It's about time: implications of chronoactivity on health and disease

Albalak, G.

3

Setting your clock: associations between timing of objective physical activity and cardiovascular disease risk in the general population.

Gali Albalak, Marjon Stijntjes, David van Bodegom, J. Wouter Jukema, Douwe E. Atsma, Diana van Heemst, Raymond Noordam

European Journal of Preventive Cardiology. *Feb 14 2023; 30(3):232-240. doi:10.1093/eurjpc/zwac239*

Abstract

Aims

Little is known about the impact of daily physical activity timing (here referred to as "chronoactivity") on cardiovascular disease (CVD) risk. We aimed to examined the associations between chronoactivity and multiple CVD outcomes in the UK Biobank.

Methods

Objective physical activity data was collected in the UK Biobank through triaxial accelerometer over a 7-day measurement period. We used K-means clustering to create clusters of participants with similar chronoactivity irrespective of the mean daily intensity of the physical activity. Multivariable-adjusted Cox proportional hazard models were used to estimate hazard ratios (HRs) comparing the different clusters adjusted for age and sex (model 1), and baseline cardiovascular risk factors (model 2). Additional stratified analyses were done by sex, mean activity level, and self-reported sleep chronotype.

Results

We included 86,657 individuals (58% female, mean age: 61.6 [SD: 7.8] years, mean BMI: 26.6 [4.5] kg/m^2). Over a follow-up period of 6 years, 3,707 incident CVD events were reported. Overall, participants with a tendency of late morning physical activity had a lower risk of incident coronary artery disease (CAD) (HR: 0.84, 95%CI: 0.77, 0.92) and stroke (HR: 0.83, 95%CI: 0.70, 0.98) compared to participants with a midday pattern of physical activity. These associations were more pronounced in women (p-value for interaction = 0.001). We did not find evidence favouring effect modification by total activity level and sleep chronotype.

Conclusion

Irrespective of total physical activity, morning physical activity was associated with lower risks of incident cardiovascular diseases, highlighting the potential importance of chronoactivity in cardiovascular disease prevention.

Introduction

Cardiovascular disease (CVD) is the most common cause of death worldwide.(1, 2) Prevalence of CVD is projected to rise even further as a result of, amongst others, physical inactivity, obesity, diabetes mellitus, hypertension, high cholesterol and lipid levels and numbers of individuals reaching old age.(1, 3, 4) Physical activity is an important stepping stone to healthy ageing and -in theory- easily adjustable for most individuals.(5, 6) In contrast, according to the World Heart Federation, physical inactivity, which is becoming increasingly prevalent in our sedentary society, is the fourth leading risk factor for mortality.(6) Moreover, sufficient physical activity can reduce cardiovascular mortality risk and improve quality of life.(3, 7) Large population-based studies have consistently shown an inverse association between increased physical activity and risk of CVD.(5, 8-12) For this reason, current guidelines and interventions aim to reduce sedentary behaviour and increase daily physical activity.

Current guidelines recommend 150-300 minutes of moderate intensity physical activity (defined as 3-6 times the intensity of rest or >1.5 metabolic equivalents) or 75-150 minutes of vigorous physical activity (defined as 6 or more times the intensity of rest or >1.5 metabolic equivalents) per week for adults and older adults to maintain and improve cardiorespiratory fitness and overall health.(13) In addition to the intensity and duration of habitual physical activity, there is increasing evidence including from mouse studies suggesting that the timing of physical activity during the day or "chronoactivity" is an independent determinant for cardiovascular disease risk.(14, 15) Recent studies in humans underlined a possible influence of the timing of physical activity and other behavioural factors (e.g. food intake and light exposure) on weight control, cardiometabolic, and cardiovascular health.(4, 14, 16-22) This new insight is the result of evolving analysis of accelerometery data enabling the identification of patterns of physical activity, and by the increasing interest and evidence on the interaction between the circadian clock and lifestyle behaviours.(23, 24) Although the amount of evidence is limited, a small number of studies showed that cardiometabolic health is not only influenced by duration, frequency, and intensity of physical activity, but is also independently affected by timing of the behaviour.(4, 14)

Despite promising results, human studies on physical activity timing were either relatively small or based on homogeneous study populations, and yet no prospective studies have been conducted.(25) Gaining novel insights on this topic, allows tailoring of current guidelines and interventions and help identify risk profiles of physical activity timing for CVD. Therefore, this study aims to examine the association between intraday patterns of physical activity and

CVD incidence in a large study population which enables additional subgroup analyses.

Methods

Study design and population

The present study was conducted using data collected from the UK Biobank population, a large, open-access, population-based, prospective cohort study including 502,490 participants aged 40-69 years when recruited in 2006-2010. Extensive phenotypic and genotypic details about its participants were and are continuously collected across the entire United Kingdom with ongoing longitudinal follow-up for many health-related outcomes. De-identified data is available for researchers that sign a material transfer agreement, undertaking to use data only for the purposes of the approved research and not to attempt to identify any participant in order to keep personal data secure.(26, 27) The UK biobank study was approved by the North-West Multi-centre Research Ethics Committee (MREC). Access for information to invite participants was approved by the Patient Information Advisory Group (PIAG) for England and Wales. All participants in the UK Biobank provided a written informed consent. The present study was accepted under project number 81423.

In a substantial subset of 103,684 participants from UK Biobank, a second assessment round was performed after follow-up during which accelerometer data for the assessment of hourly objective physical activity levels was collected. For this sample, we excluded 3,223 (3.1%) participants because of data quality issues; participants who either had less than three measurement days (27, 28), more than eight hourly acceleration values that equalled zero, or had unrealistically high acceleration values (daily mean acceleration adjusted for non-wear time bias equal to or more than 100 mg). Finally, we excluded 13,804 (13.3%) participants with CVD history which was defined as incident CVD (according to diagnosis in the Hospital Episode Statistics (HES) before the accelerometer wear period date (ranging from July 1st 2013 to December 23rd 2015), leaving a total of 86,657 (83.6%) participants for the analysis.

Assessment of physical activity

Between February 2013 and December 2015, a group of participants who had provided a valid email address were invited to wear an accelerometer for seven consecutive days. Participant email addresses were chosen randomly and participants were sent the accelerometer after accepting the invitation.

(27) As mentioned earlier, a total of 103,684 participants were included in this accelerometer subsample. Objective physical activity was assessed using the Axivity AX3 wrist-worn triaxial accelerometer, a commercial version of the Open Movement AX3 open source sensor (https://github.com/digitalinteraction/openmovement) designed by Open Lab, Newcastle University.(27, 29) The device measured triaxial acceleration data over a seven day period at 100 Hz with a dynamic range of +-8 g. Calibration of the acceleration signals was performed according to the procedure described before (29) to ensure similar output across devices.(27) Acceleration is presented in milligravity (mg) (1 mg = 0.00981 m/s^{-2}) . Thresholds for activity intensities were: inactivity, <30 mg; light, 30-99 mg; moderate, 100-399 mg; vigorous, ≥400 mg.(30) Participants were informed to wear the accelerometer continuously on their dominant wrist and to carry out their normal daily activities.

In the present study, 24 hourly means of all measurement days were used. To group participants in clusters based on physical activity timing, we used K-means clustering analysis using the 24 hourly means. In order to assure that clusters would not be a representation of participants level of physical activity intensity, yet rather depict clusters of physical activity timing, we calculated relative acceleration by dividing each hourly mean by each individual total day acceleration mean (adjusted for non-wear time bias). Prior to the clustering analysis, a Within Sum of Squares (WSS) plot was made to determine the number of clusters that should be taken into account in the K-means analysis.

Assessment of cardiovascular disease

CVD was defined as coronary artery disease (CAD) or cerebrovascular disease according to the International Classification of Diseases (ICD) edition 10. CAD cases were defined as angina pectoris (I20), myocardial infarction (MI) (I21 and I22), and acute and chronic ischemic heart disease (IHD) (I24 and I25). Stroke cases are defined as I64, ischemic stroke as I63.9 and haemorrhagic stroke as I61.9. In the present analysis, ischemic and haemorrhagic stroke were analysed jointly (as “stroke”) and ischemic stroke was analysed as a separate disease outcome. Incidence of CVD was defined as the first hospital admission or CVD-related death identified from linkages to the national death index and HES.(26) Incidence of CAD, stroke, and ischemic stroke was monitored through linkage of the self-reported data, hospital admissions data, and data collected from the general practitioner. Participants were followed until the onset of one of these diseases, loss to follow-up, death or the end of the study (January 1, 2021), whichever came first.

Covariates

Body mass index (BMI) was constructed from height and weight measured during the initial assessment centre visit. Data on smoking status, alcohol intake frequency, cholesterol and blood pressure lowering medication and chronotype were collected by questionnaire at the moment of the study inclusion in the UK Biobank. Chronotype was assessed through a single questions that asked, "Do you consider yourself to be".(26, 31)

Statistical analyses

Descriptive statistics were computed for the total population and for each chronoactivity cluster. Characteristics of the study population were presented as mean (with standard deviation, SD) for continuous data or No. (%) for categorical data.

As a first analysis, we examined the associations between hourly mean relative physical activity (in SD units) and CVD using Cox-proportional hazard model, adjusted for age and sex. For our second analysis, based on these hourly objective physical activity levels in a population free from coronary artery disease (CAD) or stroke at baseline, a data-driven K-means clustering analysis was performed to identify clusters of individuals with a similar pattern of chronoactivity independent of the mean daily physical activity level (to prevent clusters purely reflect the intensity of the daily mean physical activity). The clusters representing participants with different timing patterns determined from the WSS plot and K-means analysis were used as independent variables in multivariable-adjusted Cox-proportional hazards regression models. The cluster representing an average relative acceleration pattern which best represented the average acceleration of the entire UK Biobank population and was the largest group, was used as reference. We used two adjustment models; model 1, in which we adjusted for age and sex, and model 2, in which we additionally adjusted for baseline BMI, smoking status (never, former, or current), Townsend deprivation index (TDI),(32) the baseline self-reported use of cholesterol lowering medication, and blood pressure lowering medication. We only added characteristics to the statistical model that can clearly confound the association between physical activity timing and incident cardiovascular disease risk. Other characteristics, like blood pressure and cholesterol levels, are more likely to be mediators rather than confounding factors. We analysed these variables in a separate model as it was unclear at this moment whether these factors were confounders or possible mediators. Moreover, we conducted stratified analysis by sex, mean daily physical activity level, and by self-reported chronotype. Participants were

divided into two activity groups; less active (mean acceleration ≤27.23 mg), or more active (mean acceleration >27.23 mg). For the chronotype stratified analysis, participants were divided into two groups; morning chronotype (participants who characterized themselves as 'definitely a morning person' or 'more a morning person than an evening person'), and evening chronotype (participants who characterized themselves as 'definitely an evening person' or 'more an evening person than a morning person').

R v4.0.3 statistical software was used to perform all statistical analyses. (33) Results are presented as the hazard ratio (HR) with accompanying 95% confidence interval (CI).

Results

Participant characteristics

Participant characteristics are shown in **Table 1**. A total of 86,657 participants were included. The mean age of the total population was 61.6 (SD: 7.8) years at the accelerometer measurement period. The majority of participants were women (57.6%). On average, participants were overweight with a mean BMI of 26.6 (SD: 4.5) kg/m^2. Most participants reported themselves as morning person (56.8%). A total of 12,489 (14.4%) participants used blood pressure lowering medication.

Table 1. Participant characteristics

	Total population
No.	86,657
Age, mean (SD), years	61.6 (7.8)
Age groups, No. (%)	
40-49	21,170 (24)
50-59	32,026 (37)
60-69	33,212 (38)
70+	253 (0.3)
Female Sex , No. (%)	49,902 (58)
BMI , mean (SD), kg/m2	26.6 (4.5)
Average acceleration, mean (SD), mg	28.2 (8.7)
Sleep chronotype, No. (%)	
Morning person	49,193 (57)
Evening person	28,381 (33)
Unknown	9,083 (11)
TDI, median (IQR)	-2.5 (-3.8, -0.3)

	Total population
Smoking status, No. (%)	
Never	50,450 (58)
Previous	30,172 (35)
Current	5,812 (7)
Alcohol intake frequency, No. (%)	
Never	4,662 (5)
< 3x per week	39,249 (45)
≥ 3x per week	42,679 (49)
Familial CVD history, No. (%)	23,186 (27)
Blood pressure, mean (SD), mmHg	
Diastolic	81.7 (10.6)
Systolic	138.3 (19.2)
Cholesterol, mean (SD), mmol/L	5.8 (1.1)
HDL	1.5 (0.4)
LDL	3.6 (0.8)
Triglycerides	1.7 (1.0)
Blood pressure medication, No. (%)	12,489 (14)
Cholesterol medication, No. (%)	9,368 (11)

BMI, body mass index; TDI, Townsend deprivation index; CVD, cardio vascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Participant characteristics of study population from the UK Biobank. Data presented as number n proportion (%); mean (SD); median (25th-75th percentile).

Hourly relative physical activity in relation to incident cardiovascular disease

During follow-up, 2,911 participants developed CAD, and 796 participants developed a stroke. Risk patterns for CAD, stroke and ischemic stroke were identified by investigating their associations with the 24 standardized hourly means of relative physical activity. For the risk of CAD (**Figure 1a)**, stroke (**Figure 1b**), and ischemic stroke (**Figure 1c**), a clear pattern is visible in which high relative physical activity during the nightly hours (12:00 PM – 6:00 AM) was associated with higher risks, and high relative physical activity during morning hours (8:00-11:00 AM) was associated with lower risks.

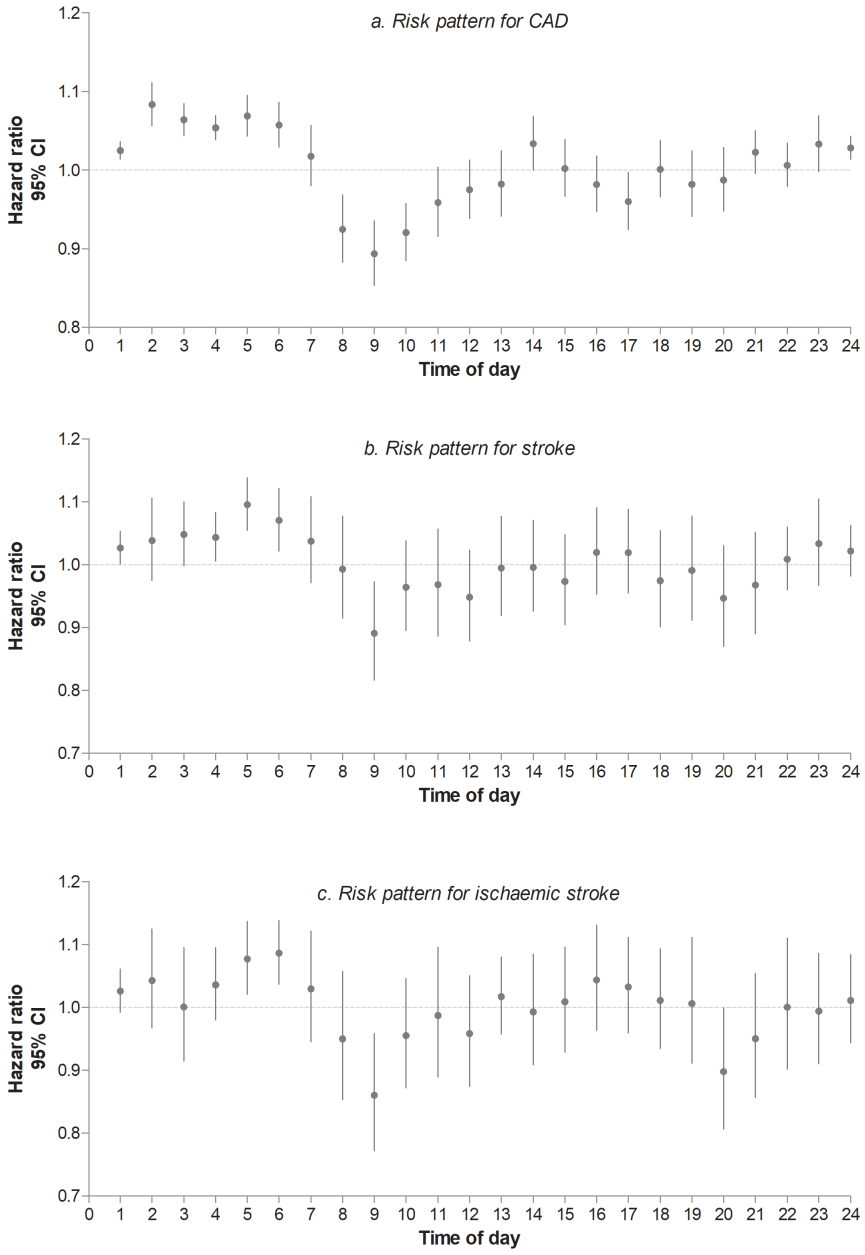


Figure 1. Hourly physical activity and cardiovascular disease risk pattern (CVD). Risk on incident CVD per mean physical activity per hour of the total study population. Adjustment model 1 was used for this analysis. A. shows the risk on incident coronary artery disease (CAD), b. shows the risk on incident stroke, and c. shows the risk on incident

ischemic stroke.

Clusters of physical activity and their characteristics

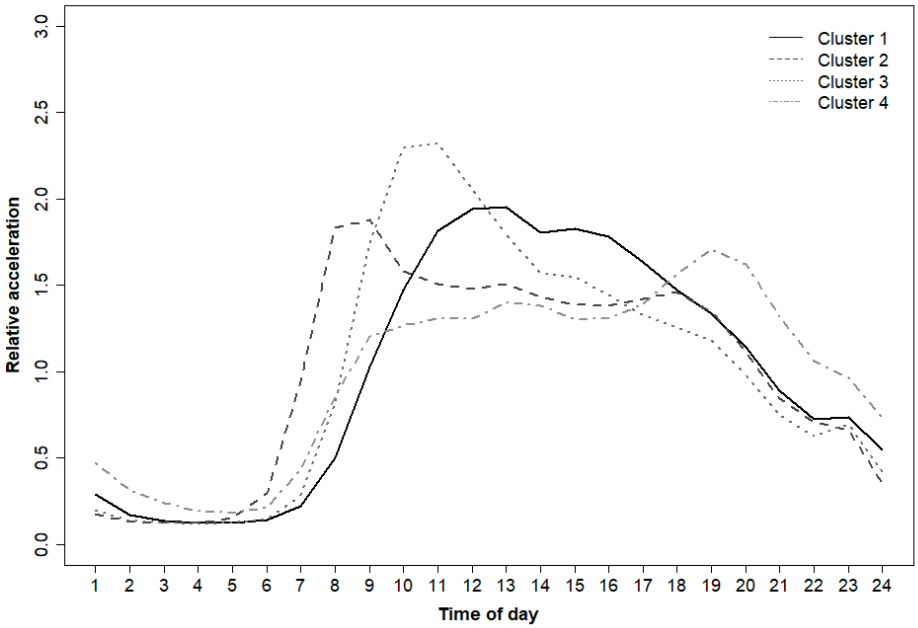


Figure 2. Pattern clusters of relative acceleration. Mean relative physical activity pattern per cluster. Relative acceleration shown in milligravity (mg). Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

Based on the WSS plot (**Supplementary figure 1**), we determined that optimal intra- and inter-cluster variance was reached with four clusters. **Figure 2** shows the chronoactivity pattern per cluster. Cluster 1, being the largest in sample size, represents an 'average pattern of physical activity' closest to the average pattern of physical activity of the entire UK Biobank acceleration subgroup (**Supplementary figure 2**). Cluster 2 represents a group of individuals with a pattern of 'early morning physical activity peak', cluster 3 represents a pattern of 'late morning physical activity peak', and cluster 4 represents a pattern of 'evening physical activity peak'. Participant characteristics per cluster are shown in **Table 2**.

Chronoactivity and cardiovascular disease

Table 2. Participant characteristics per chronoactivity cluster

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
No.	27,552	19,843	22,648	16,614
Age, mean (SD), years	63.0 (7.1)	59.2 (7.9)	64.7 (6.9)	57.7 (7.6)
Age groups, No. (%)				
40-49	4,565 (17)	6,909 (35)	2,623 (12)	7,071 (43)
50-59	10,799 (39)	7,623 (38)	7,439 (33)	6,163 (37)
60-69	12,097 (44)	5,272 (27)	12,484 (55)	3,359 (20)
70+	91 (0.3)	39 (0.2)	102 (0.5)	21 (0.1)
Female Sex , No. (%)	15,941 (58)	11,286 (57)	13,480 (60)	9,195 (55)
BMI , mean (SD), kg/m2	26.7 (4.4)	26.5 (4.6)	26.4 (4.2)	26.6 (4.8)
Average acceleration, mean (SD), mg	27.6 (8.0)	29.5 (8.7)	27.8 (8.3)	28.1 (10.0)
Sleep chronotype, No. (%)				
Morning person	13,095 (48)	14,074 (71)	14,926 (66)	7,098 (43)
Evening person	11,333 (41)	4,001 (20)	5,351 (24)	7,696 (46)
Unknown	3,124 (11)	1,756 (9)	2,371 (11)	1,819 (11)
TDI, median (IQR)	-2.6 (-3.9, -0.5)	-2.3 (-3.7, 0)	-2.8 (-4, -0.9)	-2.2 (-3.7, 0.4)
Smoking status, No. (%)				
Never	15,436 (56)	12,018 (61)	12,977 (57)	10,019 (60)
Previous	9,969 (36)	6,553 (33)	8,491 (38)	5,159 (31)
Current	2,077 (8)	1,223 (6)	1,109 (5)	1,403 (8)
Alcohol intake frequency, No. (%)				
Never	1,310 (5)	1215 (6)	1161 (5)	976 (6)
< 3x per week	14,216 (52)	9,046 (46)	11,468 (51)	7,949 (48)
≥ 3x per week	12,008 (44)	9,567 (48)	9,998 (44)	7,676 (46)
Familial CVD history, No. (%)	7,661 (28)	5,086 (26)	6,360 (28)	4,079 (24)
Blood pressure, mean (SD), mmHg				
Diastolic	81.9 (10.5)	81.5 (10.7)	82.0 (10.5)	81.0 (10.7)
Systolic	139.4 (19.1)	136.6 (18.8)	141.2 (19.5)	134.6 (18.4)
Cholesterol, mean (SD), mmol/L	5.8 (1.1)	5.7 (1.1)	5.9 (1.1)	5.7 (1.1)
HDL	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)
LDL	3.6 (0.8)	3.5 (0.8)	3.7 (0.8)	3.5 (0.8)
Triglycerides	1.4 (1, 2.1)	1.4 (1, 2)	1.4 (1, 2)	1.4 (1, 2)
Blood pressure medication, No. (%)	4,296 (16)	2,440 (12)	3,985 (18)	1,768 (11)
Cholesterol medication, No. (%)	3,349 (12)	1,723 (9)	3,016 (13)	1,280 (8)

BMI, body mass index; TDI, Townsend deprivation index; CVD, Cardio vascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Participant characteristics of study population from the UK Biobank. Characteristics show Chronoactivity cluster. Data presented as number n proportion (%); mean (SD); median (25th-75th percentile).

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

Figure 3 illustrates the associations between the clusters of physical activity timing and risk of the CVD outcome variables in the total population and provides a visual representation of the associations. After adjusting for age and sex, participants who were most active in the early morning or late morning, had a lower risk of incident CAD (HR 0.89; [95% CI 0.80, 0.99]; HR 0.84; [95% CI 0.77, 0.92], respectively) compared to the reference group (cluster 1). In addition, participants with a tendency of late morning physical activity had a 17% [HR 0.83; (95% CI 0.70, 0.98)] decreased risk of any incident stroke and a 21% decreased risk of incident ischaemic stroke [HR 0.79; (95% CI 0.64, 0.97)] compared with the reference group. The results derived from the additionally adjusted model 2 were directionally consistent, yet some of the effect sizes attenuated to some extent (**Supplementary table 1**).

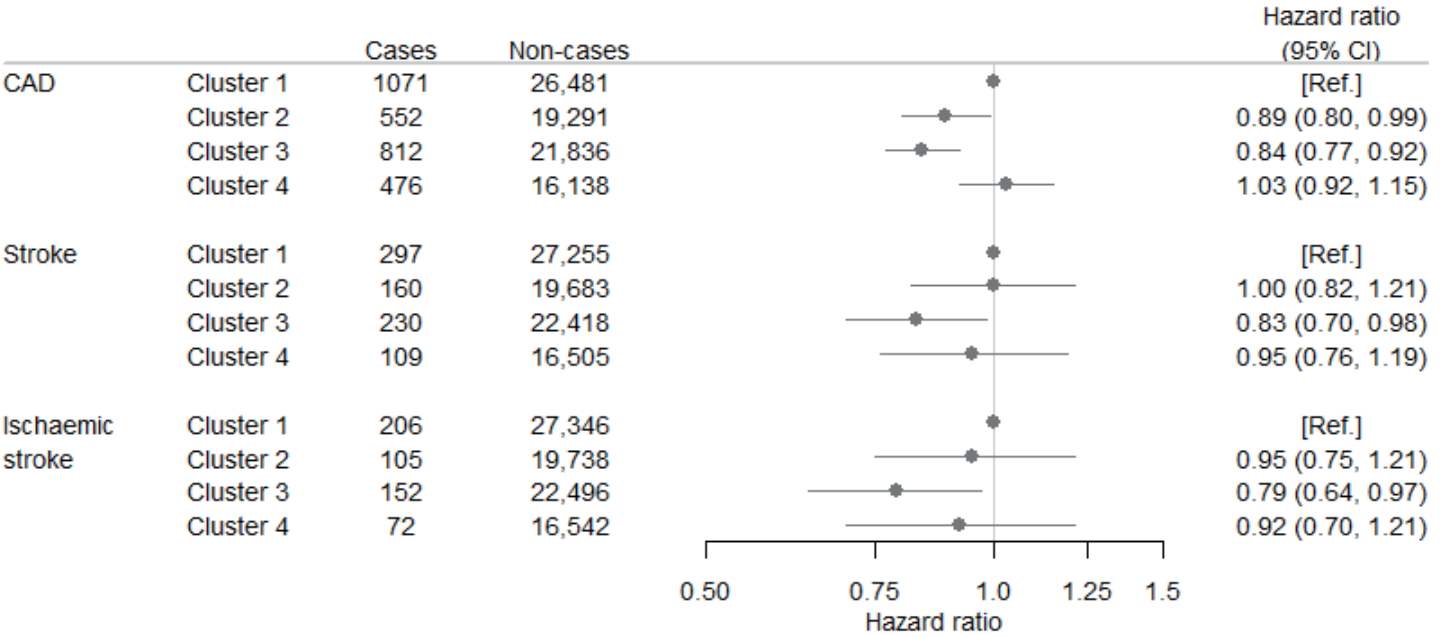


Figure 3. Associations between physical activity timing and cardiovascular disease. HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Hazard ratios for CAD, stroke, and ischemic stroke incidence for every chronoactivity cluster in the total study population. Cox- proportional hazard models were adjusted for age and sex.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

Stratified analyses

Sex

Both adjustment models showed a considerable decreased risk of CAD for women (**Supplementary figure 3** and **Supplementary table 2)** in both the early (model 1: HR 0.73; [95% CI 0.61, 0.87], model 2: HR 0.78; [95% CI 0.62, 0.97]) and late morning (model 1: HR 0.77; [95% CI 0.66, 0.89], model 2: HR 0.76; [95% CI 0.63, 0.92]) physical activity peak clusters compared to participants in the reference group. In men, we observed no difference in risk between early morning physical activity and the reference group (model 1: HR 0.99 [95% CI 0.87, 1.13], model 2: HR 1.08 [95% CI 0.92, 1.27]) for the early morning group (p-value for interaction between men and women= 0.001). We observed no evidence favouring differences in between men and women in the late morning and evening group in CAD (p-value for interaction= 0.18, 0.16, respectively).

Total physical activity

In the stratified analysis for total physical activity within each cluster (**Supplementary figure 4** and **Supplementary table 3**), the number of incident CVD cases in the less active group is considerably higher than in the more active group (2311 cases vs. 1396 cases). Less active participants who were most active in the late morning had a significantly decreased risk of CAD incident (HR 0.83; [95% CI 0.74, 0.93]) with similar results in the fully adjusted model. In the more physically active group, we observed a lower risk of CAD incident in participants with a tendency to early morning activity compared to the reference group (HR 0.82; [95% CI 0.69, 0.96]). We did not observe differences between the less- and more active groups in the association between the clusters and incident CVD (p-values for interaction>0.05; **Supplementary figure 4**).

Chronotype

Individuals with a self-reported morning chronotype who were most active in the early morning had a 14% (HR 0.86; [95% CI 0.76, 0.99]) decreased risk of CAD compared to the reference group **(Supplementary figure 5** and **Supplementary table 4)**. Additionally, individuals with a self-reported morning chronotype who were most active in the late morning, had a 16% (HR 0.84; [95% CI 0.74, 0.95]) lower risk of CAD incident compared to the reference group. Similar directions and effect sizes were noticeable for participants who were more active in the early morning. Furthermore, high physical activity in the late

morning was also associated with lower stroke incident risk (HR 0.73; [95% CI 0.58, 0.92]) in morning chronotypes. We observed a difference in risk for stroke between morning and evening chronotypes in the late morning and evening group (p-value for interaction= 0.04, 0.02 respectively).

Discussion

This prospective observational study identified several distinct physical activity timing subgroups in the general UK population, resembling participants with distinct types of chronoactivity. We found that participants who were most active in the morning, independent of their total mean daily physical activity level, had a lower risk of incident CAD and stroke.

Physical activity remains one of the most distinct cornerstones in CVD prevention.(1, 4) The present study adds to the previous evidence(4) that timing of physical activity is an additional independent contributing factor to CVD risk, and therefore adds a novel dimension to CVD risk prevention. Most notably, we observed that participants with the highest daily physical activity performed during the late morning, had a 16% decreased risk of CAD and a 17% decreased risk of stroke compared to participants who best represented the average (midday) pattern of acceleration of the UK Biobank population.

Various studies in mice and in humans were done that support the findings from our study. For example, Sato et al.(15) found that in mice, time of exercise is a critical factor to amplify the beneficial impact of exercise on metabolic pathways in skeletal muscle and systemic energy homeostasis which in turn are associated with CVD incidence.(34) Specifically, they found decreased muscle and blood glucose levels only after early active phase (i.e., in the morning) exercise.(15) In addition to this finding, Sato and colleagues also found enhanced transcription of genes involved in glycolysis in mice that exercised in the early active phase as well as a robust activation of the hypoxia-inducible factor 1α (HIF1α) pathway in skeletal muscles. This pathway contributes to activation of glycolysis rather than oxidative phosphorylation under hypoxic conditions during exercise. Next to the metabolic influence, HIF1α also coordinates circadian clock activity which makes it plausible that this pathway could be one of the underlying mechanisms of the seemingly positive effect of morning activity in humans as well.(15) In human studies, there are several studies that have shown associations between morning physical activity and better (cardio)metabolic health.(14, 22, 25, 35) For instance, an improved postprandial metabolic response, which is associated with lower CVD risk, was observed in overweight and obese men (n= 10) that performed

physical exercise in the morning.(36, 37) Additionally, middle aged to older men with diabetes mellitus type 2 (n= 2,153) who performed most moderate to vigorous physical activity in the morning were found to have the highest cardiorespiratory fitness compared with other timing groups.(4)

However, there are also a number of studies showing opposite findings. Brito et al. showed that evening exercise was associated with better heart rate recovery and decreased blood pressure than morning exercise.(38) Moreover, Savikj et al. showed that in diabetic (type II) men, afternoon exercise was more efficacious for improving blood glucose levels than morning exercise. Savikj and colleagues additionally stated that morning exercise had an acute deleterious effect on blood glucose levels.(20) This contrariety could be partly explained by 1) different study outcomes, 2) different measurement methods and definitions of physical activity, and 3) small sample sizes in which the studies have been conducted. Nevertheless, it is important to extend this new concept of chronoactivity with further research.

In the stratified analyses, we saw that the results of the total population were mostly driven by the women in the population since most associations disappeared in the stratified analyses for men. These differences might be partially explained by the sex-specific differences in disease aetiology and pathophysiology of CVD. (39) However, we were not able to specify a biological mechanism explaining these differences in the current study, as we considered this beyond the scope of the research. Furthermore, when we tested for effect modification by total mean physical activity level, we found no differences between the two groups indicating that the association between specific timing of physical activity with CVD is independent of total physical activity levels. Moreover, we did not find differences in effect between morning and evening chronotypes. In this study, evening chronotypes seem to have as much benefits from morning physical activity as morning chronotypes. Yet, studies have shown that chronotype impacts the optimal timing of physical activity. (34) We did however see higher numbers of CVD cases in the evening group which is in line with previous literature.(40) Since chronotype as well as CVD are closely linked to and strongly affected by circadian rhythm and an association would be probable, more research on this topic is recommended.

This study has several strengths including its large sample size and well-characterized cohort from UK Biobank. Moreover, physical activity was objectively collected in the UK Biobank and we used a data-driven clustering method to identify chronoactivity subgroups which is more likely to represent the natural behavioural rhythms of our participant than predefined timing periods that have been used previously. Finally, we were able to examine the

influence of chronotype on the association between chronoactivity and CVD incidence. The study also has some limitations. First, data on nutritional intake and chrononutrition (i.e., timing of nutritional intake) was not available. Since the known impact of chrononutrition on the circadian clock and the presumed interplay between timing of lifestyle behaviours,(16) it would be valuable to collect these data in future research. Second, due to the design of the UK Biobank, we were not able to retrieve the nature of the observed acceleration (e.g. leisure or work-related activity). Although having this information may lead to a better understanding of the observed association, according to the latest WHO report on physical activity, there is no effect difference between leisure of work-related activity on health.(6) Third, participants characteristics and health covariables were collected at baseline which took place some years before the accelerometer measurement period. This could likely have introduced some measurement error and misclassification of the exposure at the moment of the accelerometer assessment. Fourth, accelerometer data was only collected for seven days at baseline, and therefore does not take into account possible changes of physical activity timing during follow up. Yet, Keadle and colleagues showed high intraclass correlation for physical activity and sedentary behaviour between two 7-day accelerometer measurement periods of 2-3 years apart highlighting the reproducibility and representativeness of a 7-day measurement period.(41) Finally, some of the variables that we have corrected for in model 2, could either be mediators or confounders. Unfortunately, since these variables were collected several years before the accelerometer measurement period, we were not able to perform mediation analyses to sort this out. Therefore, they could lie in the causal pathway of the associations. Besides, given the observational nature of the study, we cannot rule out that residual confounding plays a role in our observations and we were not able to examine the causal relationship of this association. It is important to keep this in mind when interpreting the results from this study, especially those from the second adjustment model. Despite these limitations, this study adds to the rather unexplored field of research by its strengths and encourages future research on the causal relation between chronoactivity and CVD risk. When future studies are able to replicate our results as well as to demonstrate the direct health benefits of morning physical activity, societal challenges might influence the implementation of morning physical activity to benefit overall public health as previous research already demonstrated timing of physical activity already influences the participation rate in studies.(42)

In conclusion, our study showed that a greater proportion of physical activity in the morning was associated with lower CVD risk irrespective of the

average total physical activity. This study provides the first evidence from a large population-based database and presents the novel term chronoactivity as well as insights on the seemingly positive effects of morning physical activity on the risk of CVD. When being validated and extensively metabolically characterized, these present results might suggest that time-dependent physical activity interventions might be an added beneficial behavioural factor to reach maximum health benefits and to lower the risk of CVD.

References

1. WHO. About Cardiovascular Disease: World Health Organization; [cited 2021 12 April]. Available from: https://www.who.int/cardiovascular_diseases/about_cvd/en/.
2. Al-Mallah MH, Sakr S, Al-Qunaibet A. Cardiorespiratory Fitness and Cardiovascular Disease Prevention: an Update. Curr Atheroscler Rep. 2018;20(1):1.
3. Balakumar P, Maung UK, Jagadeesh G. Prevalence and prevention of cardiovascular disease and diabetes mellitus. Pharmacol Res. 2016;113(Pt A):600-9.
4. Qian J, Walkup MP, Chen SH, Brubaker PH, Bond DS, Richey PA, et al. Association of Objectively Measured Timing of Physical Activity Bouts With Cardiovascular Health in Type 2 Diabetes. Diabetes Care. 2021;44(4):1046-54.
5. Ramakrishnan R, Doherty A, Smith-Byrne K, Rahimi K, Bennett D, Woodward M, et al. Accelerometer measured physical activity and the incidence of cardiovascular disease: Evidence from the UK Biobank cohort study. PLoS Med. 2021;18(1):e1003487.
6. WHO. WHO guidelines on physical activity and sedentary behaviour: at a glance. Geneva2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/337001/9789240014886-eng.pdfdoi.
7. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. Circulation. 2021;143(8):e254-e743.
8. Sattelmair J, Pertman J, Ding EL, Kohl HW, 3rd, Haskell W, Lee IM. Dose response between physical activity and risk of coronary heart disease: a meta-analysis. Circulation. 2011;124(7):789-95.
9. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, et al. The Physical Activity Guidelines for Americans. JAMA. 2018;320(19):2020-8.
10. Shiroma EJ, Lee IM. Physical activity and cardiovascular health: lessons learned from epidemiological studies across age, gender, and race/ethnicity. Circulation. 2010;122(7):743-52.
11. Qureshi WT, Alirhayim Z, Blaha MJ, Juraschek SP, Keteyian SJ, Brawner CA, et al. Cardiorespiratory Fitness and Risk of Incident Atrial Fibrillation: Results From the Henry Ford Exercise Testing (FIT) Project. Circulation. 2015;131(21):1827-34.
12. USPSTF. Behavioral Counseling to Promote a Healthful Diet and Physical Activity for Cardiovascular Disease Prevention in Adults Without Cardiovascular Risk Factors - US Preventative Services Task Force Recommendation Statement. JAMA. 2017;318(2):167-74.
13. WHO. Global action plan on physical activity 2018-20302018.
14. Chomistek AK, Shiroma EJ, Lee IM. The Relationship Between Time of Day of Physical Activity and Obesity in Older Women. J Phys Act Health. 2016;13(4):416-8.
15. Sato S, Basse AL, Schonke M, Chen S, Samad M, Altintas A, et al. Time of Exercise Specifies the Impact on Muscle Metabolic Pathways and Systemic Energy Homeostasis. Cell Metab. 2019;30(1):92-110 e4.
16. Marinac CR, Quante M, Mariani S, Weng J, Redline S, Cespedes Feliciano EM, et al. Associations Between Timing of Meals, Physical Activity, Light Exposure, and Sleep With Body Mass Index in Free-Living Adults. J Phys Act Health. 2019;16(3):214-21.

17. de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. N Engl J Med. 2019;381(26):2541-51.

18. Willis EA, Creasy SA, Honas JJ, Melanson EL, Donnelly JE. The effects of exercise session timing on weight loss and components of energy balance: midwest exercise trial 2. Int J Obes (Lond). 2020;44(1):114-24.

19. Di Blasio A, Di Donato F, Mastrodicasa M, Fabrizio N, Di Renzo D, Napolitano G, et al. Effects of the time of day of walking on dietary behaviour, body composition and aerobic fitness in post-menopausal women. J Sports Med Phys Fitness. 2010;50(2):196-201.

20. Savikj M, Gabriel BM, Alm PS, Smith J, Caidahl K, Bjornholm M, et al. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: a randomised crossover trial. Diabetologia. 2019;62(2):233-7.

21. Ezagouri S, Zwighaft Z, Sobel J, Baillieul S, Doutreleau S, Ladeuix B, et al. Physiological and Molecular Dissection of Daily Variance in Exercise Capacity. Cell Metab. 2019;30(1):78-91 e4.

22. Albalak G, Stijntjes M, Wijsman CA, Slagboom PE, van der Ouderaa FJ, Mooijaart SP, et al. Timing of objectively-collected physical activity in relation to body weight and metabolic health in sedentary older people: a cross-sectional and prospective analysis. Int J Obes (Lond). 2021.

23. Wrobel J, Muschelli J, Leroux A. Diurnal Physical Activity Patterns across Ages in a Large UK Based Cohort: The UK Biobank Study. Sensors (Basel). 2021;21(4).

24. Gupta NJ. Lifestyle and Circadian Health: Where the Challenges Lie? Nutr Metab Insights. 2019;12:1178638819869024.

25. Schumacher LM, Thomas JG, Raynor HA, Rhodes RE, Bond DS. Consistent Morning Exercise May Be Beneficial for Individuals With Obesity. Exerc Sport Sci Rev. 2020;48(4):201-8.

26. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.

27. Doherty A, Jackson D, Hammerla N, Plotz T, Olivier P, Granat MH, et al. Large Scale Population Assessment of Physical Activity Using Wrist Worn Accelerometers: The UK Biobank Study. PLoS One. 2017;12(2):e0169649.

28. Matthews CE, Ainsworth BE, Thompson RW, Bassett DR, Jr. Sources of variance in daily physical activity levels as measured by an accelerometer. Med Sci Sports Exerc. 2002;34(8):1376-81.

29. van Hees VT, Fang Z, Langford J, Assah F, Mohammad A, da Silva IC, et al. Autocalibration of accelerometer data for free-living physical activity assessment using local gravity and temperature: an evaluation on four continents. J Appl Physiol (1985). 2014;117(7):738-44.

30. Migueles JH RA, Huber F, Sabia S, van Hees VT. GGIR: A Research Community–Driven Open Source R Package for Generating Physical Activity and Sleep Outcomes From Multi-Day Raw Accelerometer Data. Journal for Measurement of Physical Behaviour. 2019(2 (3)).

31. Knutson KL, von Schantz M. Associations between chronotype, morbidity and mortality in the UK Biobank cohort. Chronobiol Int. 2018;35(8):1045-53.

32. Foster HME, Celis-Morales CA, Nicholl BI, Petermann-Rocha F, Pell JP, Gill JMR, et al. The effect of socioeconomic deprivation on the association between an extended measurement of unhealthy lifestyle factors and health outcomes: a prospective analysis of the UK Biobank cohort. Lancet Public Health. 2018;3(12):e576-e85.

33. Team RC. R: A Language and Environment for Statistical Computing Vienna, Austria2021 [Available from: https://www.r-project.org/.

34. Hower IM, Harper SA, Buford TW. Circadian Rhythms, Exercise, and Cardiovascular Health. J Circadian Rhythms. 2018;16:7.

35. Park S, Jastremski CA, Wallace JP. Time of day for exercise on blood pressure reduction in dipping and nondipping hypertension. J Hum Hypertens. 2005;19(8):597-605.

36. Farah NM, Gill JM. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. Br J Nutr. 2013;109(12):2297-307.

37. Lefebvre PJ, Scheen AJ. The postprandial state and risk of cardiovascular disease. Diabet Med. 1998;15 Suppl 4:S63-8.

38. Brito LC, Pecanha T, Fecchio RY, Pio-Abreu A, Silva G, Mion-Junior D, et al. Comparison of morning versus evening aerobic-exercise training on heart rate recovery in treated hypertensive men: a randomized controlled trial. Blood Press Monit. 2021.

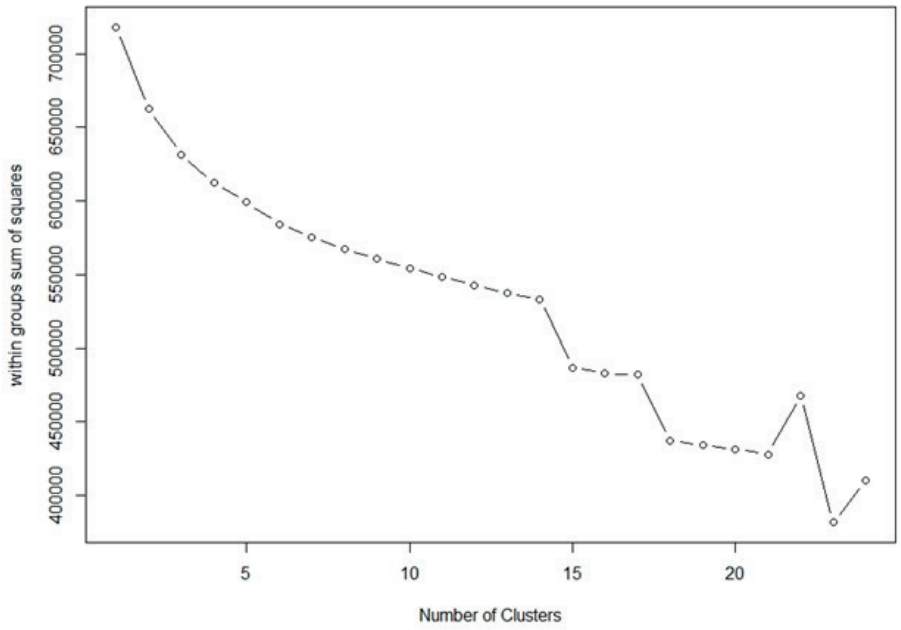
39. Gerdts E, Regitz-Zagrosek V. Sex differences in cardiometabolic disorders. Nat Med. 2019;25(11):1657-66.

40. Makarem N, Paul J, Giardina EV, Liao M, Aggarwal B. Evening chronotype is associated with poor cardiovascular health and adverse health behaviors in a diverse population of women. Chronobiol Int. 2020;37(5):673-85.

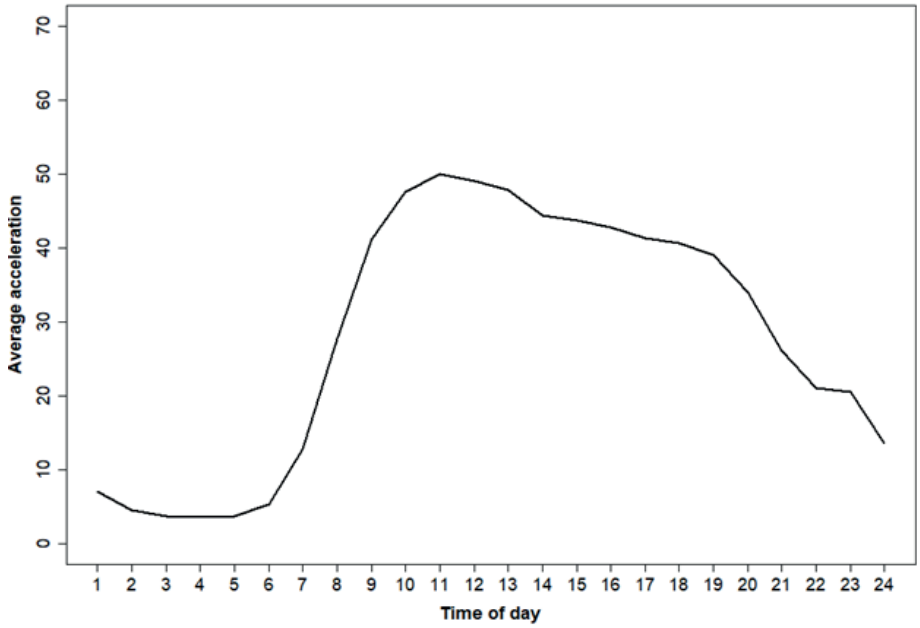
41. Keadle SK, Shiroma EJ, Kamada M, Matthews CE, Harris TB, Lee IM. Reproducibility of Accelerometer-Assessed Physical Activity and Sedentary Time. Am J Prev Med. 2017;52(4):541-8.

42. Vidal-Almela S, Czajkowski B, Prince SA, Chirico D, Way KL, Pipe AL, et al. Lessons learned from community- and home-based physical activity programs: A narrative review of factors influencing women's participation in cardiac rehabilitation. Eur J Prev Cardiol. 2020.

Appendix



Supplementary figure 1. Within Sum of Square plot. Within Sum of Squares plot to determine number of clusters for K-means clustering analysis. Finally, the number of clusters considered for clustering analysis was four.



Supplementary figure 2. Pattern of average acceleration. Pattern of mean daily acceleration of the total study group (N=86,657) shown in milligravity (mg).

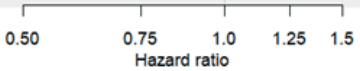
Supplementary table 1. Hazard ratios for cardiovascular disease by clusters model 2

	Cases	Non-cases	Hazard Ratio (95% CI)
CAD			
Cluster 1	1,071	26,481	[Ref.]
Cluster 2	552	19,291	0.96 (0.84, 1.09)
Cluster 3	812	21,836	0.91 (0.81, 1.02)
Cluster 4	476	16,138	1.12 (0.97, 1.28)
Stroke			
Cluster 1	297	27,255	[Ref.]
Cluster 2	160	19,683	0.96 (0.76, 1.21)
Cluster 3	230	22,418	0.81 (0.66, 1.00)
Cluster 4	109	16,505	0.94 (0.72, 1.24)
Ischaemic stroke			
Cluster 1	206	27,346	[Ref.]
Cluster 2	105	19,738	0.89 (0.67, 1.18)
Cluster 3	152	22,496	0.73 (0.57, 0.94)
Cluster 4	72	16,542	0.88 (0.63, 1.23)

HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Table shows outcomes from Cox-proportional hazard models adjusted for age, sex, BMI, smoking status, Townsend Deprivation Index, cholesterol lowering medicine and blood pressure lowering medicine (model 2). Results represent the risk of CAD, stroke and ischemic stroke compared to the reference group [Ref.] and are presented as hazard ratios with accompanying 95% confidence interval.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

		Sex	Cases	Non-cases	Hazard ratio (95% CI)	p-value for interaction
CAD	Cluster 1	Women	429	15,512	[Ref.]	
		Men	642	10,969	[Ref.]	
	Cluster 2	Women	167	11,119	0.73 (0.61, 0.87)	
		Men	385	8172	0.99 (0.87, 1.13)	0.001
	Cluster 3	Women	313	13,167	0.77 (0.66, 0.89)	
		Men	499	8669	0.89 (0.79, 1.00)	0.18
	Cluster 4	Women	163	9032	0.98 (0.82, 1.18)	
		Men	313	7106	1.06 (0.92, 1.22)	0.16
Stroke	Cluster 1	Women	131	15,810	[Ref.]	
		Men	166	11,445	[Ref.]	
	Cluster 2	Women	65	11,221	0.99 (0.73, 1.33)	
		Men	95	8462	1.01 (0.78, 1.30)	0.75
	Cluster 3	Women	99	13,381	0.77 (0.59, 1.00)	
		Men	131	9037	0.88 (0.70, 1.10)	0.55
	Cluster 4	Women	44	9151	0.96 (0.68, 1.36)	
		Men	65	7354	0.95 (0.71, 1.27)	0.79
Ischaemic stroke	Cluster 1	Women	90	15,851	[Ref.]	
		Men	116	11,495	[Ref.]	
	Cluster 2	Women	42	11,244	0.96 (0.67, 1.39)	
		Men	63	8494	0.95 (0.70, 1.30)	0.77
	Cluster 3	Women	66	13,414	0.73 (0.53, 1.01)	
		Men	86	9082	0.83 (0.63, 1.09)	0.73
	Cluster 4	Women	26	9169	0.88 (0.57, 1.37)	
		Men	46	7373	0.94 (0.67, 1.34)	0.43



Supplementary figure 3. Associations between physical activity timing and cardiovascular disease stratified by sex. HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Hazard ratios for CAD, stroke, and ischaemic stroke incidence for every chronoactivity cluster stratified for sex. Cox- proportional hazard models were adjusted for age. P-values for interaction shown, p<0.05 indicates an effect interaction between men and women

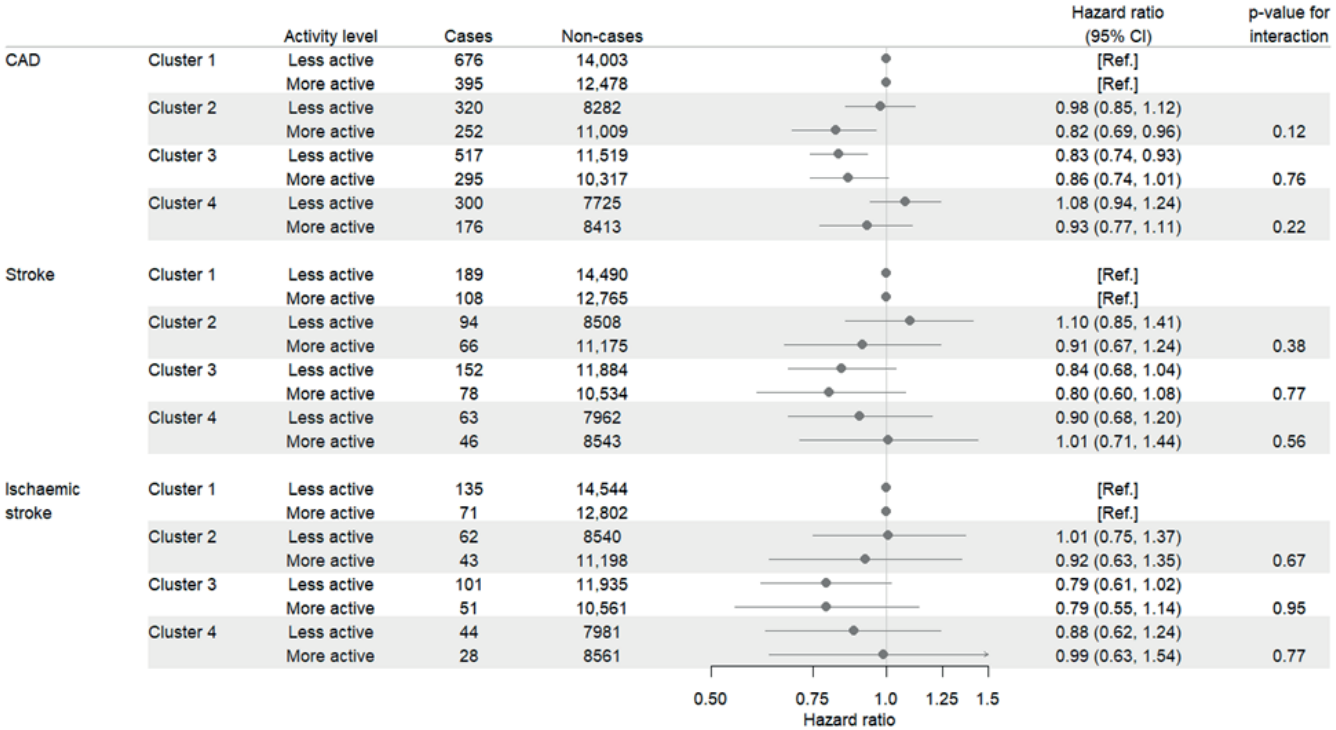
Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

Supplementary table 2. Hazard ratios for cardiovascular disease by clusters, stratified analysis for sex

	Group	Cases	Non-cases	Model 2 HR (95% CI)
CAD				
Cluster 1	Women	429	15,512	[Ref.]
	Men	642	10,969	[Ref.]
Cluster 2	Women	167	11,119	0.78 (0.62, 0.97)
	Men	385	8,172	1.08 (0.92, 1.27)
Cluster 3	Women	313	13,167	0.76 (0.63, 0.92)
	Men	499	8,669	1.01 (0.88, 1.17)
Cluster 4	Women	163	9,032	1.03 (0.81, 1.29)
	Men	313	7,106	1.18 (0.99, 1.40)
Stroke				
Cluster 1	Women	131	15,810	[Ref.]
	Men	166	11,445	[Ref.]
Cluster 2	Women	65	11,221	0.85 (0.59, 1.22)
	Men	95	8,462	1.06 (0.78, 1.45)
Cluster 3	Women	99	13,381	0.65 (0.47, 0.89)
	Men	131	9,037	0.97 (0.73, 1.28)
Cluster 4	Women	44	9,151	0.90 (0.59, 1.36)
	Men	65	7,354	0.99 (0.69, 1.42)
Ischaemic stroke				
Cluster 1	Women	90	15,851	[Ref.]
	Men	116	11,495	[Ref.]
Cluster 2	Women	42	11,244	0.73 (0.42, 1.24)
	Men	63	8,494	0.96 (0.65, 1.41)
Cluster 3	Women	66	13,414	0.56 (0.38, 0.83)
	Men	86	9,082	0.90 (0.64, 1.26)
Cluster 4	Women	26	9,169	0.82 (0.53, 1.27)
	Men	46	7,373	1.01 (0.66, 1.54)

HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Table shows outcomes from Cox-proportional hazard models adjusted for age, sex, BMI, smoking status, Townsend Deprivation Index, cholesterol lowering medicine and blood pressure lowering medicine (model 2) from stratified analyses for sex. Results represent the risk of CAD, stroke and ischemic stroke compared to the reference group [Ref.] and are presented as hazard ratios with accompanying 95% confidence interval.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.



Supplementary figure 4. Associations between physical activity timing and cardiovascular disease stratified by total physical activity. HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Hazard ratios for CAD, stroke, and ischaemic stroke incidence for every chronoactivity cluster stratified for mean activity level. Cox- proportional hazard models were adjusted for age and sex. P-values for interaction shown, $p<0.05$ indicates an effect interaction between the less- and more active group.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

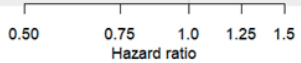
Supplementary table 3. Hazard ratios for cardiovascular disease by clusters , stratified analysis for activity level

	Group	Cases	Non-cases	Model 2 HR (95% CI)
CAD				
Cluster 1	Less active	676	14,003	[Ref.]
	More active	395	12,478	[Ref.]
Cluster 2	Less active	320	8,282	0.99 (0.84, 1.17)
	More active	232	11,009	0.93 (0.76, 1.14)
Cluster 3	Less active	517	11,519	0.89 (0.77, 1.03)
	More active	295	10,317	0.95 (0.78, 1.14)
Cluster 4	Less active	300	7,725	1.16 (0.98, 1.38)
	More active	176	8,413	1.04 (0.83, 1.31)
Stroke				
Cluster 1	Less active	189	14,490	[Ref.]
	More active	108	12,765	[Ref.]
Cluster 2	Less active	94	8,508	0.98 (0.72, 1.34)
	More active	66	11,175	0.94 (0.65, 1.35)
Cluster 3	Less active	152	11,884	0.81 (0.63, 1.06)
	More active	78	10,534	0.81 (0.57, 1.15)
Cluster 4	Less active	63	7,962	0.92 (0.65, 1.30)
	More active	46	8,543	0.97 (0.63, 1.51)
Ischaemic stroke				
Cluster 1	Less active	135	14,544	[Ref.]
	More active	71	12,802	[Ref.]
Cluster 2	Less active	62	8,540	0.84 (0.57, 1.23)
	More active	43	11,198	0.98 (0.63, 1.54)
Cluster 3	Less active	101	11,935	0.71 (0.52, 0.97)
	More active	51	10,561	0.80 (0.52, 1.22)
Cluster 4	Less active	44	7,981	0.81 (0.53, 1.23)
	More active	28	8,561	1.02 (0.59, 1.74)

HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Table shows outcomes from Cox-proportional hazard models adjusted for age, sex, BMI, smoking status, Townsend Deprivation Index, cholesterol lowering medicine and blood pressure lowering medicine (model 2) from stratified analyses for total physical activity level. Results represent the risk of CAD, stroke and ischemic stroke compared to the reference group [Ref.] and are presented as hazard ratios with accompanying 95% confidence interval.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

		Chronotype	Cases	Non-cases	Hazard ratio (95% CI)	p-value for interaction
CAD	Cluster 1	Morning	517	12,578	[Ref.]	
		Evening	432	10,901	[Ref.]	
	Cluster 2	Morning	393	13,681	0.86 (0.76, 0.99)	
		Evening	101	3900	0.93 (0.75, 1.16)	0.31
	Cluster 3	Morning	537	14,389	0.84 (0.74, 0.95)	
		Evening	193	5158	0.86 (0.73, 1.02)	0.80
	Cluster 4	Morning	206	6892	1.01 (0.86, 1.19)	
		Evening	215	7481	1.05 (0.88, 1.24)	0.73
Stroke	Cluster 1	Morning	156	12,939	[Ref.]	
		Evening	119	11,214	[Ref.]	
	Cluster 2	Morning	124	13,950	0.96 (0.76, 1.21)	
		Evening	25	3976	0.95 (0.61, 1.47)	0.61
	Cluster 3	Morning	147	14,779	0.73 (0.58, 0.92)	
		Evening	56	5295	0.88 (0.64, 1.21)	0.04
	Cluster 4	Morning	56	7042	1.02 (0.75, 1.38)	
		Evening	37	7659	0.73 (0.50, 1.07)	0.02
Ischaemic stroke	Cluster 1	Morning	105	12,990	[Ref.]	
		Evening	85	11,248	[Ref.]	
	Cluster 2	Morning	81	13,993	0.94 (0.70, 1.26)	
		Evening	19	3982	1.02 (0.62, 1.70)	0.64
	Cluster 3	Morning	101	14,825	0.74 (0.56, 0.98)	
		Evening	33	5318	0.73 (0.48, 1.09)	0.11
	Cluster 4	Morning	37	7061	1.02 (0.70, 1.49)	
		Evening	24	7672	0.68 (0.43, 1.07)	0.06



Supplementary fi*gure 5*. Associations between physical activity timing and cardiovascular disease stratified by chronotype. HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Hazard ratios for CAD, stroke, and ischaemic stroke incidence for every chrono-activity cluster stratified for chronotype. Cox- proportional hazard models were adjusted for age and sex. P-values for interaction shown, p<0.05 indicates an effect interaction between morning and evening chronotypes

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.

Supplementary table 4. Hazard ratios for cardiovascular disease by clusters, stratified analysis for sleep chronotype

	Group	Cases	Non-cases	Model 2 HR (95% CI)
CAD				
Cluster 1	Morning	517	12,578	[Ref.]
	Evening	432	10,901	[Ref.]
Cluster 2	Morning	393	13,681	1.00 (0.84, 1.18)
	Evening	101	3,900	0.94 (0.71, 1.24)
Cluster 3	Morning	537	14,389	0.98 (0.84, 1.14)
	Evening	193	5,158	0.91 (0.74, 1.11)
Cluster 4	Morning	206	6,892	1.17 (0.96, 1.44)
	Evening	215	7,481	1.06 (0.86, 1.31)
Stroke				
Cluster 1	Morning	156	12,939	[Ref.]
	Evening	119	11,214	[Ref.]
Cluster 2	Morning	124	13,950	0.92 (0.69, 1.23)
	Evening	25	3976	0.88 (0.52, 1.50)
Cluster 3	Morning	147	14,779	0.74 (0.56, 0.98)
	Evening	56	5,295	0.72 (0.48, 1.07)
Cluster 4	Morning	56	7,042	1.13 (0.78, 1.62)
	Evening	37	7,659	0.59 (0.36, 0.95)
Ischaemic stroke				
Cluster 1	Morning	105	12,990	[Ref.]
	Evening	85	11,248	[Ref.]
Cluster 2	Morning	81	13,993	0.92 (0.64, 1.32)
	Evening	19	3,982	0.91 (0.50, 1.67)
Cluster 3	Morning	101	14,825	0.75 (0.54, 1.05)
	Evening	33	5,318	0.53 (0.32, 0.89)
Cluster 4	Morning	37	7,061	1.13 (0.72, 1.78)
	Evening	24	7,672	0.48 (0.26, 0.89)

HR, Hazard Ratio; CAD, coronary artery disease; CI, confidence interval. Table shows outcomes from Cox-proportional hazard models adjusted for age, sex, BMI, smoking status, Townsend Deprivation Index, cholesterol lowering medicine and blood pressure lowering medicine (model 2) from stratified analyses for chronotype. Results represent the risk of CAD, stroke and ischemic stroke compared to the reference group [Ref.] and are presented as hazard ratios with accompanying 95% confidence interval.

Definitions cluster: cluster 1; average pattern of acceleration close to the average pattern of absolute acceleration of the total UK Biobank population, cluster 2; 'early morning peak', cluster 3; 'late morning peak', cluster 4; 'evening peak'.