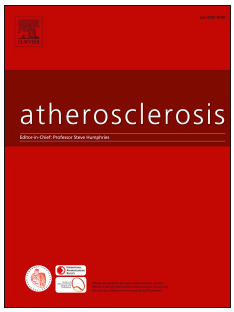

$CD34^+$ progenitors are predictive of mortality and are associated with physical activity in cardiovascular disease patients

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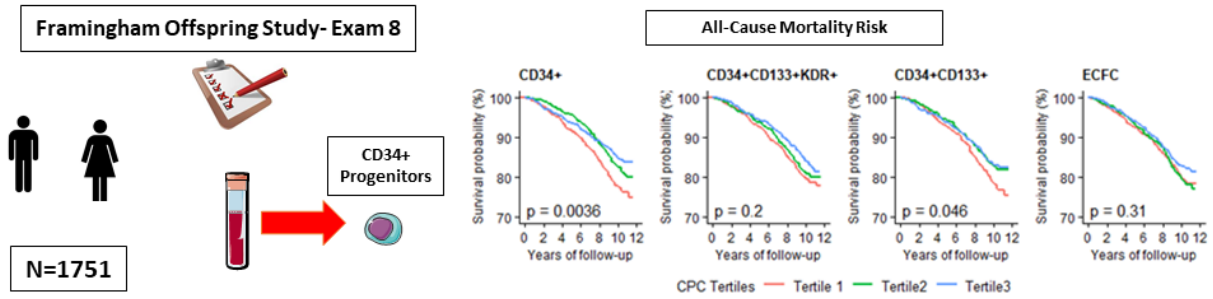
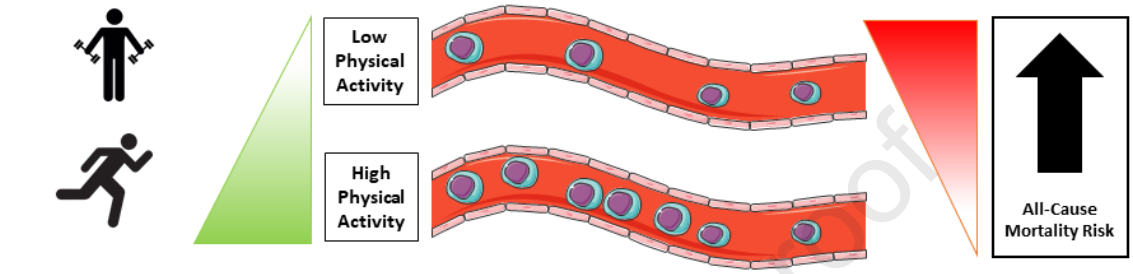
Credit Author Statement

M.R conceived and designed the research. D.M, M.R and J.D undertook statistical analysis of the data. M.R and D.M interpreted results of the experiments, prepared figures and drafted the manuscript; all authors edited and revised the manuscript; all authors approved the final version of the manuscript.





CD34+ progenitors are predictive of mortality and are associated with physical activity in cardiovascular disease patients

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Abstract

Background and aims: Circulating progenitor cells (CPCs) play an important role in vascular repair and can influence cardiovascular (CV) health and longevity. Exercise is known to modulate these cells via mobilization from the bone marrow. The primary aims of this study were to evaluate the association of CPCs with mortality and explore the association between physical activity (PA) and CPCs.

Methods: 1,751 individuals from the Framingham Offspring cohort (66 ± 9 years [40-92 years], 54% female) were included in the study. CPCs ($CD34^+$, $CD34^+CD133^+$, $CD34^+CD133^+KDR^+$) were measured by flow cytometry. Multivariable Cox regression analyses were performed to investigate relationship of CPCs with future CV event and mortality. Multivariate regression analyses were performed to determine the relationship between self-reported PA and CPC counts.

Results: Following adjustment for standard risk factors, there was an inverse association between $CD34^+$ CPCs and all-cause mortality (hazard ratio (HR) per unit increase in $CD34^+$, 0.79; 95% CI 0.64 – 0.98, p=0.036). $CD34^+CD133^+$ CPCs were inversely associated with CV mortality (HR 0.63, 95% CI 0.44 – 0.91, p=0.013). Associations of $CD34^+$ and $CD34^+CD133^+$ with mortality were strongest in participants with pre-existing CVD. PA was associated with $CD34^+$ CPCs only in CVD participants (PA Index: β=0.176, p=0.003; moderate-to-vigorous [MVPA]: β=0.159, p=0.007). This relationship was maintained after adjustment for confounding variables.

Conclusions: A higher number of $CD34^+$ and $CD34^+$ $CD133^+$ CPCs was inversely associated with all-cause and CV mortality. These associations were strongest in

participants with CVD. PA is independently associated with $CD34^+$ CPCs in individuals with CVD only, suggestive of greater benefit for this population group.

1. Introduction

Circulating progenitor cells (CPC) are a heterogenous group of cells which have tissue regenerative potential. A number of studies have shown that $CD34^+$ CPCs and several subsets of $CD34^+$ cells (such as $CD34^+CD133^+/KDR^+$) can participate in vascular repair and growth [1–3], and may be associated with vascular endothelial function [4,5]. Therefore, these cells may reflect vascular integrity and have been used as biomarkers of vascular repair [6]. $CD34^+$ CPCs are a diverse group of progenitors, consisting of both hematopoietic and non-hematopoietic CPCs [7], with CD133 and KDR often used as more definitive antigen markers for endothelial progenitor cells (EPC) [8].

A low number of these CPCs is associated with vascular dysfunction [4,9] and subsequent greater cardiovascular (CV) risk [10,11]. Observational studies have shown that individuals with cardiovascular disease (CVD) exhibit lower number and angiogenic function of these CPCs [12], reflecting reduced vascular repair capacity. Studies have demonstrated that in individuals hospitalized with heart failure [13], or with acute coronary syndromes [14], low number of $CD34^+$ CPCs predicts earlier mortality in these patients compared to patients with high numbers of $CD34^+$ CPCs, which suggests impaired vascular repair capacity in those with higher mortality risk. Whilst there are no studies that have investigated the role of $CD34^+$ CPCs and associated subsets in predicting clinical endpoints in a heterogeneous human population, there is evidence to suggest that these CPCs are reflective of subclinical atherosclerotic risk in an apparently healthy population [12].

Lifestyle behaviors can significantly affect CV health. Smoking [15], physical inactivity [16] and obesity [17] are associated with perturbed vascular health, leading to greater risk of mortality. Physical activity, known for its effect on improving vascular function [18,19] may do so in part via modulating CPC content and/or function. Studies investigating acute [20–23] and chronic exercise training [24,25] have demonstrated that progenitor cells can be mobilized into peripheral blood compartment in humans, where they can exert their vaso-reparative functions. However, the efficacy of exercise training to promote progenitor cell number has been argued, with recent evidence demonstrating little or no change in CPC number in humans after exercise training [6]. As yet, there is no evidence from large cohorts investigating the association between physical activity and CPCs, with subsequent patient subgroup (CVD *vs* CVD-free) analysis to determine if physical activity is more strongly associated with CPCs in either population.

The primary aim of this study was to investigate the prognostic potential of $CD34^+$ CPCs on all-cause and CV mortality, with the secondary aim to investigate the relationship between self-reported physical activity on CPCs in a large cohort. It was hypothesized that circulating $CD34^+$ CPCs and subpopulations would predict mortality, and that these cells are associated with self-reported physical activity levels.

2. Materials and methods

2.1 Study sample

The Framingham Heart Study (FHS) is a longitudinal community-based cohort set up in 1948 under the direction of the National Heart, Lung, and Blood Institute (NHLBI) aimed to determine factors that contribute to the onset and progression of cardiovascular disease (CVD) [26]. Subsequently, an Offspring cohort was included from 1971 [27]. Participants (n=3,002) in the Framingham Offspring cohort who attended the 8th examination cycle (2004-2008) were eligible for our retrospective investigation, with n=1,751 included in the study due to availability of key data (circulating progenitor cells, self-report physical activity levels, follow-up data; see **Figure 1**). Participant characteristics are shown in **Table 1**.

This study complies with the Declaration of Helsinki. Ethical approval for all data collection and research purposes was granted by Boston University Medical Centre, and written informed consent was obtained for the collection and use of the data available for secondary investigators. Edinburgh Napier University Research Ethics and Integrity Committee approved the use of the secondary dataset for the purposes of the study.

2.2 Clinical assessment

All participants underwent a clinical and risk factor assessment including assessment of blood pressure, height and body mass. Fasting blood samples were drawn for

quantification of glucose, glycated hemoglobin (HbA1c), total cholesterol, and high density lipoprotein cholesterol (HDL-C), and triglycerides.

2.3 Quantification of circulating progenitor cells

Blood samples were collected from participants in the fasted state to quantify CPC counts. Blood samples were centrifuged and the peripheral blood mononuclear cells (PBMCs) were isolated for cell phenotyping as previously described [28]. PBMCs were stained with anti-CD34 FITC, anti-CD133 APC and anti-KDR-PE antibodies (all BD Biosciences). $CD34^+$ cells were gated for subsequent expression of CD133 and finally KDR. Total progenitor cells are defined as $CD34^+$ cells, and EPCs are defined as $CD34^+CD133^+$ and $CD34^+CD133^+KDR^+$ cells. Analysis of flow cytometry files were performed using FlowJo analysis software (Treeestar, Inc.) and reviewed by investigators blinded to the identity of the participants.

2.4 Endothelial cell colony forming cells (ECFC)

In 1653 participants, PBMCs were also used to assess endothelial cell colony forming cells (ECFC). PBMCs were cultured on fibronectin-coated tissue culture plates (BD Biosciences) and cultured for 7 days. After 7 days of culture, the number of colonies in each well was counted by a single blinded individual. ECFC number was reported as average number of colonies per well up to 12 wells.

2.5 Mortality and event incidence

Follow up (average: 9 ± 2 years; total: 15,587 person follow-up years) was conducted for primary end points of all-cause and CV death. Cause of death was determined through medical history, review of medical records, death certificate, interview of next of kin, and review of the National Death Index. CV death was defined as death attributed to ischemic cause (fatal myocardial infarction, stroke). CV event risk was only assessed in individuals with no pre-existing CVD or CV event occurring before exam 8 (n = 1467). CV event or incident CVD was assessed using the standard Framingham Heart Study criteria and included the following: new-onset angina, fatal and non-fatal MI or stroke, heart failure or intermittent claudication.

2.6 Self-reported physical activity levels

Self-reported sleep, sitting time, light, moderate and heavy activity were determined using a physical activity questionnaire employed by the Framingham Heart Study. The number of hours of certain activity per week was collected. A composite score was calculated (physical activity index; PAI), for each participant by weighting a 24 h activity recall. Participants were asked to report the number of hours in a typical day spent sleeping (weighting factor [WF] = 1) and in sedentary (WF = 1.1), slight (WF = 1.5), moderate (WF = 2.4), and heavy activities (WF = 5) [29]. PAI was subsequently calculated by adding the products of the hours spent at each activity domain and their weighting factor based on the oxygen requirements for said activity [30].

2.7 Statistical analysis

Continuous variables were assessed for normality by assessing histograms and Q-Q plots. Data for $CD34^+$, $CD34^+CD133^+KDR^+$, $CD34^+CD133^+$ and PAI were natural log transformed and EFCFs were square root transformed. Appropriate data transformations were applied when relevant prior to further statistical analysis. Participants with missing data were excluded and thus complete-case analyses were performed. All participants were categorized into tertiles for each CPC measure for event and mortality risk analyses using Kaplan-Meier curve and log-rank analyses. Subsequent Cox proportional hazards regression analyses were performed, utilizing transformed continuous data for CPC. Cox proportional hazards regressions were performed unadjusted and adjusted for age, sex, BMI, PAI, CVD and diabetes status, smoking status. To investigate the effects of CVD status, the data set was split and analyses repeated for those free of CVD at exam 8 (n = 1467) and those with a CVD diagnosis prior to exam 8 (n = 284). Proportional hazards assumptions for each of the Cox models were evaluated by plots of Schoenfeld residuals.

To assess the influence of physical activity on CPC counts, linear regression analyses were performed to assess the relationship between CPC counts and PAI. A subset of physical activity, moderate + heavy activity time, was also investigated. Unadjusted and adjusted analyses are displayed. Data analyses were carried out using RStudio Team (2019, RStudio: Integrated Development for R. RStudio, Inc, Boston, MA: http://www.rstudio.com/). *p*-values of <0.05 were considered statistically significant.

3. Results

3.1 Relationship between CPC Counts and adverse events

3.1.1 All-cause mortality

Kaplan Meier curves based on tertiles of CPC counts and all-cause mortality are shown in **Figure 2 A-D**. In unadjusted Cox proportional hazard models, increases in $CD34^+$ and $CD34^+CD133^+$ CPCs were significantly associated with a decreased risk of death ($p<0.001$, $p=0.001$; **Table 2**). Following adjustment, increases in $CD34^+$ remained significantly associated with a decreased risk of death ($p=0.036$). Whilst there was a trend for $CD34^+CD133^+$ on all-cause mortality, this did not reach statistical significance ($p=0.07$). No significant associations were observed for all-cause mortality for $CD34^+CD133^+KDR^+$ EPCs or ECFC (all $p>0.05$; **Table 2**).

3.1.2 Cardiovascular mortality

Kaplan Meier curves based on tertiles of CPC counts and CV mortality are shown in **Figure 2 E-H**. In unadjusted Cox proportional hazard models, increases in $CD34^+$ and $CD34^+CD133^+$ CPCs were significantly associated with a decreased risk of CV death ($p=0.008$, $p=0.006$; **Table 2**). Following adjustment, $CD34^+CD133^+$ CPCs were significantly associated with a decreased risk of CV death ($p=0.013$). Whilst there was a trend for $CD34^+$ on CVD mortality, this did not reach statistical significance ($p=0.055$). No other significant associations were observed for CVD mortality (all $p>0.05$; **Table 2**).

3.2 Relationship between CPC counts and adverse events- influence of CVD status

3.2.1 All-cause mortality

Kaplan Meier curves based on tertiles of CPC counts and all-cause mortality for those free of CVD and those with CVD at exam 8 are shown in **Supplementary Figure 1**. Unadjusted and adjusted Cox proportional hazard models for CPC counts are displayed in **Table 3**. Following adjustment, increases in $CD34^+$ and $CD34^+CD133^+$ CPCs were significantly associated with a decreased risk of death in those with CVD at exam 8 (p=0.032, p =0.003). No other significant associations were observed for all-cause mortality (all p>0.05; **Table 3**).

3.2.2 Cardiovascular mortality

Kaplan Meier curves based on tertiles of CPC counts and CV mortality for those free of CVD and those with CVD at exam 8 are shown in **Supplementary Figure 1.** Unadjusted and adjusted Cox proportional hazard models for CPC counts and CV mortality are displayed in **Table 3.** In unadjusted and adjusted Cox proportional hazard models, increases in $CD34^+$ and $CD34^+CD133^+$ CPCs were significantly associated with a decreased risk of CV mortality in the CVD present at exam 8 group (all p<0.05, **Table 3**). No other significant associations were observed for CV mortality in either of the sub-groups (all p>0.05).

3.2.3 Cardiovascular events

Cox proportional hazard analysis was performed in the population free of CVD for incidence of future CV events. ECFCs were significantly associated with a decreased

risk of future CV events (p=0.046, **Supplementary Table 1**). There was no association between CPC counts and CV event risk for all other measures (all p>0.05).

3.3 Association of physical activity with CPC counts

To assess the association between physical activity and CPC counts, both unadjusted and adjusted linear regressions were performed. In unadjusted and adjusted analyses, PAI and moderate + heavy activity hours were not associated with any CPC subset or with ECFC units. However, in the CVD group, after adjusting for confounders, both PAI and moderate + heavy activity time were positively associated with $CD34^+$ CPCs and were the only significant predictors of the number of these cells (**Table 4** and **Supplementary Table 2**). Physical activity was not associated with $CD34^+CD133^+$, $CD34^+CD133^+KDR^+$ CPCs or ECFC counts, both in univariate and multivariate analyses. Light activity time was not significantly associated with $CD34^+$, $CD34^+CD133^+$, $CD34^+CD133^+KDR^+$ or ECFC counts (all p>0.05).

4. Discussion

Our main findings were that $CD34^+$ and $CD34^+CD133^+$ CPCs were significant predictors of all-cause and CV mortality in the Framingham Offspring cohort, driven primarily by the strength of this association in individuals with CVD. Additionally, increase in self-reported physical activity is positively associated with higher $CD34^+$ CPCs in our CVD cohort after adjustment for confounders, a relationship not evident in our CVD-free cohort. Together, these findings suggest that the observed protection

of increased $CD34^+$ CPCs on mortality in a diseased population is partly driven by the physical activity levels of individuals.

Several small studies have investigated the prognostic potential of CPCs as biomarkers of vascular repair for predicting incident risk of all-cause and/or CV death. These studies have demonstrated that these cells can predict mortality or clinical end-points in several disease populations, for example patients with coronary artery disease [31], acute coronary syndromes [14], heart failure [13], or type 2 diabetes [32]. Our data support these observations, with $CD34^+$ and $CD34^+CD133^+$ CPCs predictive of all-cause and CV mortality. Interestingly, this association was absent in the CVD-free population and driven mainly by a strong association with mortality in individuals with pre-diagnosed CVD, suggestive that the prognostic potential of these cells is much stronger in disease populations, and offers little predictive potential, if any, in apparently healthy populations. Interestingly, $CD34^+CD133^+KDR$ putative EPCs and ECFCs showed no predictive ability for all-cause or CV death in our study.

In the largest study investigating the role of CPCs on incident risk prediction in a CVD cohort, Patel and colleagues [31] observed that, like our study, only $CD34^+$ and $CD34^+CD133^+$ CPCs were predictive of mortality. In 2 cohorts, each over 400 patients (n=905 pooled), Patel et al. [31] showed that increases in both these progenitor subsets showed a significant inverse association with all-cause and CV-mortality, and that $CD34^+CD133^+KDR^+$ cells, like our data, showed no association with mortality, with Werner et al. [33] also demonstrating little prognostic potential for KDR^+ EPCs on all-cause death, MI and stroke, however, they did show a

significant association with CV mortality, which was defined as death from acute MI, CAD, or congestive heart failure. It is likely that differences in definition of CV death between these studies may explain the different findings. Our data in >1700 individuals, however, specifically shows that the associations of $CD34^+$ and $CD34^+CD133^+$ with all-cause and CV mortality are driven by their prognostic strength in individuals with CVD, and not those who are CVD-free. It is likely that these cells play a more important role in CVD when the vascular system is in a state of constant damage, and that a lower number of these cells in these patients reflects exhaustion of the progenitor cell pool. Interestingly, our data indicated that ECFC numbers were predictive of future CV event incidence in CVD-free participants, potentially emphasizing the possibly more sensitive cell culture measures of vascular repair as opposed to flow cytometric measures. However, ECFCs showed no other association with all-cause or CV mortality in either population group.

Both $CD34^+$ and $CD133^+$ progenitor cells have vascular regenerative capabilities [2,34–36]. These cells, reported initially to have pro-angiogenic capabilities due to the potential to differentiate into endothelial cells [3], most probably work in a paracrine manner, through secretion of vasoactive and proangiogenic factors, such as VEGF and other pro-angiogenic cytokines [36]. Due to their potential vasculo-reparative capacities, clinical studies have been undertaken to assess their efficacy as cellular therapies to promote recovery of blood flow in myocardial infarction and stroke studies. Clinical studies showing implantation or injection of these cell types show promise in repair of damaged myocardium in animal models [1] and in some human studies [37,38], however, due to the expense and research and development required

to optimize this cellular therapy, other non-pharmaceutical interventions may be more effective in promoting endogenous vascular repair for clinical benefit.

In addition, given the reduced number [39,40] of CPCs in individuals with CVD, and the predictive association with mortality [31], it is pertinent to find therapies to augment production, mobilization and function of these progenitor cells. Exercise and physical activity have the potential to mobilize $CD34^+$ cells into the circulation as evident from acute exercise studies showing transient increases in CPCs in both healthy [20,21,41,42]and diseased populations [43], although the response to acute exercise is somewhat diminished in CVD patients [44]. Long-term physical exercise and physical activity show promise in increasing number and/or function of these CPCs [45–47], potentially through promoting bone marrow production of progenitor cell subsets (although the origin of EPCs has been a topic of debate recently [48]) or via reducing inflammatory or pro-apoptotic stimuli in the circulation [49], thus enhancing survival of these cells in our body. Our data support the use of physical activity to promote or maintain $CD34^+$ CPC number in humans. High levels of self-reported physical activity were associated with reduced risk of all-cause mortality (**Supplementary Tables 3 and 4**), and they were associated with a higher number of $CD34^+$ CPCs, which were also associated with mortality, but only in individuals with CVD, and not in our CVD-free group.

Together these findings suggest that the observed protection of increased $CD34^+$ CPCs on mortality in a diseased population is partly driven by the physical activity levels of individuals. These findings may be clinically relevant as they are supportive of exercise-based cardiac rehabilitation and suggest an area for future interventions.

Whilst both acute aerobic and resistance exercise can promote progenitor cell release and improve pro-angiogenic function, long-term resistance exercise training studies are lacking and thus warranted, specifically in a CVD cohort.

4.1 Limitations

The participants in this study self-reported physical activity levels, and thus, to confirm our findings, studies that include accelerometer-derived physical activity levels are required. This will allow researchers to more accurately assess the influence of light, moderate and strenuous activity, as well as inactivity, on measures of vascular repair and regeneration, key to maintenance of CV health. Additionally, repeated longitudinal measures of physical activity, CPCs and other clinical markers would provide more robust evidence for the relationship between physical activity and these markers of vascular repair. It must be noted that these findings are associative, and do not necessarily imply causality, however, there are several studies demonstrating the positive impact of exercise and physical activity on CPCs [45–47]. Another consideration is the quantification of rare cells by flow cytometry. $CD34^+$ CPCs are typically between 0.001 and 0.01% of total circulating mononuclear cells [50], meaning accurate quantification can be problematic [51]. This limitation is compounded when investigating subpopulations, including $CD34^+KDR^+$ CPCs, which are even fewer in number, therefore the predictive strength of these cells is reduced, despite evidence showing their positive impact on the vasculature.

4.2 Conclusions

Our study demonstrated that $CD34^+$ and $CD34+CD133^+$ CPCs are predictive of mortality in a large cohort, but with more prognostic potential in individuals with

399 CVD. For the first time, we have provided data that shows physical activity is
400 associated with significantly greater $CD34^+$ CPCs in a CVD population, with no
401 relationship in a non-CVD population. Exercise and physical activity may promote
402 vascular health and longevity in CVD patients via modulating $CD34^+$ CPC number.
403

404 **Declaration of competing interests**

405 The authors declare that they have no known competing financial interests or personal
406 relationships that could have appeared to influence the work reported in this paper.
407

408 **Authors contributions**

409 M.R conceived and designed the research. D.M, M.R and J.D undertook statistical
410 analysis of the data. M.R and D.M interpreted results of the experiments, prepared
411 figures and drafted the manuscript; all authors edited and revised the manuscript; all
412 authors approved the final version of the manuscript.
413

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Figure legends

Figure 1. Flow chart of the Framingham Offspring Cohort and the participants included in this study.

MI- myocardial infarction, HF- heart failure, IC- intermittent claudication.

Figure 2. Kaplan Meier survival curve's for the relationship between CPC tertile group ($CD34^+$ n=1751, $CD34^+CD133^+$ n=1630, $CD34^+CD133^+KDR^+$ n=1751, ECFC n=1649) and all-cause mortality (A-D) and cardiovascular mortality (E-H) (Tertile 1 = Low count, Tertile 2 = Moderate count, Tertile 3 = High count).

Statistical significance was set at p <0.05 derived from Cox proportional hazard regressions.

715

716 **Tables**

717 **Table 1.** Participant characteristics

	All (n=1751)	CVD-free (n=1467, 84%)	CVD (n=284, 16%)	*p*-value
Age (years)	66 ± 9 [40-92]	65 ± 9 [40-90]	72 ± 9 [51-92]	<0.001
Female (n, %)	940, 53.7%	822, 56%	118, 41.5%	-
BMI ($kg \cdot m^2$)	28.4 ± 5.3 [13.8-54.2]	28.2 ± 5.3 [13.8-54.2]	29.2 ± 5.2 [18.4-45.1]	0.007
Systolic blood Pressure (mmHg)	129 ± 18 [76-204]	129 ± 18 [76-204]	131 ± 19 [90-198]	0.070
Diastolic blood Pressure (mmHg)	74 ± 11 [34-122]	75 ± 10 [34-122]	70 ± 11 [40-108]	<0.001***
Fasting glucose (mg/dL)	107.0 ± 24.1 [36-327]	106 ± 23 [58-327]	113 ± 28 [36-292]	<0.001***
Total cholesterol (mg/dL)	186.0 ± 37.5 [71-322]	190 ± 35.6 [96-322]	164 ± 39.4 [71-289]	<0.001***
HDL-cholesterol (mg/dL)	57.4 ± 18.2 [21-147]	58.8 ± 18.3 [21-152]	50.3 ± 15.8 [23-130]	<0.001***
Triglycerides (mg/dL)	118.0 ± 70.6 [30-976]	115 ± 67.2 [30-976]	133 ± 84.4 [40-583]	<0.001
Smokers (n, %)	206, 11.8%	170, 12%	36, 13%	
Hypertensive (n, %)	989, 56.5%	831, 57%	158, 56%	-
CD34+ CPCs (% MNCs)	0.0873 ± 0.0492 [0.011-0.490]	0.0875 ± 0.0483 [0.0110-0.490]	0.0860 ± 0.0538 [0.0160-0.370]	0.201
CD34+CD133+ CPCs (% MNCs)	0.0402 ± 0.0366 [0.0020-0.6090]	0.0407 ± 0.0380 [0.0020-0.6090]	0.0375 ± 0.0286 [0.0040-0.2420]	0.290
	(n=1630, 55% Female)	(n=1356, 58% Female)	(n=274, 41% Female)	

CD34+CD133+KDR+ (% MNCs)	0.0040 ± 0.0037 [0.0001-0.0470]	0.0040 ± 0.0037 [0.0001-0.0470]	0.0041 ± 0.0039 [0.0002-0.0261]	0.648
ECFC (number of colonies)	43 ± 31 [0-196]	43 ± 31 [0-196]	42 ± 32 [0-178]	0.470
	(n=1649, 53% female)	(n=1387, 56% female)	(n=262, 41% female)	

718 Data are mean ± SD [range]. $CD34^{+}CD133^{+}$ (in 1630 participants).

719 ECFC- endothelial colony forming cells (in 1649 participants). * p <0.05, *** p

720 <0.001, independent samples T-test.

721

722 **Table 2.** CPC Counts and mortality risk

CPC subset	Outcome	Model	No. of events/ No. at risk	HR	HR 95% CI	*p* value
CD34$^+$ CPCs	All-cause mortality	Unadjusted	326/1751	0.64	0.52 - 0.79	<0.001***
		Adjusted[a]	326/1751	0.79	0.64 - 0.98	0.036*
	CVD mortality	Unadjusted	71/1751	0.54	0.35 - 0.85	0.008**
		Adjusted[a]	71/1751	0.64	0.41 - 1.01	0.055
CD34$^+$CD133$^+$KDR$^+$ EPCs	All-cause mortality	Unadjusted	326/1751	0.88	0.76 - 1.01	0.066
		Adjusted[a]	326/1751	0.93	0.80 - 1.07	0.300
	CVD mortality	Unadjusted	71/1751	1.02	0.76 - 1.38	0.888
		Adjusted[a]	71/1751	1.09	0.81 - 1.47	0.579
CD34$^+$CD133$^+$ CPCs	All-cause mortality	Unadjusted	303/1630	0.76	0.65 - 0.90	0.001**
		Adjusted[a]	303/1630	0.86	0.72 - 1.01	0.07
	CVD mortality	Unadjusted	65/1630	0.61	0.43 - 0.87	0.006**
		Adjusted[a]	65/1630	0.63	0.44 - 0.91	0.013*
ECFC	All-cause mortality	Unadjusted	321/1649	0.96	0.92 – 1.04	0.11
		Adjusted[a]	321/1649	0.98	0.94 – 1.03	0.393
	CVD mortality	Unadjusted	71/1649	0.97	0.88 – 1.07	0.505
		Adjusted[a]	71/1649	0.99	0.90 – 1.10	0.893

723 HR – hazard ratio, CI – confidence intervals.

724 [a]Model adjusted for age, sex, BMI, PAI, smoking status, diabetes status, hypertension

725 and previous CVD diagnosis.

726 *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ derived from Cox proportional hazard regressions

Table 3. CPC Counts and risk of death for all participants split by CVD diagnosis at exam 8

CPC Subset	Outcome	Model	CVD free at exam 8				CVD diagnosis by exam 8			
			No. of events/ No. at risk	HR	HR 95% CI	*P* value	No. of events/ No. at risk	HR	HR 95% CI	*P* value
CD34$^+$ CPCs	All-cause mortality	Unadjusted	211/1467	0.70	0.54 - 0.91	0.008**	115/284	0.57	0.41 - 0.81	0.002**
		Adjusted[a]	211/1467	0.89	0.67 - 1.18	0.424	115/284	0.68	0.48 - 0.97	0.032*
	CVD mortality	Unadjusted	34/1467	0.65	0.34 - 1.26	0.203	37/284	0.49	0.26 - 0.91	0.023*
		Adjusted[a]	34/1467	0.84	0.41 - 1.70	0.619	37/284	0.53	0.28 - 0.99	0.048*
CD34$^+$CD133$^+$KDR$^+$	All-cause mortality	Unadjusted	211/1467	0.90	0.76 - 1.08	0.258	115/284	0.85	0.68 - 1.06	0.149
EPCs		Adjusted[a]	211/1467	0.92	0.76 - 1.11	0.385	115/284	0.94	0.75 - 1.18	0.586
	CVD mortality	Unadjusted	34/1467	0.86	0.56 - 1.33	0.492	37/284	1.17	0.79 - 1.72	0.442
		Adjusted[a]	34/1467	0.80	0.49 - 1.28	0.350	37/284	1.28	0.86 - 1.90	0.229
CD34$^+$CD133$^+$ CPCs	All-cause mortality	Unadjusted	196/1364	0.85	0.69 - 1.04	0.11	107/266	0.61	0.46 - 0.82	0.001**
		Adjusted[a]	196/1364	0.99	0.80 - 1.23	0.943	107/266	0.64	0.48 - 0.86	0.003**
	CVD mortality	Unadjusted	31/1364	0.78	0.47 - 1.30	0.344	34/266	0.45	0.27 - 0.76	0.003**
		Adjusted[a]	31/1364	0.86	0.50 - 1.49	0.588	34/266	0.42	0.24 - 0.72	0.002**
ECFC	All-cause mortality	Unadjusted	205/1391	0.96	0.91 – 1.02	0.179	116/262	0.99	0.92 – 1.07	0.782
		Adjusted[a]	205/1391	0.97	0.92 – 1.02	0.261	116/262	1.01	0.93 – 1.09	0.828
	CVD mortality	Unadjusted	34/1391	1.03	0.90 – 1.19	0.655	37/262	0.93	0.82 – 1.07	0.317
		Adjusted[a]	34/1391	1.03	0.90 – 1.19	0.633	37/262	0.95	0.82 – 1.09	0.437

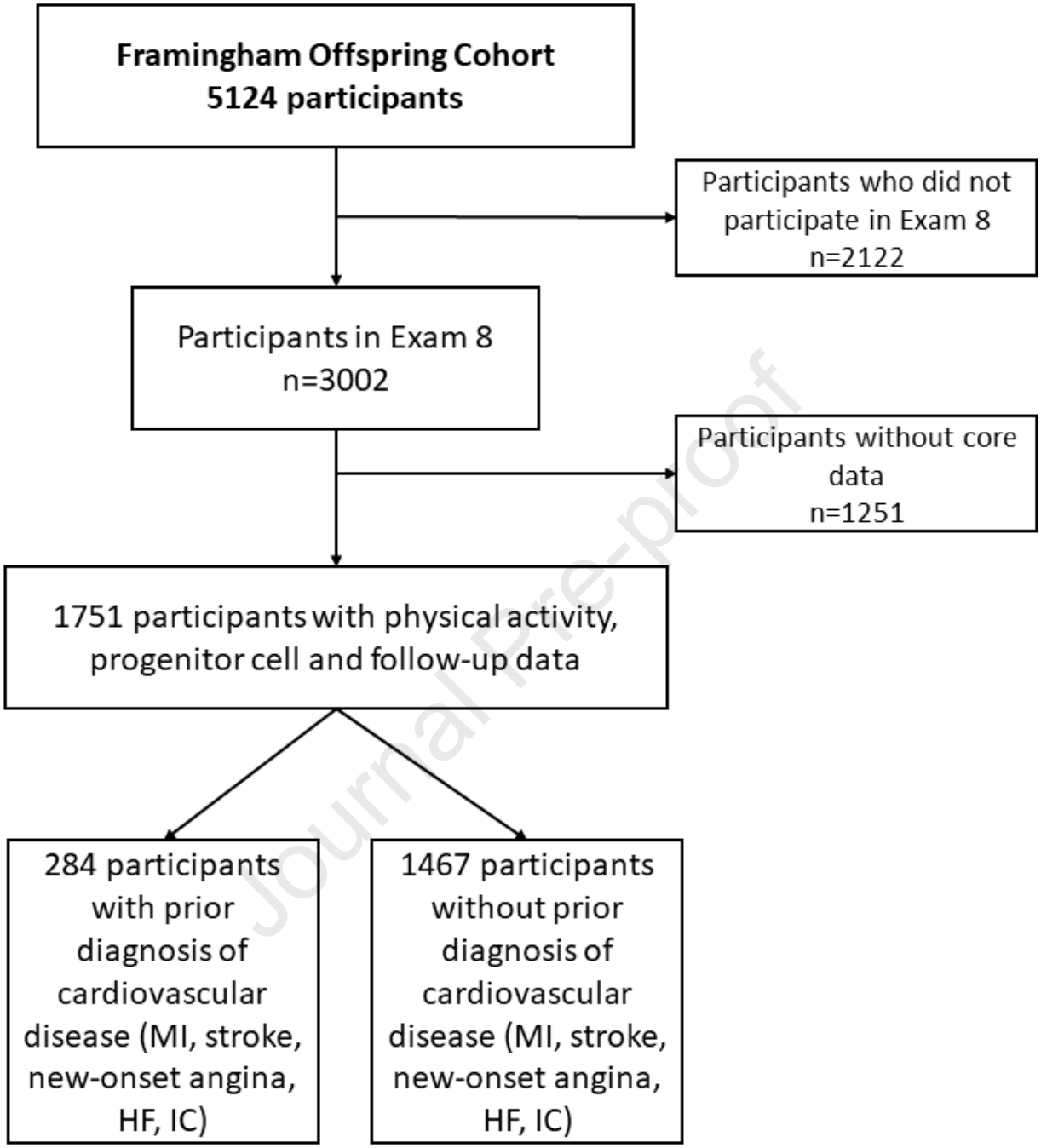
HR- hazard ratio, CI- confidence intervals.

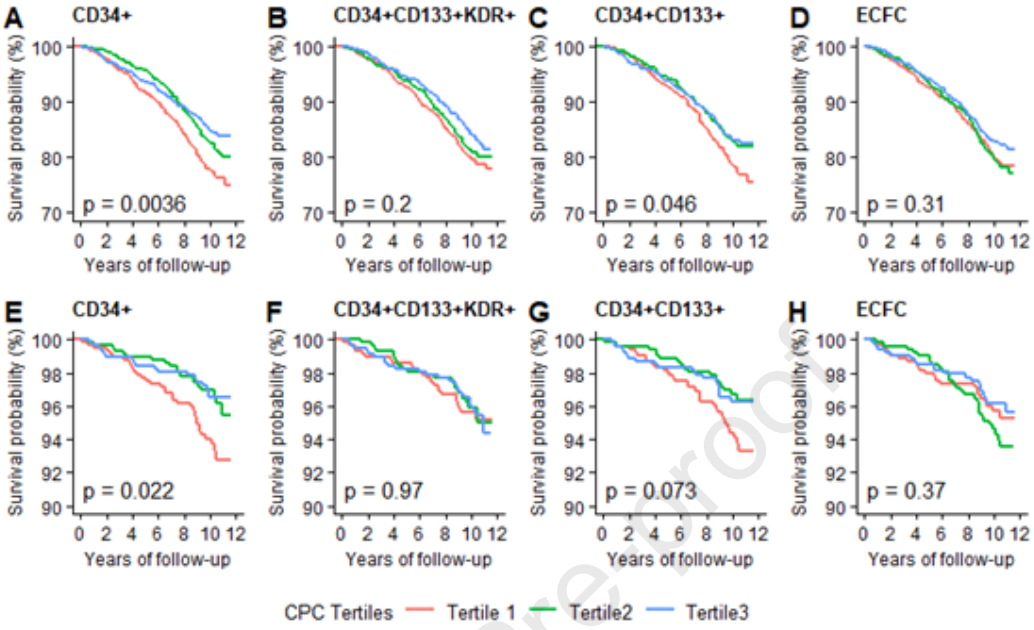
[a]Model adjusted for age, sex, BMI, PAI, smoking status, diabetes status, hypertension. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ derived from Cox proportional hazard regressions.

731 **Table 4.** Association between physical activity index and CPC Counts

CPC subset	Outcome	All	CVD-free	CVD
		ß, *T*-value, *p*-value	ß, *T*-value, *p*-value	ß, *T*-value, *p*-value
CD34$^+$	Unadjusted	0.023, 0.967, 0.334	-0.013, -0.486, 0.627	0.176, 3.009, 0.003**
	Adjusted[a]	0.008, 0.322, 0.748	-0.021, -0.813, 0.416	0.153, 2.461, 0.014*
CD34$^+$CD133$^+$	Unadjusted	-0.014, -0.567, 0.571	-0.04, -1.495, 0.135	0.115, 1.893, 0.059
	Adjusted[a]	-0.022, -0.872, 0.383	-0.042, -1.574, 0.116	0.107, 1.616, 0.107
CD34$^+$CD133$^+$KDR$^+$	Unadjusted	0.022, 0.901, 0.368	0.002, 0.086, 0.932	0.108, 1.817, 0.07
	Adjusted[a]	0.019, 0.775, 0.439	0.005, 0.194, 0.846	0.080, 1.269, 0.205
ECFC	Unadjusted	0.005, 0.197, 0.844	-0.005, -0.201, 0.841	0.049, 0.785, 0.433
	Adjusted[a]	-0.006, -0.241, 0.809	-0.015, -0.535, 0.592	0.036, 0.537, 0.592

732 [a]Model adjusted for age, sex, BMI, smoking status, diabetes status and hypertension.
733 For "All" adjustment also includes previous CVD diagnosis
734 ECFC- endothelial colony forming cells.
735 *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ derived from Cox proportional hazard
736 regressions.





CD34+ progenitors are predictive of mortality and are associated with physical activity in cardiovascular disease patients

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Highlights:

- Circulating CD34+ progenitor cells are biomarkers of endothelial regenerative capacity
- Lower levels of these cells are predictive of cardiovascular and all-cause mortality, specifically in those with underlying or pre-diagnosed cardiovascular disease
- Self-reported physical activity is positively associated with these CD34+ progenitor cells independent of other known risk factors, but only in individuals with pre-existing cardiovascular disease which may help to explain the role of physical activity in reducing future event risk

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: