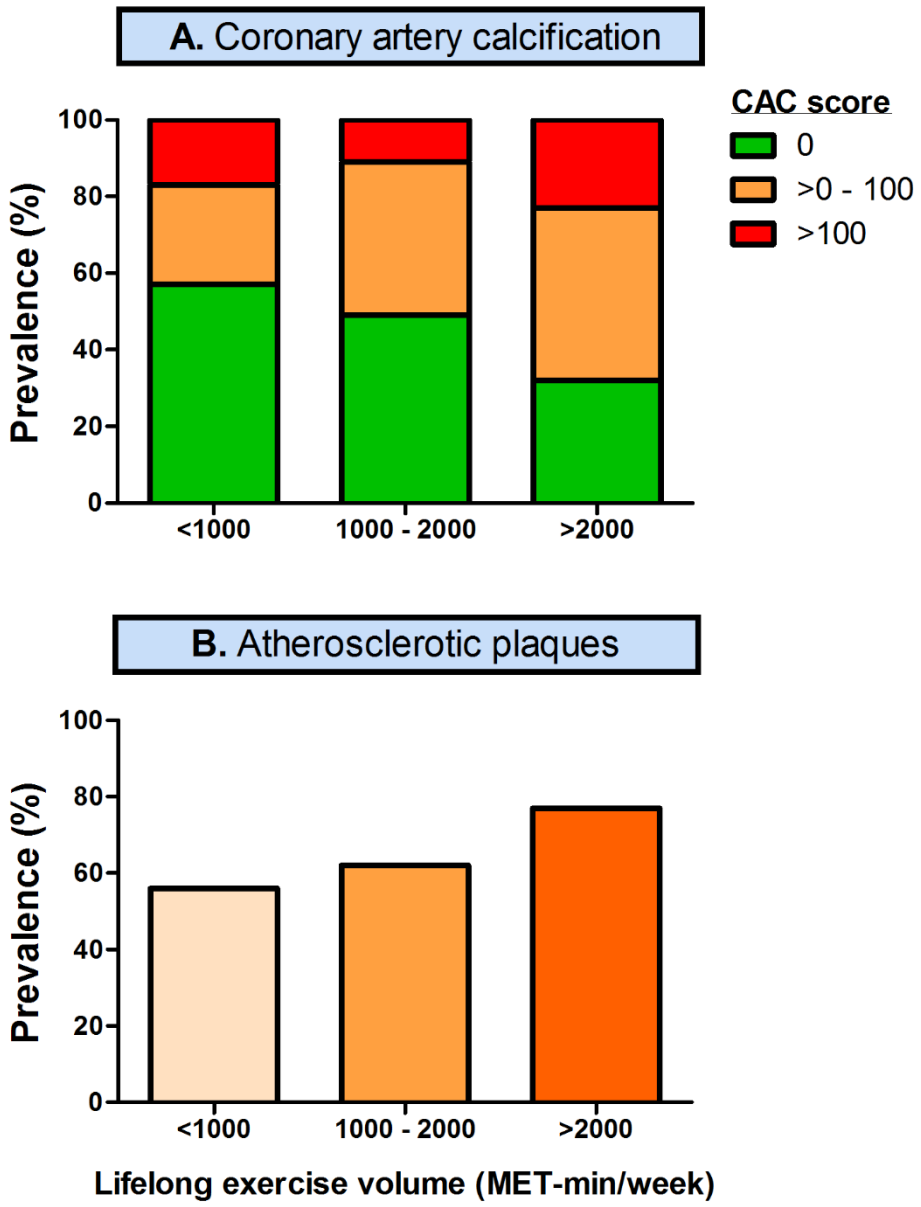


Figure 2. Prevalence of coronary artery calcification and atherosclerotic plaques across lifelong exercise volume groups. Data were derived from CT and CTCA scans for assessment of CAC and atherosclerotic plaques (n=284). **Panel A** shows a comparison of CAC score categories across exercise volume groups. A significant difference in CAC score categories (p=.006) was found across exercise volume groups, with higher CAC scores in the >2000 MET-min/week group. The >2000 MET-min/week group had an adjusted odds ratio of 3.2 (95%CI: 1.6-6.6) for CAC scores >0 compared to the <1000 MET-min/week group. **Panel B** shows a significant increase of atherosclerotic plaque prevalence across exercise volume groups (p=.013) with an adjusted odds ratio of 3.3 (95%CI: 1.6-7.1) for presence of plaque for the >2000 MET-min/week compared to the <1000 MET-min/week group. CAC = coronary artery calcification; CT = computed tomography; CTCA = computed tomography coronary angiography; MET = metabolic equivalent of task.


A. Coronary artery calcification
CAC score
0
>0 - 100
>100
Prevalence (%)
100
80
60
40
20
0
<1000
1000 - 2000
>2000
B. Atherosclerotic plaques
Prevalence (%)
100
80
60
40
20
0
<1000
1000 - 2000
>2000
Lifelong exercise volume (MET-min/week)

1

Figure 3. Plaque characteristics across the lifelong exercise volume groups in participants with CTCA evidence of coronary atherosclerosis (n=182). The >2000 MET-min/week group had fewer mixed plaques (**panel A**) and more often only calcified plaques (**panel B**). These data suggest that plaque morphology is different across exercise volume groups, which may translate to a lower risk for major adverse cardiac events for the most active exercisers, despite their higher prevalence of coronary atherosclerosis. CAC = coronary artery calcification; CTCA = computed tomography coronary angiography; MET = metabolic equivalent of task.

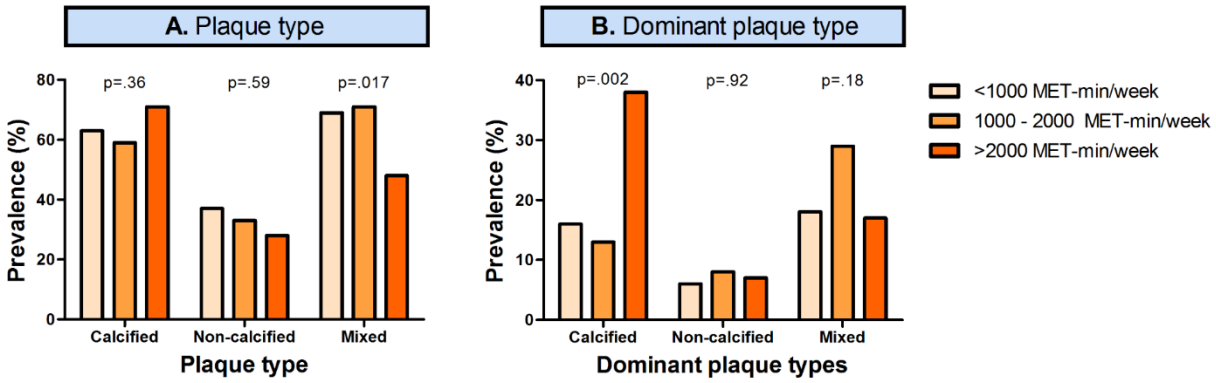


Table 1. A comparison of participant and coronary artery calcification characteristics across exercise volume groups.

	Lifelong exercise volume (MET-min/week)			
	<1000 (n=88)	**1000-2000 (n=121)**	**>2000 (n=75)**	**P-value**
Participant characteristics				
Age, years	54.4 (6.1)	54.8 (6.3)	55.9 (6.9)	.35
Systolic BP, mmHg	128 (11)	130 (15)	129 (12)	.63
Diastolic BP, mmHg	80 (8)	80 (9)	80 (8)	.82
Height, cm	183 (7)	183 (6)	181 (7)	.11
Weight, kg	84 (11)	83 (10)	80 (9) †	.029*
BMI, kg/m^2	25.3 (2.9)	24.8 (2.8)	24.5 (2.3)	.14
BSA, m^2	2.06 (.16)	2.05 (.14)	2.00 (.13) †	.025*
Exercise tolerance, Watt	298 (44)	319 (47)†	321 (48)†	.001*
Total Cholesterol, mmol/l	5.36 (.87)	5.31 (.88)	5.44 (.96)	.63
Statin, n (%)	6 (7%)	2 (2%)	7 (9%)	.048*
Current smokers, n (%)	7 (8%)	5 (4%)	2 (3%)	.26
Former smoker, n (%)	32 (36%)	43 (36%)	33 (44%)	.46
Never smoker, n (%)	49 (56%)	73 (60%)	40 (53%)	.60
Pack years of smoking, n	0 [0-8]	0 [0-7]	0 [0-8]	.81
Antihypertensive, n (%)	7 (8%)	7 (6%)	6 (8%)	.78

Diabetes, n (%)	1 (1%)	1 (1%)	2 (3%)	.55
Family history of CHD, n (%)	29 (33%)	35 (29%)	25 (33%)	.75
CAC Characteristics				
CAC, Agatston Units	0 [0-43.5]	.8 [0-26.5]	9.4 [0-60.9]†‡	.019*
CAC=0, n (%)	50 (57%)	60 (50%)	24 (32%)	.005*
Area, mm2	0 [0-16.81]	.8 [0-10.8]	4.3 [0-20.3]†	.025*
Density, au	3.0 [1.9-3.5]	2.6 [1.6-3.2]	3.0 [2.0-3.4]	.25
Regions of interest, n	0 [0-3]	1 [0-3]	2 [2-5] †‡	.014*
Lifelong Exercise Characteristics				
Years of exercise§, n	27 [13-37]	36 [30-41]†	40 [35-47]†‡	<.001*
Sessions/week, n	0.9 [.7-1.4]	2.1 [1.7-2.5]†	3.3 [2.7-4.5]†‡	<.001*
Duration/session, hrs	1.4 [1.1-1.8]	1.4 [1.2-1.7]	1.7 [1.5-2.0]†‡	<.001*
Exercise duration/week, hrs	1.5 [.9-1.9]	3.0 [2.4-3.6]†	5.7 [4.6-7.3]†‡	<.001*
MET-min/week, au	669 [405-802]	1443 [1189-1672]†	2724 [2295-3526]†‡	<.001*
MET-hours/week, au	11.2 [6.7-13.4]	24.1 [19.8-27.9]†	45.4 [38.2-58.8]†‡	<.001*
Light intensity, (%)	0 [0-0]	0 [0-0]	0 [0-0]	.47
Moderate intensity, (%)	11 [0-39]	6 [0-23]	0 [0-14]†	.035*
Vigorous intensity, (%)	52 [21-89]	56 [22-86]	67 [32-87]	.66
Very vigorous intensity, %	2 [0-50]	18 [0-53]	23 [3-54]†	.036*
Lifetime MET-hours, au	24257 [13541-30410]	52280 [42458-61098]†	104208 [81539-137010]†‡	<.001*

Au, arbitrary units; BMI, body mass index; BP, Blood pressure; BSA, body surface area; CAC, coronary artery calcification; CHD, coronary heart disease; MET, Metabolic Equivalent of Task. Data is presented as mean (SD), n (%) or median [interquartile range]. * = P-value <.05. † = pairwise comparison, significantly different from <1000 MET-min/week.‡= pairwise comparison, significantly different from 1000-2000 MET-min/week. §= since age 12.

1

2

Table 2. Unadjusted and multivariable-adjusted associations between lifelong exercise volumes and presence of coronary artery calcification (CAC>0).

	Unadjusted		***Model 1****		***Model 2**†*		***Model 3**‡*	
	Odds Ratio (95% CI)	***P-value***	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***
Presence of CAC								
MET-hrs/week	1.02 (1.01 – 1.04)	.003	1.02 (1.00 – 1.04)	.014	1.02 (1.01 – 1.04)	.012	1.02 (1.01 – 1.04)	.006
Exercise intensity								
Moderate intensity (hrs/week)	1.09 (0.88 – 1.37)	.43	1.06 (0.84 – 1.33)	.65	1.01 (0.79 – 1.30)	.91	1.03 (0.80 – 1.32)	.81
Vigorous intensity (hrs/week)	1.16 (1.01 – 1.32)	.031	1.12 (0.97 – 1.28)	.13	1.12 (0.97 – 1.29)	.13	1.13 (0.97 – 1.31)	.12
Very vigorous intensity (hrs/week)	1.35 (1.06 – 1.71)	.014	1.35 (1.06 – 1.72)	.016	1.41 (1.10 – 1.81)	.008	1.47 (1.14 – 1.91)	.003

Exercise volume groups								
<1000 MET-min/week	Reference		Reference		Reference		Reference	
1000 – 2000 MET-min/week	1.34 (0.77 – 2.32)	.30	1.33 (0.75 – 2.35)	.33	1.45 (0.80 – 2.63)	.22	1.62 (.88 – 2.97)	.12
>2000 MET-min/week	2.80 (1.47 – 5.32)	.002	2.69 (1.38 – 5.23)	.004	2.93 (1.46 – 5.86)	.002	3.20 (1.56 – 6.57)	.001

MET, Metabolic Equivalent of Task. Each exposure (exercise volume and exercise intensity) was entered separately into the different models. *Adjusted for age. † Additionally adjusted for body mass index, systolic blood pressure, ever smoked, use of antihypertensive, total cholesterol and family history of coronary heart disease. ‡ Additionally adjusted for use of statin and diabetes.

Table 3. Unadjusted and multivariable-adjusted associations between lifelong exercise volumes and CTCA evidence of coronary atherosclerosis.

	Unadjusted		***Model 1****		***Model 2**†*		***Model 3**‡*	
	Odds Ratio (95% CI)	***P-value***	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***
Presence of plaque								
MET-hrs/week	1.02 (1.00 – 1.03)	.021	1.02 (1.00 – 1.03)	.06	1.02 (1.00 – 1.04)	.033	1.02 (1.00 – 1.04)	.015
Exercise intensity								
Moderate intensity (hrs/week)	1.11 (0.86 – 1.43)	.41	1.08 (0.84 – 1.39)	.56	1.05 (0.81 – 1.38)	.70	1.07 (0.81 – 1.40)	.64
Vigorous intensity (hrs/week)	1.10 (0.96 – 1.26)	.18	1.06 (0.92 – 1.23)	.40	1.08 (0.93 – 1.26)	.33	1.08 (0.93 – 1.27)	.32
Very vigorous intensity (hrs/week)	1.38 (1.06 – 1.80)	.015	1.38 (1.06 – 1.79)	.017	1.46 (1.11 – 1.92)	.007	1.56 (1.17 – 2.08)	.002

Exercise volume groups								
<1000 MET-min/week	Reference		Reference		Reference		Reference	
1000 – 2000 MET-min/week	1.30 (0.74 – 2.27)	.36	1.28 (0.73 – 2.27)	.39	1.49 (0.81 – 2.71)	.20	1.62 (.88 – 2.99)	.12
>2000 MET-min/week	2.72 (1.37 – 5.39)	.004	2.60 (1.29 – 5.24)	.007	2.99 (1.44 – 6.23)	.003	3.35 (1.57 – 7.14)	.002

MET, Metabolic Equivalent of Task. Each exposure (exercise volume and exercise intensity) was entered separately into the different models. *Adjusted for age. † Additionally adjusted for body mass index, systolic blood pressure, ever smoked, use of antihypertensive, total cholesterol and family history of coronary heart disease. ‡ Additionally adjusted for use of statin and diabetes.

Supplemental Table 1. Frequency of sports participation

Type of sport	**Frequency (%)**		**MET-score***
	Total	**Dominant sport**	
Athletics	41 (14%)	10 (4%)	6.7
Badminton	14 (5%)	1 (0%)	5.5
Baseball	4 (1%)	1 (0%)	5
Basketball	18 (6%)	2 (1%)	6.5
Bowling	1 (0%)	1 (0%)	3
Boxing	4 (1%)	0 (0%)	8.7
Cycling	169 (60%)	81 (29%)	6.8/7.5/8.5/10
Dancing	2 (1%)	0 (0%)	5
Diving	2 (1%)	0 (0%)	7
Fitness/health club/strength	54 (19%)	13 (5%)	3.5/4.5/5.5/7.3/7.8
Golf/cricket	12 (4%)	6 (2%)	4.8
Gymnastics	13 (5%)	1 (0%)	3.8/6
Handball	8 (3%)	2 (1%)	8
Hockey	36 (13%)	11 (4%)	7.8

Horse riding	6 (2%)	2 (1%)	5.5
Judo/Karate/Jujutsu	20 (7%)	1 (0%)	5.3
Korfball	7 (3%)	2 (1%)	6.5
Motor cross	5 (2%)	0 (0%)	4
Mountain climbing	2 (1%)	1 (0%)	6.6
Paragliding	2 (1%)	0 (0%)	1.8
Rowing	21 (7%)	4 (1%)	9.2
Rugby	4 (1%)	2 (1%)	6.3/8.3
Running	158 (56%)	72 (25%)	6/7/9.3/11.8
Sailing/windsurfing	8 (3%)	3 (1%)	3/4.5/5
Shooting sport	1 (0%)	1 (0%)	4.3
(Water) Skiing/snowboard	9 (3%)	1 (0%)	5.3/6
Soccer	109 (38%)	29 (10%)	7
(Inline) Speed skating	35 (12%)	1 (0%)	7.5/9
Squash	8 (3%)	1 (0%)	9.7
Swimming	28 (10%)	3 (1%)	8/10
Table tennis	10 (4%)	2 (1%)	4

Tennis	78 (28%)	10 (4%)	5/7.3
Triathlon	12 (4%)	5 (2%)	9.8
Ultimate Frisbee	2 (1%)	0 (0%)	8
Volleyball	22 (8%)	3 (1%)	4/6
Walking	6 (2%)	2 (1%)	4.3
Water polo	17 (6%)	10 (4%)	10

MET: Metabolic Equivalent of Task; *MET-score can differ based on level of competition and specific type of sport (e.g. cycling can be racing/spinning/touring/etc.)

Supplemental Table 2. Participant characteristics of the total study population as well as athletes with and without coronary artery calcification.

	Total population (n=284)	Without CAC (n=134)	With CAC (n=150)	P-value
Participant Characteristics				
Age, years	55.0 (6.5)	53.2 (5.7)	56.6 (6.8)	<.001*
Systolic BP, mmHg	129 (13)	127 (13)	131 (13)	.003*
Diastolic BP, mmHg	80 (8)	79 (8)	81 (8)	.033*
Height, cm	182 (7)	183 (7)	182 (6)	.35
Weight, kg	83 (10)	82 (10)	83 (10)	.66
BMI, kg/m^2	24.9 (2.7)	24.7 (2.6)	25.1 (2.8)	.22
BSA, m^2	2.04 (.14)	2.04 (.14)	2.04 (.14)	.98
Exercise tolerance, Watt	313 (47)	313 (45)	313 (50)	.96
Total cholesterol, mmol/l	5.4 (0.9)	5.2 (0.9)	5.5 (0.9)	.004*
Statin, n (%)	15 (5%)	1 (1%)	14 (9%)	.001*
Current smokers, n (%)	14 (5%)	8 (6%)	6 (4%)	.44
Former smoker, n (%)	108 (38%)	42 (31%)	66 (44%)	.03*
Never smoker, n (%)	162 (57%)	84 (63%)	78 (52%)	.07
Pack years of smoking, n	0 [0-8]	0 [0-7]	0 [0-8]	.21
Antihypertensive, n (%)	20 (7%)	6 (5%)	14 (9%)	.11
Diabetes, n (%)	4 (1%)	2 (2%)	2 (1%)	.91
Family history of CHD, n (%)	89 (31%)	34 (25%)	55 (37%)	.041*

CAC Characteristics				
CAC, Agatston Units	.9 [0-42.1]	-	35.8 [9.3-145.8]	-
Area, mm2	.9 [0-15.0]	-	13.0 [4.5-44.1]	-
Density, au	2.8 [1.8-3.4]	-	2.8 [1.8-3.4]	-
Regions of interest, n	1 [1-4]	-	3 [1-10]	-
Lifelong Exercise Characteristics				
Years of exercise training†, n	36 [27-42]	35 [23-39]	37 [31-45]	<.001*
Sessions/week, n	1.9 [1.3-2.8]	1.8 [1.2-2.6]	2.0 [1.5-3.0]	.019*
Duration/session, hrs	1.5 [1.2-1.8]	1.5 [1.2-1.8]	1.5 [1.3-1.8]	.20
Exercise duration/week, hrs	2.9 [1.9-4.4]	2.6 [1.7-3.8]	3.1 [2.1-4.7]	.012*
MET-min/week, au	1356 [851-2030]	1225 [749-1782]	1528 [997-2248]	.003*
MET-hours/week, au	22.6 [14.2-33.8]	20.4 [12.48-29.7]	25.5 [16.6-37.5]	.003*
Light intensity, (%)	0 [0-0]	0 [0-0]	0 [0-0]	.18
Moderate intensity, (%)	4 [0-27]	4 [0-24]	6 [0-31]	.58
Vigorous intensity, (%)	59 [23-87]	66 [27-90]	55 [20-82]	.19
Very vigorous intensity, %	16 [0-53]	13 [0-51]	19 [0-54]	.39
Lifetime MET-hours, au	50290 [32431-76462]	41526 [26716-65149]	56259 [34682-89679]	<.001*

Au, arbitrary units; BMI, body mass index; BP, Blood pressure; BSA, body surface area; CAC, coronary artery calcification; CHD, coronary heart disease; MET, Metabolic Equivalent of Task. Data is presented as mean (SD), n (%) or median [interquartile range]. *=P-value <.05. †= since age 12.

Supplemental Table 3. A comparison of participant and coronary artery calcification (CAC) characteristics across exercise volume groups in participants with CAC>0.

	Lifelong exercise volume (MET-min/week)			
	<1000 (n=38)	**1000-2000 (n=61)**	**>2000 (n=51)**	**P-value**
CAC, Agatston Units	69.6 [13.8-331.5]	24.1 [6.5-85.5]	39.2 [8.4-159.0]	.20
Area, mm2	22.0 [7.8-99.3]	10.5 [3.7-30.0]	12.4 [4.1-53.0]	.21
Density, au	3.0 [1.9-3.5]	2.6 [1.6-3.2]	3.0 [2.0-3.4]	.25
Regions of interest, n	5 [2-16]	3 [1-7]	4 [2-10]	.20
Location of CAC, n (%)				
Left anterior descending	34 (90%)	53 (87%)	43 (84%)	.78
Ramus circumflexus	13 (34%)	23 (38%)	20 (39%)	.89
Right coronary artery	17 (45%)	22 (36%)	21 (41%)	.68
Proximal segments*	32 (84%)	49 (80%)	39 (77%)	.66

Au, arbitrary units; CAC, coronary artery calcification; Data is presented as median [interquartile range] or n (%). * = proximal segments defined as segments 1,5,6 and 11 [1].

Supplemental Table 4. A comparison of plaque characteristics across exercise volume groups in participants with computed tomography coronary angiography evidence of atherosclerotic plaques.

	Lifelong exercise volume (MET-min/week)			
	<1000 (n=49)	**1000-2000 (n=75)**	**>2000 (n=58)**	**P-value**
Presence of plaques				
Calcified, n (%)	31 (63%)	44 (59%)	41 (71%)	.36
Non-Calcified, n (%)	18 (37%)	25 (33%)	16 (28%)	.59
Mixed (both), n (%)	34 (69%)	53 (71%)	28 (48%)	.017*
Mixed < 130 HU, n (%)	21 (43%)	25 (33%)	12 (21%)	.046*
Mixed >130 HU, n (%)	20 (41%)	37 (49%)	23 (40%)	.47
Dominant plaque types				
ONLY Calcified, n (%)	8 (16%)	10 (13%)	22 (38%)	.002*
ONLY Non-Calcified, n (%)	3 (6%)	6 (8%)	4 (7%)	.92
ONLY Mixed (both), n (%)	9 (18%)	22 (29%)	10 (17%)	.18
ONLY Mixed >130 HU, n (%)	3 (6%)	10 (13%)	5 (9%)	.39
ONLY Mixed <130 HU, n (%)	5 (10%)	7 (9%)	3 (5%)	.58
Location of plaques				
Left anterior descending, n (%)	47 (96%)	67 (89%)	49 (85%)	.16
Ramus circumflexus, n (%)	18 (37%)	26 (35%)	24 (41%)	.73

Right coronary artery, n (%)	24 (49%)	29 (39%)	27 (47%)	.47
Proximal segments[†] , n (%)	43 (88%)	64 (85%)	45 (78%)	.32

HU = Hounsfield Units; [*]=P-value <.05. [†]= = proximal segments defined as segments 1,5,6 and 11[1].