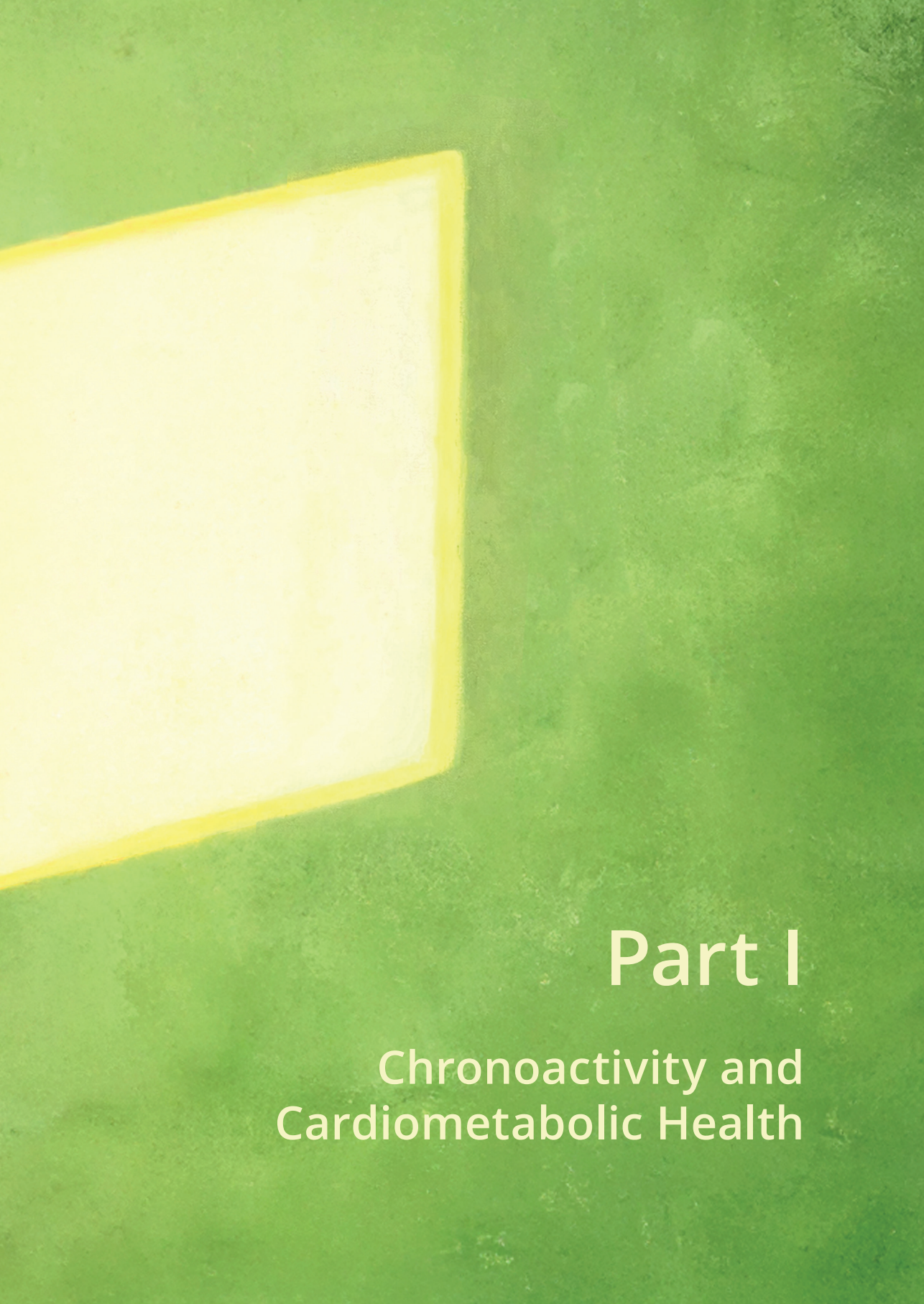
It's about time: implications of chronoactivity on health and disease

Albalak, G.



Part I

Chronoactivity and Cardiometabolic Health

2

Timing of objectively-collected physical activity in relation to body weight and metabolic health in sedentary older people: a cross-sectional and prospective analysis.

Gali Albalak, Marjon Stijntjes, Caroline A. Wijsman, P. Eline Slagboom, Frans J. van der Ouderaa, Simon P. Mooijaart, Diana van Heemst and Raymond Noordam.

International Journal of Obesity. *Nov 15 2021; doi:10.1038/s41366-021-01018-7*

Abstract

Background

Little is known about the impact of timing as opposed to frequency and intensity of daily physical activity on metabolic health. Therefore, we assessed the association between accelerometery-based daily timing of physical activity and measures of metabolic health in sedentary older people.

Methods

Hourly mean physical activity derived from wrist-worn accelerometers over a 6-day period was collected at baseline and after 3 months in sedentary participants from the Active and Healthy Ageing study. A principal component analysis (PCA) was performed to reduce the number of dimensions (e.g. define periods instead of separate hours) of hourly physical activity at baseline and change after follow-up. Cross-sectionally, a multivariable-adjusted linear regression analysis was used to associate the principal components, particularly correlated with increased physical activity in data-driven periods during the day, with body mass index (BMI), fasting glucose and insulin, HbA1c and the homeostatic model assessment for insulin resistance (HOMA-IR). For the longitudinal analyses, we calculated the hourly changes in physical activity and change in metabolic health after follow-up.

Results

We included 207 individuals (61.4% male, mean age: 64.8 [SD 2.9], mean BMI: 28.9 [4.7]). Higher physical activity in the early morning was associated with lower fasting glucose (−2.22%, 95% CI: −4.19, −0.40), fasting insulin (−13.54%, 95%CI: −23.49,−4.39), and HOMA-IR (−16.07%, 95%CI: −27.63, −5.65). Higher physical activity in the late afternoon to evening was associated with lower BMI (−2.84%, 95% CI: −4.92, −0.70). Higher physical activity at night was associated with higher BMI (2.86%, 95% CI: 0.90, 4.78), fasting glucose (2.57%, 95% CI: 0.70, 4.30), and HbA1c (2.37%, 95% CI: 1.00, 3.82). Similar results were present in the prospective analysis.

Conclusions

Specific physical activity timing patterns were associated with more beneficial metabolic health, suggesting particular time-dependent physical activity interventions might maximize health benefits.

Introduction

Insufficient physical activity, which occurs frequently in our ageing and sedentary society (1-3), can aggravate misalignment of circadian rhythms that might consequently lead to obesity, diabetes mellitus, cardiovascular disease, cognitive decline, and premature mortality.(3-8) In 2019, the prevalence of overweight and obesity was 50.1% and 14.7% respectively in Dutch adults and even 57.9% and 16.8% respectively in adults aged 65 years and older.(9) Therefore, many current guidelines and interventions aim to increase physical activity to promote healthy living and decrease the obesity-associated disease burden.

Currently, many studies are focused on duration, intensity and frequency of physical activity, and international guidelines recommend 150 to 300 minutes of moderate-intensity physical activity per week to maintain good health.(10) However, emerging evidence underlines a possible influence of timing of physical activity on weight control and (cardio)metabolic health.(8, 11) Although timing of physical activity is an aspect yet to be explored in further detail, studies comprising timing of other Zeitgebers (e.g., timing of nutritional intake and light exposure) have shown associations with weight loss.(8, 12-15) These studies particularly demonstrate that the impact on weight loss and perhaps on other aspects of metabolic health is not limited to the (average) amount of nutritional intake but also takes into account timing of behaviour. For example, de Cabo *et al*. showed the temporal effects on timing of meals across the day by intermittent fasting e.g. consuming all meals in an 8 hour period.(16) The very few studies examining the impact of physical activity timing on health show that for example women who are less active in the morning, have an increased risk of obesity compared to women who were most active before noon.(11)

As a result of evolving technology of accelerometery, more extensive analysis on for example timing of physical activity has been enabled.(17-21) However, recent studies are mainly focused on weight loss leaving other parameters of metabolic health uncharted.(8, 11, 22) Moreover, these studies applied a cross-sectional design which limits the validity of the findings. We hypothesized that particular timing of physical activity is associated with a more beneficial adiposity level and metabolic health status. Therefore, in the present study, we assessed the association between timing of physical activity and (changes in) weight and measures of metabolic health in a post-hoc analysis in relatively sedentary older people who were encouraged to increase their physical activity levels over a 3-month follow-up period.(23)

Methods

Ethical considerations

The Medical Ethical Committee of the Leiden University Medical Centre approved this study. Written informed consent was obtained from all study participants. The Active and Healthy Ageing (AGO) study was registered in the Dutch Trial Register (http://www.trialregister.nl) as NTR3045.

Study setting and population

This post-hoc study embedded in the AGO-study, was conducted between 2011 and 2012. Originally, this randomized controlled trial was designed to study the effect of a 3-month web-based intervention program which was designed to increase physical activity levels in sedentary older adults. A more detailed description of the study setting and selection of study population is published elsewhere.(23) In short, individuals were eligible for the study inclusion when they were 1) between 60 and 70 years of age, 2) had no history of diabetes mellitus or use of glucose-lowering medication, 3) had no disabilities compromising increase in physical activity, and 4) were in the possession of a personal computer with access to the internet. All eligible individuals were screened for the presence of a sedentary lifestyle with the General Practice Physical Activity Questionnaire (GPPAQ). Individuals within the GPPAQ categories 'inactive', 'moderately inactive', and 'moderately active' were grouped together as 'inactive' which was defined as having less than 3 hours of exercise and cycling combined weekly.(23) Only inactive individuals were included in the study after meeting the respective inclusion criteria. In total, 235 individuals were successfully included and randomly assigned to either the intervention program or the control arm of the AGO study in which they were randomly allocated. Individuals in the intervention arm received a commercially available web-based physical activity program (DirectLife, Philips, Consumer Lifestyle, Amsterdam, the Netherlands).(23) The control group was put on a waiting list and did not receive any specific instructions regarding daily physical activity.

In the present study, all participants with more than or equal to three (≥ 3) consecutive days of valid and complete wrist accelerometer data at baseline and after 3-month follow-up, and data on outcomes of interest to this study available in either the intervention or control group were included. Of the 235 participants that were included in the AGO intervention study, 14 participants were excluded in the present analyses due to missing accelerometery data either at baseline or at follow-up. After further (visual) inspection of the

accelerometer output data, 12 participants who did not meet the criterium of either ≥3 measurement days (n=5), an interrupted measurement period (n=1), or a non-correctable mislabelling of body location of the accelerometer (n=6) were excluded. Finally, we removed two participants without any clinical data, leaving a total of 207 participants (intervention group: n=105) for analyses.

As the effect of the intervention on daily mean physical activity was minimal (23), and no meaningful differences were observed in timing at follow-up between the intervention and control arm (**Supplementary Figures 1 and 2**), no distinction was made between the intervention and control group in the main analyses. We are aware of the role of confounding factors that might cause differences between participants from the intervention and control group (i.e. change of diet due to the intervention). Nonetheless, since our group is rather small, we analysed all participants that met acceleration wear time criteria jointly for sake of power, and performed sensitivity analyses of the prospective data in the intervention and control group separately.

Physical activity assessment

Physical activity was objectively measured at baseline and after 3-month follow-up using a wrist- and ankle-worn tri-axial accelerometer (GENEActiv, Kimbolton, Cambs, United Kingdom) for an average of six consecutive days. During this measurement period, the devices were worn for 24 hours per day on the right wrist and ankle. For this study, the ankle data was not used since the used data extraction program is validated for wrist-worn accelerometers (20), Wearing time started on a random weekday and the device was returned to the research centre by standard mail. Measurement frequency on the devices was set at 85.7 Hz and raw acceleration values in “g” were recorded continuously.

Raw acceleration from the GENEActiv was then processed with the GGIR package (v. 2.0-0, https://cran.r-project.org/web/packages/GGIR/index.html) which is a validated and commonly used tool to process raw accelerometery data from wrist-worn accelerometers. (20, 24) This package includes automatic calibration, abnormality detection and multiple data smoothening steps. Acceleration was summarized with metric Euclidean Norm Minus One with values set to zero (ENMO) and is shown in milligravity (mg) (1 mg = 0.00981 m/s^{-2}). 24 hourly means were calculated based on all measurement days for both baseline and after 3-months follow-up. Additionally, minutes spent in activity intensities were calculated to give insight in activity levels of the study population. Daytime physical activity was defined as physical activity during waking hours as calculated by GGIR. Wake time activity was defined as all

waking activity during a 24-hour period (daytime activity + waking activity during sleep period time). Thresholds for activity intensities were: inactivity, <30 mg; light, 30-99 mg; moderate, 100-399 mg; vigorous, ≥400 mg.

Body composition assessment

Data on anthropometrics was collected at baseline and after 3-months follow-up. Body height (in meters) was measured without shoes using a stadiometer and body weight (in kilograms) was assessed without shoes using a measurement scale. Body mass index (BMI in kg/m^2) was calculated from body weight and height and fat percentage (in kg) was assessed by bio-electrical impedance (BIA) analysis using a commercial portable device with hand-to-foot single frequency measurement (Biostat 1500, Euromedix, Leuven, Belgium).(23)

Measurements of metabolic variables/biochemical assessments

Fasting blood samples were drawn from each participant at both visits in the morning. Samples were transferred to the laboratory within two hours, divided into single-use aliquots, and frozen at -80 °C. All serum measurements were performed in one batch after completion of the entire study with fully automated equipment. Fasting glucose (mmol/L) was determined using the Modular P2 analyser (Roche, Almere, the Netherlands), fasting serum insulin (mU/L) using immunoassay by Immulite 2500 (DPC, Los Angeles, CA, USA), and glycated haemoglobin (HbA1c in mmol/mol Hb) was determined by high performance liquid chromatography (Primus Ultra2, Trinity Biotech Company, Kansas City, MO, USA). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by multiplying fasting glucose in mmol/L with fasting serum insulin in mU/L and dividing by 22.5.(25) For the longitudinal analysis, the changes in all outcome variables were used as dependent variables.

Statistical analyses

Characteristics of the study population were presented as median (with interquartile range, IQR) for numerical data or N (%) for categorical data.

A principal component analysis (PCA) was performed to reduce the number of dimensions (e.g., define periods instead of separate hours) of hourly physical activity. This analysis produces uncorrelated summary variables of hourly physical activity capturing periods of physical activity. These new 'compatibility' scores characterize an individual's behaviour. For the cross-

sectional analysis, we used the hourly mean acceleration data from the first measurement period. For the longitudinal analysis, we calculated the change of hourly mean acceleration between baseline and 3-months follow-up by subtracting the follow-up means from the baseline means ($\Delta = ENMO_{visit2} - ENMO_{visit1}$). Moreover, change in all outcome variables (BMI, fat percentage, fasting glucose, fasting insulin, HbA1c, and HOMA-IR) was calculated by subtracting the follow-up measurement from the baseline measurement ($\Delta = Value_{visit2} - Value_{visit1}$). Principal components with an Eigenvalue ≥1 were considered relevant. The rotated component matrix (rotation method: Varimax, Kaiser Normalization) was examined and clusters were established by interpreting this matrix. We considered the hours that had a factor loading of ≥ 0.4 in the interpretation of the component.

Subsequently, multivariable linear regression analyses were carried out to assess the associations between the calculated physical activity clusters and all outcome variables. All associations were adjusted for age and sex. This analysis was done for the cross-sectional data as well as the longitudinal data. For the longitudinal analysis, the changes in the outcome variables were used as dependent variables. All outcome variables, baseline variables and changes, were log transformed prior to the analysis to approximate a normal distribution. Subsequently, data was retransformed into a percentage difference in order to facilitate the clinical interpretation of the results. Additionally, to examine whether the found associations were explained by weight or weight loss after 3 months of follow up, we corrected for BMI in the analyses for fasting glucose, fasting insulin and HOMA-IR. The longitudinal analyses were performed for the total study population as well for the intervention and control group separately, but we acknowledge that sample size became limited.

All statistical analyses were performed with SPSS Statistics v24 (IBM Corp, Armonk, NY, USA). Results were reported as the difference in outcome measure per SD increase in the principal component with accompanied 95% confidence interval (CI).

Results

Participant characteristics

Participant characteristics are shown in **Table 1**. The median age of the total population was 65 (IQR: 62.2-67) years. The majority of participants were men (61.4%). On average, participants were overweight with a median BMI of 28 kg/m^2 (IQR: 25.7-31.1), and spent approximately 660 (SD: 89.6) minutes inactive.

Table 1. Characteristics of the study population at baseline

Characteristics	Baseline	Changes after follow-up Δ
N	207	207
Age, (years)	65 (62.2-67)	
Women (n,%)	80 (38.6)	
Body composition		
BMI (kg/m^2)	28 (25.7-31.1)	-0.2 (-0.8-0.1)
Fat percentage (kg)	34.4 (30.1-42.5)	-0.3 (-1.6-1.1)
Biochemistry		
Fasting venous glucose (mmol/L)	5.6 (5.2-6.1)	-0.2(-0.2-0.1)
Fasting insulin (mU/L)	11.1 (7.6-17.3)	-0.9 (-3.5-1.2)
HbA1c (mmol/mol Hb)	34.9 (33.1-36.8)	-0.2 (-1.1-0.6)
HOMA-IR	2.8 (2.8-4.4)	-0.3 (-1.1-0.3)
Physical activity		
Daytime		
Inactivity (min)	660 (89.6)	-1.6 (72.7)
Light (min)	238.1 (55.6)	4.8 (48.3)
Moderate (min)	82.3 (35.7)	10.7 (28.11)
Vigorous (min)	1.5 (0.6-2.9)	0.5 (3)
Wake time		
Inactivity (min)	712.3 (90.6)	-0.1 (71)
Light (min)	243.3 (55.6)	5.1 (48)
Moderate (min)	83.6 (36.2)	10.6 (27.9)
Vigorous (min)	1.5 (0.6-2.9)	0.5 (3.1)

Abbreviations: n, number of participants; BMI, body mass index; HOMA-IR, homeostatic assessment model for insulin resistance. Physical activity data represents the 24-hour mean from the complete measurement period (3-6 days). Daytime physical activity was defined as physical activity during waking hours as calculated by GGIR. Wake time activity was defined as all waking activity during a 24 hour period (daytime activity + waking activity during sleep period time). Thresholds for activity intensities were: inactivity, <30 mg; light, 30-99 mg; moderate, 100-399 mg; vigorous, ≥400 mg. Follow-up data are presented as the change of variables from baseline to follow-up (Δ= Valuevisit2 – Valuevisit1). Follow-up measurements were done 12 weeks after baseline. Data presented as number n proportion (%); mean (SD); median (25th-75th percentile).

Cross-sectional examination of physical activity timing and metabolic health characteristics

Based on the baseline hourly physical activity assessments, five largely nonoverlapping, correlated (>0.4) components with an Eigenvalue ≥1 were identified with consecutive periods of hourly physical activity (**Supplementary Table 1**). Notably, between 0:00 and 5:00 (defined as 'night'), between 4:00 and 9:00 (early morning), between 8:00 and 16:00 (morning and afternoon), between 15:00 and 22:00 (late afternoon to evening), and between 22:00 and 1:00 (late evening) (**Figure 1a**).

Results of the baseline analyses are presented in **Figure 2**. Higher physical activity at night was associated with higher BMI (2.86%, 95%CI: 0.90, 4.78), higher fasting glucose (2.57%, 95%CI: 0.70, 4.30), and higher HbA1c levels (2.37%, 95%CI: 1.00, 3.82). We found that 6 participants were active at night (defined as having one hourly average acceleration between 0:00 and 5:00 higher than or equal to 30 mg). To ensure these participants did not influence the latter results, we did additional sensitivity analyses in which these participants were excluded (**Supplementary Table 2**). This analysis showed that the results did not change notably. Furthermore, participants who were more active in the early morning had a lower fat percentage (-2.33%, 95%CI: -4.19, -0.50), lower fasting glucose levels (-2.22%, 95%CI: -4.19, -0.40), lower fasting insulin (-13.54%, 95%CI: -23.49, -4.39), and lower insulin resistance (-16.07%, 95%CI: -27.63, -5.65). Higher activity in the morning and afternoon, was associated with a lower BMI (per 1 SD increase in physical activity component: -2.22%, 95%CI: -4.29, -0.20), lower fasting insulin (-9.97%, 95%CI: -19.72, -1.01), and lower HOMA-IR (-10.96%, 95%CI: -22.02, -0.80). However, the association with fasting insulin diminished after adjusting for BMI (**Supplementary Table 3**). Higher physical activity in the late afternoon and evening was associated with lower BMI and fat percentage (-2.84%, 95%CI: -4.92, -0.70; -1.82%, 95%CI: -3.77, 0.00, respectively), but not with insulin, Hb1Ac and HOMA-IR. Similar results were observed for higher physical activity in the late evening (BMI: -2.02%, 95%CI: -3.98, 0.00, fat percentage: -1.82%, 95%CI: -3.67, -0.10). The association observed with fasting insulin remained after adjusting for BMI (**Supplementary Table 3**).

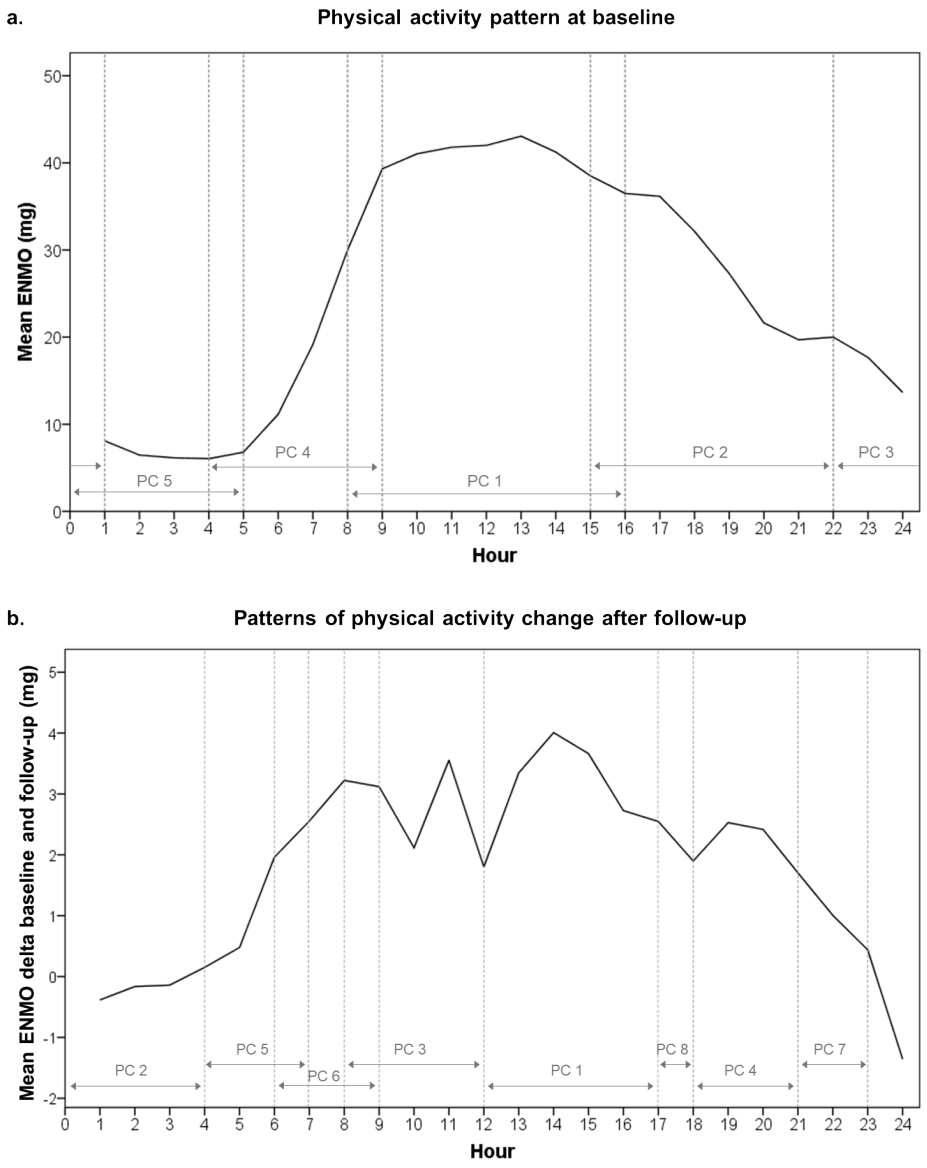


Figure 1. Characteristics in (changes in) physical activity in the study population. A. Shows the average pattern of acceleration at baseline accompanied by principal components (PC) of physical activity timing. B. Shows the average pattern of physical activity change after 3 months of follow-up accompanied by principal components of change in physical activity timing. The x-axis presents the 24 daily hours (from midnight to midnight). The y-axis presents the mean acceleration calculated through Euclidean norm minus one (ENMO) and presented as milligravity (mg).

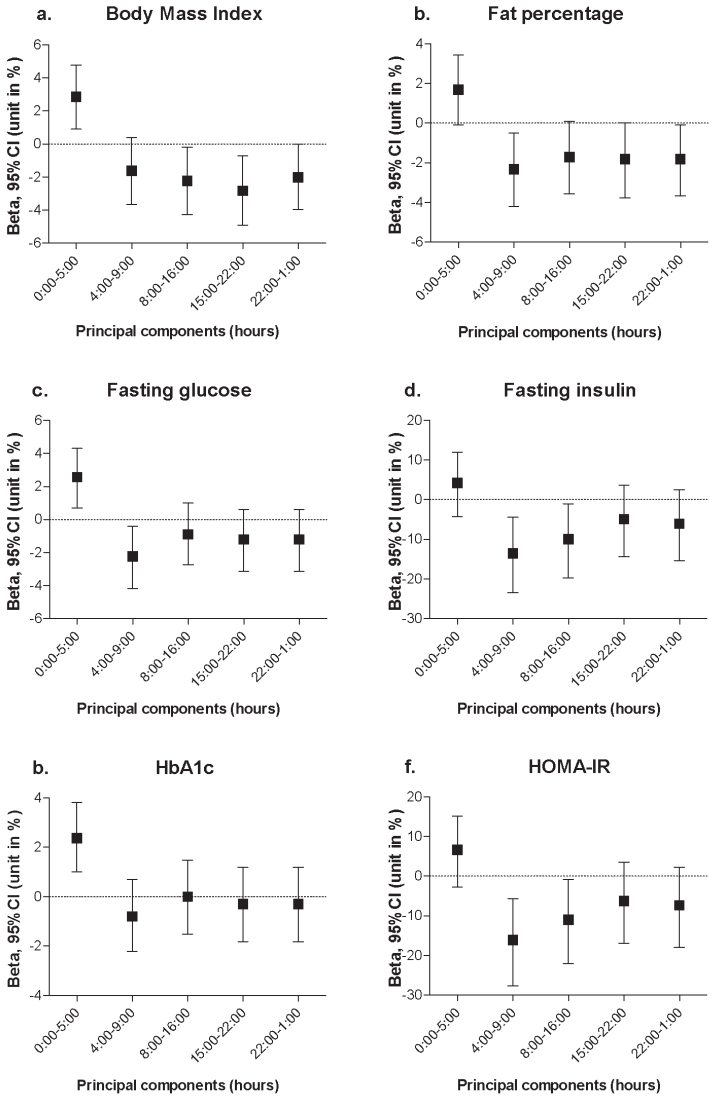


Figure 2. The associations between the individual variables of metabolic health and the principal components (PC) representing the baseline physical activity. The associations between periods of timing of physical activity (derived with principal component analyses) and the individual variables of metabolic health results are displayed as the percentage difference in outcome (with accompanying 95% confidence interval) per SD increase in physical activity in the specific principal component (e.g. physical activity period). Definitions principal components: 0:00–5:00, night; 4:00–9:00, early morning; 8:00–16:00, morning and afternoon; 15:00–22:00 late afternoon to evening; 22:00–1:00 late evening. As outcomes, we studied: body mass index (a), fat percentage (b), fasting glucose (c), fasting insulin (d), HbA1c (e), and the homeostatic model assessment for insulin resistance (HOMA-IR, f).

Longitudinal examination of physical activity timing and metabolic health characteristics

Table 1 also shows the difference in characteristics after 3 months of follow-up. Notably, a median improvement in almost all metabolic parameters was seen after three months of follow-up (e.g. Δ Fasting insulin=-0.9 mU/L IQR: -3.5-1.2).

In the PCA on the change in physical activity, eight components with an Eigenvalue ≥1 were identified (**Supplementary table 4**). The values of the eight components correlated with changes in physical activity representing the following periods; between 0:00 and 4:00 (night), between 4:00 and 7:00 (late night/early morning), between 6:00 and 9:00 (early morning), between 8:00 and 12:00 (late morning), between 12:00 and 17:00 (afternoon), between 17:00 and 18:00 (late afternoon), between 18:00 and 21:00 (early evening), and between 21:00 and 23:00 (late evening) (**Figure 1b**).

Results from the multivariable-adjusted linear regressions are shown in **Figure 3**. Participants with increased physical activity at night showed a higher increase in HbA1c levels after 3 months of follow up (0.90%, 95%CI: 0.20, 1.59). Similar to the baseline analysis, these results were apparent in all outcome variables. Increased physical activity in the late night/early morning was associated with a greater decrease of BMI and fat percentage during follow-up (-0.40%, 95%CI: -0.80, 0.00, -1.41%, 95%CI: -2.53, -0.30). Participants who became more active in the late morning had a greater decline in levels of fasting insulin and insulin resistance (-7.68%, 95%CI: -14.22, -1.61), -7.90, 95%CI: -15.14, -1.01, respectively). The association with fasting insulin remained similar after adjusted for change in BMI (**Supplementary table 5**). Participants with a higher increase in physical activity in the afternoon between baseline and follow-up, had a greater decrease in the percentage of body fat (per 1 SD physical activity increase component: -1.11%, 95%CI: -2.22, 0.00). Furthermore, participants with higher increase in physical activity in the early evening (-0.50%, 95%CI: -0.90, -0.10) and late evening (-0.50%, 95%CI: -0.90, -0.10) had a greater decrease in BMI after three months of follow-up. No clear associations were found for increased physical activity in the early morning and in the late afternoon.

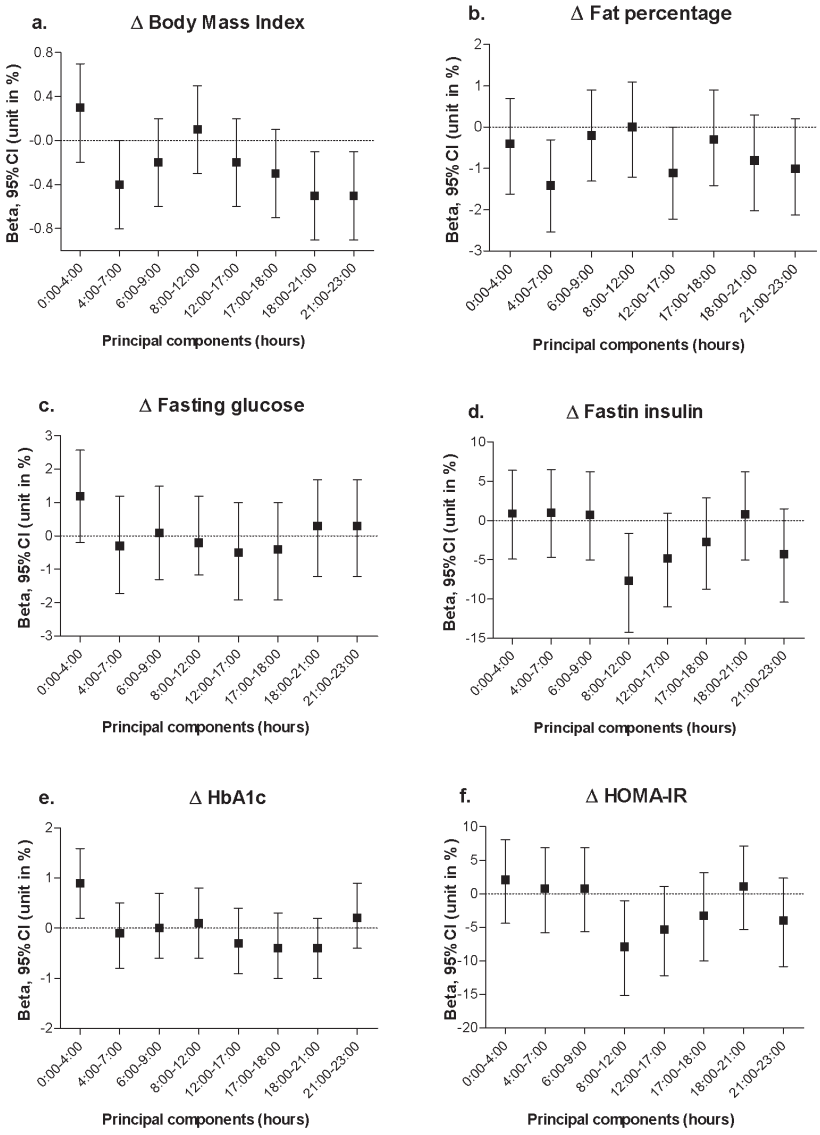


Figure 3. The associations between the individual variables of metabolic health and the longitudinal principal components (PC) representing change in timing of physical activity. The represented timing periods are shown in the legend on the bottom. Results are displayed as SD (in %) with accompanying 95% confidence interval. Definitions principal components: 0:00–4:00, night; 4:00–7:00, late night/ early morning; 6:00–9:00, early morning; 8:00–12:00, late morning; 12:00–17:00, afternoon; 17:00–18:00, late afternoon; 18:00–21:00, early evening; 21:00–23:00, late evening. As outcomes, we studied the changes in the following measures: body mass index (a), fat percentage (b), fasting glucose (c), fasting insulin (d), HbA1c (e), and the homeostatic model assessment for insulin resistance (HOMA-IR, f).

Discussion

The overall aim of the present study was to examine the associations between the timing of objectively-collected physical activity and metabolic health measures in sedentary older people. This observational prospective cohort study identified principal components that reflect specific patterns of timing and change of timing of physical activity. Furthermore, we found that increased physical activity in the morning was associated with lower BMI, fasting glucose, fasting insulin, and insulin resistance. On the other hand, we found that increased physical activity at night was associated with higher BMI, fasting glucose, and HbA1c.

One of our findings was that most of the daytime components were associated with lower BMI. The majority of previous literature favours morning physical activity in relation to weight loss and body composition.(8, 11, 26, 27) Nonetheless, evidence is ambiguous. A study with a small sample size (n=29) found that obese postmenopausal women that had a self-selected walk in the evening had a greater decrease in fat mass than women who walked in the morning.(28) Our results are most in line with the majority of evidence showing that increased activity in the morning has positive metabolic health outcomes.(8, 11, 26, 27) However, we did find associations between increased physical activity in the afternoon and evening in relation to reduced BMI and insulin resistance as well. An explanation for these differences is that the beneficial effects of certain timing on health and performance might differ between chronotypes.(29, 30) Early birds are possibly more inclined to perform and benefit more from higher physical activity in the morning than night owls and vice versa. This preference might be associated with other behavioral chronotype features, such as night-time snacking in night-owls.(31) Unpropitiously, we were unable to identify chronotypes to perform subgroup analyses as data on chronotypes was not collected in our study. Future studies on physical activity timing and metabolic health should consider chronotype and sleep patterns as possible interacting or confounding factors.

Moreover, our findings showed that increased physical activity at night brought inversed associations compared with high physical activity at daytime. The results indicated that participants with higher nightly physical activity at baseline or increased nightly physical activity during follow-up, had a higher BMI, higher fasting glucose levels and increased HbA1c. An explanation is that the observed increased physical activity at night time is merely an indicator for insomnia or poor sleep quality which is proven to have a causal relation with poor metabolic health and hormonal levels.(32, 33) An alternative explanation might be found in the extensive evidence on circadian rhythms and their

influence on metabolic health. More recently, studies have focused on how timing of certain behavioural exposures (i.e. Zeitgebers) including physical activity affects circadian rhythms.(8, 30, 34) These studies show that exposure to this behavioural factor late at night seems to alter the alignment between circadian rhythms in the suprachiasmatic nucleus and peripheral tissues which can offset metabolic processes such as glucose metabolism and insulin resistance that can eventually lead to weight gain.(7, 8, 29, 34, 35)

We were able to perform longitudinal analyses to examine the effect of change in physical activity timing on metabolic health. We found that participants who, after three months of follow-up, became more active in the morning had a substantial decrease in their fasting insulin levels and insulin resistance. A study on diurnal patterns of glucose metabolism in normoglycaemic older individuals, showed that postprandial glucose response was lower in the morning compared to afternoon and, notably, evening glucose response.(36) This suggests higher insulin response efficiency and/or lower insulin resistance in the morning. Together with our findings, this might indicate that increasing physical activity in the morning, when insulin sensitivity is highest, is most beneficial for overall diurnal glucose homeostasis.

This study has a number of strengths and limitations to address. To our best of knowledge, this study was the first that was able to examine the longitudinal association between timing of physical activity as well as change of timing over the course of three months with metabolic outcomes. Additionally, we assessed timing of physical activity using objective measurement methods and metabolic variables. A major limitation of previous research on physical activity behaviour is that conventional measurement methods have not been sufficiently enriched to be able to investigate all aspects of physical activity, including timing. Finally, we used a data-driven dimension reduction analysis to map timing in this specific data whereas previous studies have used predefined periods of timing (morning: 6:00-12:00, noon: 12:00-18:00 etc.).(11, 27, 37) These predefined periods might not always correspond with daily life timing since this is highly dependent of one's occupational activities. Although this principal component analysis adds statistical power which is beneficial when working with a smaller data set, the utilization of such analysis can complicate the clinical interpretation of the result. A limitation of our study was that, since this was a post-hoc analysis some years after study inclusion, we were not able to collect additional data on health behaviours such as eating behaviour. Therefore, we were not able to control for possible important unmeasured confounding factors. However, we believe that the influence of residual confounders is minimalized due to the relatively short follow-up time of three months in the

present study, especially in the prospective analysis. Nevertheless, we advise that future studies should take possible confounders and behavioural factors into account. Finally, we had a rather small and homogeneous sample with respect to health status and sedentary behaviour.

In conclusion, the present study contributes to the current knowledge in the literature indicating that timing of physical activity, more specifically, high physical activity in the morning, is associated with several measures of improved metabolic health. Furthermore, the present study provides novel insight on the association between increased morning physical activity and fasting insulin and insulin resistance. Our results suggest that time-dependent physical activity interventions might be required to reach maximum health benefits. Advantageously, influencing this Zeitgeber by changing an individual's physical activity timing, is a rather effortless intervention to improve said individual's metabolic health.

References

1. Kohl HW, 3rd, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, et al. The pandemic of physical inactivity: global action for public health. Lancet. 2012;380(9838):294-305.
2. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.
3. WHO. Global recommendations on physical activity for health. Switserland2010.
4. Sun F, Norman IJ, While AE. Physical activity in older people: a systematic review. BMC Public Health. 2013;13:449.
5. Braskie MN, Boyle CP, Rajagopalan P, Gutman BA, Toga AW, Raji CA, et al. Physical activity, inflammation, and volume of the aging brain. Neuroscience. 2014;273:199-209.
6. Notthoff N, Reisch P, Gerstorf D. Individual Characteristics and Physical Activity in Older Adults: A Systematic Review. Gerontology. 2017;63(5):443-59.
7. Lewis P, Korf HW, Kuffer L, Gross JV, Erren TC. Exercise time cues (zeitgebers) for human circadian systems can foster health and improve performance: a systematic review. BMJ Open Sport Exerc Med. 2018;4(1):e000443.
8. 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.
9. Statistiek CBv. Overgewicht, Cijfer en Context. In: zorg Ve, editor. 2020.
10. 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.
11. 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.
12. Reid KJ, Santostasi G, Baron KG, Wilson J, Kang J, Zee PC. Timing and intensity of light correlate with body weight in adults. PLoS One. 2014;9(4):e92251.
13. Garaulet M, Gomez-Abellan P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134:44-50.
14. Garaulet M, Gomez-Abellan P, Alburquerque-Bejar JJ, Lee YC, Ordovas JM, Scheer FA. Timing of food intake predicts weight loss effectiveness. Int J Obes (Lond). 2013;37(4):604-11.
15. Dashti HS, Scheer F, Saxena R, Garaulet M. Timing of Food Intake: Identifying Contributing Factors to Design Effective Interventions. Adv Nutr. 2019;10(4):606-20.
16. de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. N Engl J Med. 2019;381(26):2541-51.
17. Lu TC, Fu CM, Ma MH, Fang CC, Turner AM. Healthcare Applications of Smart Watches. A Systematic Review. Appl Clin Inform. 2016;7(3):850-69.
18. Piwek L, Ellis DA, Andrews S, Joinson A. The Rise of Consumer Health Wearables: Promises and Barriers. PLoS Med. 2016;13(2):e1001953.

19. Jim HSL HA, Brownstein NC, Barata A, Dicker AP, Knoop H, Gonzalez BD, et al. Innovations in Research and Clinical Care Using Patient-Generated Health Data. CA Cancer J Clin. 2020;70(3):182–99.

20. 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)).

21. van Kuppevelt D, Heywood J, Hamer M, Sabia S, Fitzsimons E, van Hees V. Segmenting accelerometer data from daily life with unsupervised machine learning. PLoS One. 2019;14(1):e0208692.

22. 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.

23. Wijsman CA, Westendorp RG, Verhagen EA, Catt M, Slagboom PE, de Craen AJ, et al. Effects of a web-based intervention on physical activity and metabolism in older adults: randomized controlled trial. J Med Internet Res. 2013;15(11):e233.

24. 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.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

26. 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.

27. Brooker PG, Gomersall SR, King NA, Leveritt MD. The feasibility and acceptability of morning versus evening exercise for overweight and obese adults: A randomized controlled trial. Contemp Clin Trials Commun. 2019;14:100320.

28. 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.

29. Henson J, Rowlands AV, Baldry E, Brady EM, Davies MJ, Edwardson CL, et al. Physical behaviors and chronotype in people with type 2 diabetes. BMJ Open Diabetes Res Care. 2020;8(1).

30. Facer-Childs ER, Boiling S, Balanos GM. The effects of time of day and chronotype on cognitive and physical performance in healthy volunteers. Sports Med Open. 2018;4(1):47.

31. Vera B, Dashti HS, Gomez-Abellan P, Hernandez-Martinez AM, Esteban A, Scheer F, et al. Modifiable lifestyle behaviors, but not a genetic risk score, associate with metabolic syndrome in evening chronotypes. Sci Rep. 2018;8(1):945.

32. Dekker SA, Noordam R, Biermasz NR, de Roos A, Lamb HJ, Rosendaal FR, et al. Habitual Sleep Measures are Associated with Overall Body Fat, and not Specifically with Visceral Fat, in Men and Women. Obesity (Silver Spring). 2018;26(10):1651-8.

33. Camila Hirotsu ST, Monica Levy Andersen. Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. Sleep Science. 2015;8(3):143-52.

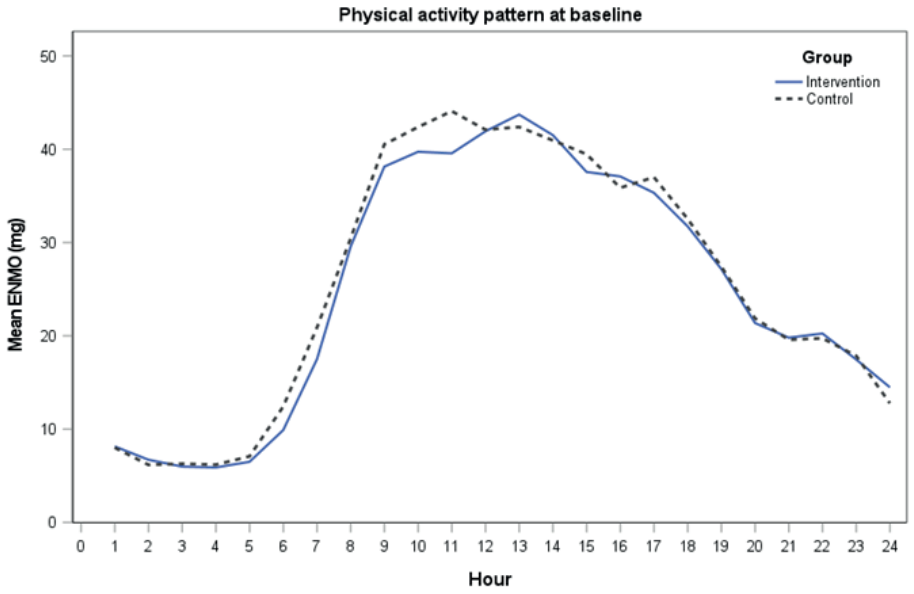
34. Hower IM, Harper SA, Buford TW. Circadian Rhythms, Exercise, and Cardiovascular Health. J Circadian Rhythms. 2018;16:7.

35. Stenvers DJ, Scheer F, Schrauwen P, la Fleur SE, Kalsbeek A. Circadian clocks and insulin resistance. Nat Rev Endocrinol. 2019;15(2):75-89.

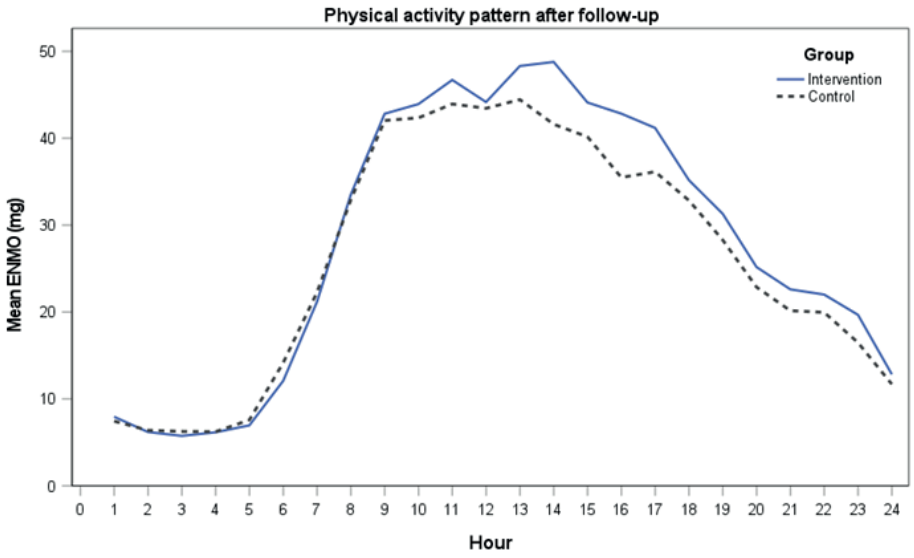
36. Akintola AA, Noordam R, Jansen SW, de Craen AJ, Ballieux BE, Cobbaert CM, et al. Accuracy of Continuous Glucose Monitoring Measurements in Normo-Glycemic Individuals. PLoS One. 2015;10(10):e0139973.

37. 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.

Appendix



Supplementary figure 1. Shows the average pattern of physical activity of the 7-day measurement period at baseline. Intervention and control group of the AGO-study were analysed and are presented separately. The x-axis presents the 24 daily hours. The y-axis presents the mean acceleration calculated through Euclidean norm minus one (ENMO) and presented as milligravity (mg).



Supplementary figure 2. Shows the average pattern of physical activity of the 7 day measurement period after three months of follow-up. Intervention and control group of the AGO-study were analysed and are presented separately. The x-axis presents the 24 daily hours. The y-axis presents the mean acceleration calculated through Euclidean norm minus one (ENMO) and presented as milligravity (mg).

Supplementary table 1. Rotated Component Matrix from the cross-sectional principal component analysis

	PC 1	PC 2	PC 3	PC 4	PC 5
	Factor loading component (R)				
Mean ENMO 0:00-1:00	-0.012	0.185	0.577	-0.245	0.547
Mean ENMO 1:00-2:00	0.028	0.117	0.318	-0.290	0.667
Mean ENMO 2:00-3:00	-0.001	0.103	0.167	-0.065	0.825
Mean ENMO 3:00-4:00	-0.089	0.054	-0.052	0.194	0.751
Mean ENMO 4:00-5:00	-0.066	-0.004	-0.207	0.429	0.472
Mean ENMO 5:00-6:00	-0.001	-0.012	0.017	0.813	0.136
Mean ENMO 6:00-7:00	0.022	0.153	0.022	0.830	-0.063
Mean ENMO 7:00-8:00	0.357	0.211	-0.006	0.661	-0.204
Mean ENMO 8:00-9:00	0.692	0.109	-0.067	0.434	-0.116
Mean ENMO 9:00-10:00	0.735	0.128	-0.231	0.217	0.002
Mean ENMO 10:00-11:00	0.800	0.216	-0.045	0.115	-0.008
Mean ENMO 11:00-12:00	0.683	0.358	-0.031	-0.091	0.053
Mean ENMO 12:00-13:00	0.724	0.131	0.215	-0.091	-0.082
Mean ENMO 13:00-14:00	0.681	0.172	0.342	-0.055	-0.025
Mean ENMO 14:00-15:00	0.540	0.347	0.342	0.007	-0.006
Mean ENMO 15:00-16:00	0.455	0.424	0.367	0.001	0.040
Mean ENMO 16:00-17:00	0.278	0.575	0.394	0.168	0.063
Mean ENMO 17:00-18:00	0.306	0.656	0.146	0.141	0.031
Mean ENMO 18:00-19:00	0.187	0.785	0.201	0.087	0.028
Mean ENMO 19:00- 20:00	0.164	0.860	-0.006	0.052	-0.010
Mean ENMO 20:00-21:00	0.161	0.738	0.144	0.056	0.145
Mean ENMO 21:00-22:00	0.192	0.556	0.246	-0.032	0.233
Mean ENMO 22:00-23:00	0.082	0.341	0.748	0.101	-0.040
Mean ENMO 23:00-24:00	0.011	0.207	0.841	-0.064	0.246

Abbreviations: PC, principal component; ENMO, Euclidean norm minus one. Rotated component matrix, output from the principal component analysis of the cross-sectional data; hourly physical activity at baseline. All principal components with an Eigenvalue ≥1 are shown with accompanying factor loadings. Hours with a factor loading score of ≥0.400 were deemed relevant and were included in the delimitation and definition of the principal component. These factor loadings are showed in bold. Rotation Method was Varimax with Kaiser Normalization

Supplementary table 3. Cross-sectional linear regression adjusting for BMI

	Fasting glucose	Fasting insulin
	Beta (95%CI)	Beta (95%CI)
0:00-5:00	1.49 (-0.20, 3.15)	-1.92 (-9.75, 5.35)
4:00-9:00	-1.71 (-3.36, 0.00)	-9.64 (-17.94, -1.92)*
8:00-16:00	-0.10 (-1.82, 1.69)	-4.81 (-12.98, 2.66)
15:00-22:00	-0.20 (-2.02, 1.49)	1.19 (-6.61, 8.33)
22:00-1:00	-0.50 (-2.22, 1.19)	-1.71 (-9.31, 5.45)

Abbreviations: BMI, body mass index; CI, confidence interval. Results are presented as the percentage difference in outcome variable (with 95% confidence interval) per SD increase in physical activity in a certain period during the day (as derived with the principal component analysis). Analyses were adjusted for age, sex and BMI. Definitions principal components: 0:00-5:00, night; 4:00-9:00, early morning; 8:00-16:00, morning and afternoon; 15:00-22:00 late afternoon to evening; 22:00-1:00 late evening.

* indicates a significant association (p <0.05)

Supplementary table 2. Sensitivity analysis for the night component in de cross-sectional analysis

	0:00-5:00
	Beta (95%CI)
BMI	3.83 (1.59, 6.11)
Fat percentage	2.76 (0.70, 4.78)
Fasting glucose	3.05 (0.90, 5.16)
Fasting insulin	5.07 (-5.13, 14.27)
HbA1c	3.05 (1.29, 4.69)
HOMA-IR	7.97 (-3.25, 17.88)

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; CI, confidence interval. Results are presented as the percentage difference in outcome variable (with 95% confidence interval) per SD increase in physical activity during the 'night' component (from 0:00-5:00 as derived with the principal component analysis). Analyses were adjusted for age and sex.

Supplementary table 4. Rotated Component Matrix from the longitudinal principal component analysis

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
	Factor loading component (R)							
Δ Mean ENMO 0:00-1:00	-0.089	0.710	0.113	0.010	0.053	-0.252	-0.095	-0.233
Δ Mean ENMO 1:00-2:00	-0.138	0.761	-0.034	-0.021	-0.047	-0.037	-0.010	-0.178
Δ Mean ENMO 2:00-3:00	0.006	0.784	-0.060	-0.136	0.032	0.158	-0.056	0.123
Δ Mean ENMO 3:00-4:00	0.125	0.507	-0.038	0.105	0.208	0.191	-0.030	0.244
Δ Mean ENMO 4:00-5:00	0.051	0.032	0.186	-0.018	0.651	0.118	-0.148	-0.035
Δ Mean ENMO 5:00-6:00	0.011	0.100	-0.083	0.152	0.819	0.024	0.124	-0.018
Δ Mean ENMO 6:00-7:00	0.108	-0.019	-0.176	-0.035	0.444	0.636	0.122	0.180
Δ Mean ENMO 7:00-8:00	0.124	0.048	0.128	0.110	0.068	0.819	0.061	-0.019
Δ Mean ENMO 8:00-9:00	0.127	0.027	0.622	0.027	-0.154	0.540	0.018	-0.007
Δ Mean ENMO 9:00-10:00	0.016	-0.047	0.810	0.078	-0.053	0.052	-0.047	-0.018
Δ Mean ENMO 10:00-11:00	0.276	-0.010	0.593	0.098	0.249	-0.016	-0.002	0.197
Δ Mean ENMO 11:00-12:00	0.376	0.024	0.543	-0.233	0.142	-0.118	0.109	0.075
Δ Mean ENMO 12:00-13:00	0.682	-0.105	0.234	-0.075	0.086	0.101	0.051	-0.179
Δ Mean ENMO 13:00-14:00	0.772	-0.046	0.181	0.078	0.007	0.094	0.007	-0.193
Δ Mean ENMO 14:00-15:00	0.719	-0.001	0.026	0.174	-0.005	0.140	-0.076	-0.050
Δ Mean ENMO 15:00-16:00	0.660	0.021	0.008	0.056	0.104	-0.019	0.185	0.338
Δ Mean ENMO 16:00-17:00	0.551	-0.016	0.056	0.225	-0.043	0.013	0.086	0.296
Δ Mean ENMO 17:00-18:00	0.280	0.196	0.154	0.283	-0.206	0.168	0.128	0.536
Δ Mean ENMO 18:00-19:00	0.096	-0.029	0.042	0.771	-0.081	0.199	0.008	-0.024
Δ Mean ENMO 19:00- 20:00	0.188	-0.071	-0.045	0.768	0.176	-0.123	0.027	0.184
Δ Mean ENMO 20:00-21:00	0.057	0.026	0.116	0.544	0.392	0.011	0.367	-0.005
Δ Mean ENMO 21:00-22:00	0.011	-0.067	-0.065	0.128	0.082	-0.046	0.846	0.139
Δ Mean ENMO 22:00-23:00	0.129	-0.093	0.068	-0.017	-0.108	0.218	0.771	-0.194
Δ Mean ENMO 23:00-24:00	0.262	0.298	-0.094	0.012	-0.049	0.007	0.145	-0.621

Abbreviations: PC, principal component; ENMO, Euclidean norm minus one. Rotated component matrix, output from the principal component analysis of the longitudinal data; change in hourly physical activity between baseline and 3 months of follow-up. All principal components with an Eigenvalue ≥1 are shown with accompanying factor loadings. Hours with a factor loading score of ≥0.400 were deemed relevant and

were included in the delimitation and definition of the principal component. These factor loadings are showed in bold. Rotation Method was Varimax with Kaiser Normalization.

Supplementary table 5. longitudinal linear regression adjusting for BMI

	Fasting glucose	**Fasting insulin**
	Beta (95%CI)	Beta (95%CI)
0:00-4:00	1.29 (-0.20, 2.66)	0.50 (-5.34, 5.92)
4:00-7:00	-0.40 (-1.92, 1.00)	2.08 (-3.56, 7.50)
6:00-9:00	0.00 (-1.41, 1.49)	1.09 (-4.60, 6.48)
8:00-12:00	-0.20 (-1.61, 1.29)	-7.68 (-14.11, -1.61)*
12:00-17:00	-0.50 (-1.92, 0.90)	-4.60 (-10.63, 1.00)
17:00-18:00	-0.50 (-2.02, 0.90)	-2.12 (-8.00, 3.44)
18:00-21:00	0.10 (-1.41, 1.49)	2.08 (-3.56, 7.50)
21:00-23:00	0.10 (-1.41, 1.59)	-2.84 (-8.87, 2.86)

Abbreviations: BMI, body mass index; CI, confidence interval. Results are presented as the percentage difference in outcome variable (with 95% confidence interval) per SD increase in physical activity in a certain period during the day (as derived with the principal component analysis). Analyses were adjusted for age, sex and BMI. Definitions principal components: 0:00-4:00, night; 4:00-7:00, late night/early morning; 6:00-9:00, early morning; 8:00-12:00, late morning; 12:00-17:00, afternoon; 17:00-18:00, late afternoon; 18:00-21:00, early evening; 21:00-23:00, late evening.

* indicates a significant association ($p < 0.05$)