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Associations of between- and within-day patterns of physical activity accumulation with arterial stiffness and indices of microvascular health—Evidence from The Maastricht study

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Abstract

While physical activity (PA) is understood to promote vascular health, little is known about whether the daily and weekly patterns of PA accumulation associate with vascular health. Accelerometer-derived (activPAL3) 6- or 7-day stepping was analyzed for 6430 participants in The Maastricht Study (50.4% women; 22.4% Type 2 diabetes mellitus (T2DM)). Multivariable regression models examined associations between stepping metrics (average step count, and time spent slower and faster paced stepping) with arterial stiffness (measured as carotid–femoral pulse wave velocity (cfPWV)), and several indices of microvascular health (heat-induced skin hyperemia, retinal vessel reactivity and diameter), adjusting for confounders and moderators. PA pattern metrics were added to the regression models to identify associations with vascular health beyond that of stepping metrics. Analyses were stratified by T2DM status if an interaction effect was present.

IMEDOS; Janssen-Cilag B. V.; Novo Nordisk Farma B. V.; Sanofi-Aventis Netherlands B. V.

Average step count and time spent faster paced stepping was associated with better vascular health, and the association was stronger in those with compared to those without T2DM. In fully adjusted models a higher step count inter-daily stability was associated with a higher (worse) cfPWV in those without T2DM (std $\beta = 0.04$, $p = 0.007$) and retinal venular diameter in the whole cohort (std $\beta = 0.07$, $p = 0.002$). A higher within-day variability in faster paced stepping was associated with a lower (worse) heat-induced skin hyperemia in those with T2DM (std $\beta = -0.31$, $p = 0.008$). Above and beyond PA volume, the daily and weekly patterns in which PA was accumulated were additionally associated with improved macro- and microvascular health, which may have implications for the prevention of vascular disease.

KEYWORDS

arterial stiffness, between-day variability, inter-daily stability, microvascular function, physical activity pattern, step count, vascular health, within-day variability

1 | INTRODUCTION

The inverse association between habitual physical activity (PA) and risk of cardiovascular and metabolic diseases is well established.[1] A growing body of evidence points to the influence of PA on vascular health and structure as a mechanism underpinning this association.[2] Our recent meta-analysis of 18 observational studies showed that higher average daily PA was associated with a lower carotid–femoral pulse wave velocity (cfPWV) as an indicator of reduced arterial stiffness in ostensibly healthy (asymptomatic) populations.[3] However, summarizing PA data into aggregated daily totals, ignores the different ways that people accumulate their activity across a day or week.

Accelerometers collect high resolution, time stamped data allowing researchers to move beyond simple aggregate PA totals, and to capture different patterns of daily and weekly PA accumulation. Figure 1 shows real data from four cohort study participants who each accumulated an average of 10 000 steps/day. Based on these aggregated daily totals, these four participants could be viewed as having exactly the same habitual PA profile. However, the pattern of PA accumulation between days of the week (Participants 1 and 2), and across hours of each day (Participants 3 and 4) are very different. At present, it is not known whether variation in these different patterns of PA accumulation modify the observed association between aggregate daily totals of PA volume and vascular health.[3]

Experimental studies have shown that endothelial function is acutely altered by very small physical movements such as fidgeting,[4] or by small breaks in sedentary time.[5] It is therefore possible that the influence of a given volume of PA on vascular health may not be uniform, rather it may depend on different patterns of rest and PA. To date, research investigating differences in patterns of habitual PA accumulation and associations with vascular health is sparse. To our knowledge, only one existing study has considered the association between PA and vascular health in a way that was not limited only to summary measures of average PA volume. Using data from The Maastricht study, Vandercappellen et al.[6] began to examine the between-day pattern of moderate vigorous activity (≥110 steps/min) by categorizing participants as inactive (<75 min/week), insufficiently active (75–150 min/week), "weekend warrior" (>150 min/week in ≤2 sessions), and regularly active (>150 min/week in ≥3 sessions). They found that compared to those who were inactive, participants who were insufficiently active, regularly active or "weekend warriors" all had a more favorable vascular health, with no difference between the regularly active or the "weekend warriors." When looking at downstream cardiovascular events in the UK Biobank study, Kurshid et al.[7] similarly found that those who completed ≥150 min/week through being "active regularly" or "weekend warriors" both had lower risk of cardiovascular events compared to those who were inactive.[7] However, grouping people based on combinations of duration, intensity and frequency of PA makes it difficult to isolate the effect of PA pattern from overall PA volume, of which we know is beneficially associated with vascular health.

The aim of this study therefore is to evaluate the independent association of the pattern of habitual PA accumulation, beyond that of overall volume of PA, on indices of vascular health. Specifically, the between-day pattern, within-day pattern, and the stability of the daily pattern from one day to the next, will be evaluated. Additionally, given that previous research has shown the relationship

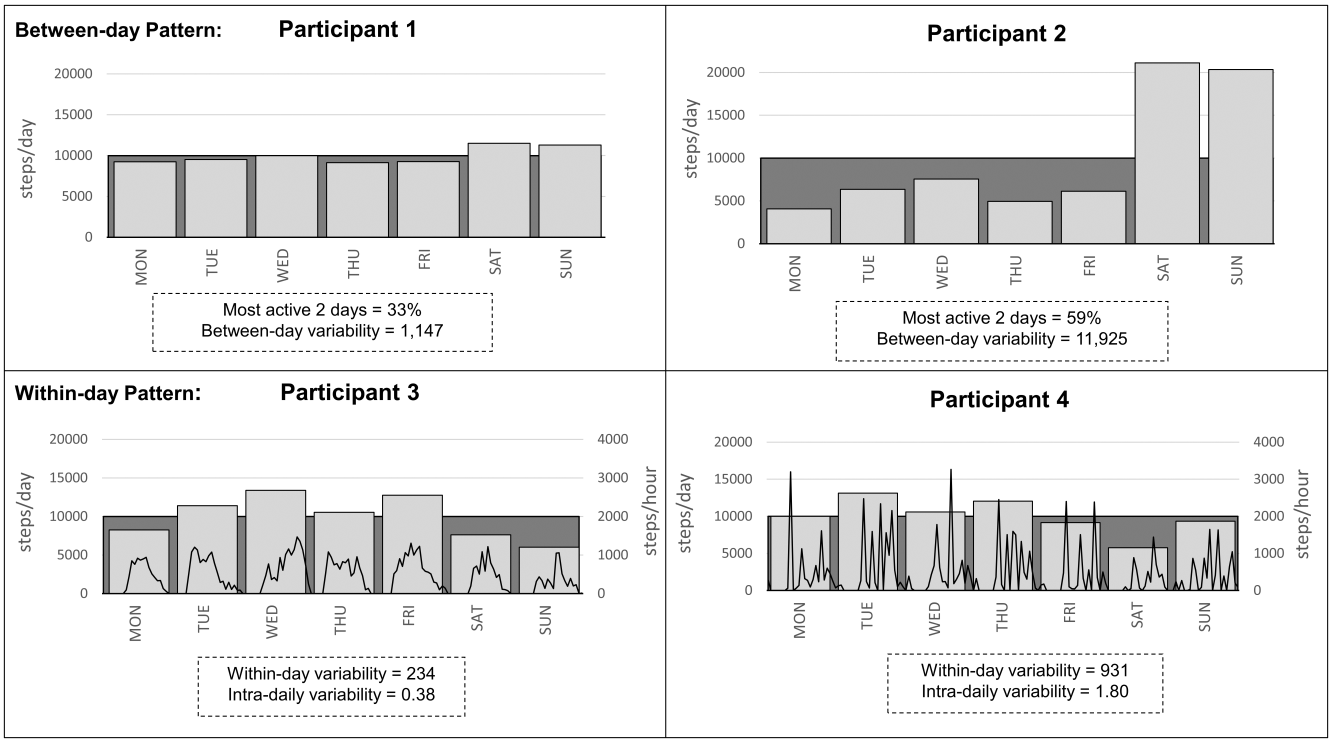

FIGURE 1 Graphical representation of real data from The Maastricht Study illustrating how for a given volume of PA, the way in which PA is accumulated can vary considerably between days of the week and between hours of the day. Dark gray continuous bars represent the average daily step count (all four participants accumulate on average 10 000 steps per day). Lighter gray individual bars represent the absolute daily step count. Black continuous line represents the steps per hour (note the alternate y-axis on right). Participant 1 accumulates approximately 10 000 steps/day on each day with very little variation between different days of the week. Summarizing this participant as achieving on average 10 000 steps/day is therefore fairly accurate. Participant 2 however accumulates the majority of their weekly activity in just 2 days of the week with over 20 000 steps on those days, and much lower levels of activity on the remaining days leading to a higher proportion of activity accumulated in the most active 2 days, and a higher between-day variability. Participant 3 has a fairly continuous pattern of activity accumulation across hours of the day with no large peaks or dips in activity during day time hours, giving a low within-day and inter-daily variability. Participant 4 however has a very fragmented pattern of activity accumulation within each day with some large peaks, followed by dips in activity repeated across hours of the day (shown by the black line) leading to a much higher within-day variability and intra-daily variability.

between PA and vascular health is stronger in those with Type 2 diabetes mellitus (T2DM),[6,8] interactions with T2DM status will be investigated.

2 | METHODS

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously.[9] In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of T2DM and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for reasons of efficiency. The present report includes cross-sectional data from 9188 participants who completed the baseline survey between November 2010 and October 2020. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131 088-105 234-PG). All participants gave written informed consent.

2.1 | Measurement of physical activity

PA was measured using the activPAL3 accelerometer (PAL Technologies Ltd, Glasgow, UK). The activPAL is a small lightweight (15 g) triaxial accelerometer which

records acceleration in the vertical, anteroposterior, and mediolateral axes. The activPAL has an excellent analytical validity in a laboratory setting in comparison to the gold-standard direct observation.[10] The activPAL was initialized and attached to the right front thigh in the first clinic visit. Participants were asked to wear the accelerometer for 8 consecutive days without removing it. Data from the first day were excluded (due to activities performed as part of the clinic visit) and data from the final wear day providing ≤14 waking hours of data were excluded from the analysis. Full compliance with the accelerometer protocol was defined as ≥6 days of continuous 24 h accelerometer wear. The full accelerometer processing and wake time algorithm has been described in detail by van der Berg et al., (2016).[11]

All activPAL data were uploaded using the activPAL proprietary software and data exported using the event analysis, in which activity events are classified (lying, sitting, standing, and stepping) and the time spent within that activity/posture computed. The internal equation used by the activPAL predicts activity intensity from step rate assuming a linear relationship between METs and step rate. Where possible, step rate is calculated as an average over 10 strides (equivalent to 20 individual steps).[12] A bout of stepping with a step rate of <20 steps/min was not classified as walking as this is likely to reflect posture change while standing. Step count and time spent faster and slower paced stepping were summarized into hourly time windows. A threshold of 100 steps/min was considered as "faster paced stepping" estimated to be of a moderate intensity PA.[13] Slower paced stepping was determined as stepping at a step rate below 100 steps/min, or specifically 20–99 steps/min due to the devices inability to measure steps below 20 steps/min.[12] PA pattern metrics were derived from the hourly and daily summaries for step count and time spent faster paced stepping as described below.

2.2 | Metrics evaluating the between-day patterns of stepping

Examples of high and low between-day pattern of PA are illustrated in Figure 1, Participants 1 and 2. Each daily step count was expressed as a percentage of total weekly step count. For those with 6 days accelerometer wear, an average daily step count was multiplied by 7 to get a total weekly activity equivalent to those with 7 days of PA data. From this, the most active two consecutive days were summed to obtain a continuous value of the proportion of total weekly step count accumulated in the most active two consecutive days. This variable was included as an alternative method to the commonly reported "weekend warrior" variable. Typically, the "weekend warrior" variable dichotomizes participants into categories based on whether they achieve more or less than 50% of their weekly PA in 2 days. Categorizing participants in this way is problematic due to the possibility of participants with very similar profiles (for example with 49% and 50% of activity on two most active days) being separated into different groups for analysis. The allocation of a continuous score of %PA for each participant in the current analysis overcomes this limitation.

Between-day variability (BDV) in PA was calculated as the standard deviation (SD) of differences in total step count between consecutive days of the measurement week (i.e., SD of 6 or 7 differences (for those with 6 or 7 days of PA data): between Monday and Tuesday, Tuesday and Wednesday, Wednesday and Thursday, etc.).

2.3 | Metrics evaluating the within-day pattern of stepping

Examples of high and low within-day pattern of PA are illustrated in Figure 1, Participants 3 and 4. Within-day variability (WDV) was calculated as the SD of differences between total step counts in consecutive hours of each consecutive day (i.e., SD of 144 or 168 differences (for those with 6 or 7 days of PA data, respectively): between 10 a.m. and 11 a.m. on Monday, between 11 a.m. and 12 p.m. on Monday, etc.).

Intra-daily variability (IV) is a measure of the hourly variation in step count. This gives an indication of how fragmented the activity pattern is across hours of the day. More frequent alterations between sedentary and activity bouts across hours of the day lead to a higher intra-daily variability. The intra-daily variability was calculated using the following formula[14]:

$$IV = \frac{\sum_{i=2}^{N}\left(X_i - X_{i-1}\right)^2 N}{(N-1)\sum_{i=1}^{N}\left(X_i - X_m\right)^2} \quad (1)$$

whereby N is the total number of hourly step count measures in the measurement period (24 h × 7 days a week =168, or 144 for those with 6 days of PA data). X_m is the average step count per hour, across all hours of all days (i.e., One mean of 144 or 168 values). X_i is the raw hourly data (144 or 168 individual values). Values range from 0 to 2.

2.4 | Metrics evaluating the stability of PA across days

Inter-daily stability (IS) is a measure of the regularity of PA pattern across days of the week, that is, the extent to

which the patterns of hourly step counts of individual days resemble each other. Examples of a high and low inter-daily stability are illustrated in Figure 2 and can be calculated using the following formula[14]:

$$IS = \frac{\sum_{h=1}^{p}\left(X_h - X_m\right)^2 N}{(p)\sum_{i=1}^{N}\left(X_i - X_m\right)^2} \quad (2)$$

whereby N is the total number of hourly step count measures in the measurement period (24 h × 7 days a week = 168, or 144 for those with 6 days of PA data) and p is the number of hourly step count measures per day.[24] X_m is the average step count per hour, across all hours of all days (i.e., One mean of 168 or 144 values). X_h is the average step count across different days for each hour of the day (i.e., mean of 7 or 6 values, to give 24 mean hourly values). X_i is the raw hourly data (168 or 144 individual values). Values range from 0 to 1.

2.5 | Measurement of vascular health

The specific methodology for obtaining vascular health measurements in The Maastricht Study have been described in detail previously.[15–17] In brief, all vascular measures were completed in one or two study visits. Prior to the assessment participants were asked to refrain from smoking, drinking alcohol, or caffeine-containing beverages for at least 3 h. A light low-fat meal (breakfast or lunch) was allowed if taken at least 90 min prior to the start of the measurements. Participants were asked to rest for 10 min in the supine position in a dark, quiet, temperature-controlled room (21–23°C) before commencing vascular measurements. Talking and sleeping was not allowed during the examination. All measurements were done by a trained vascular technician who was unaware of the participants clinical or activity status.

The vascular health measurements completed were as follows:

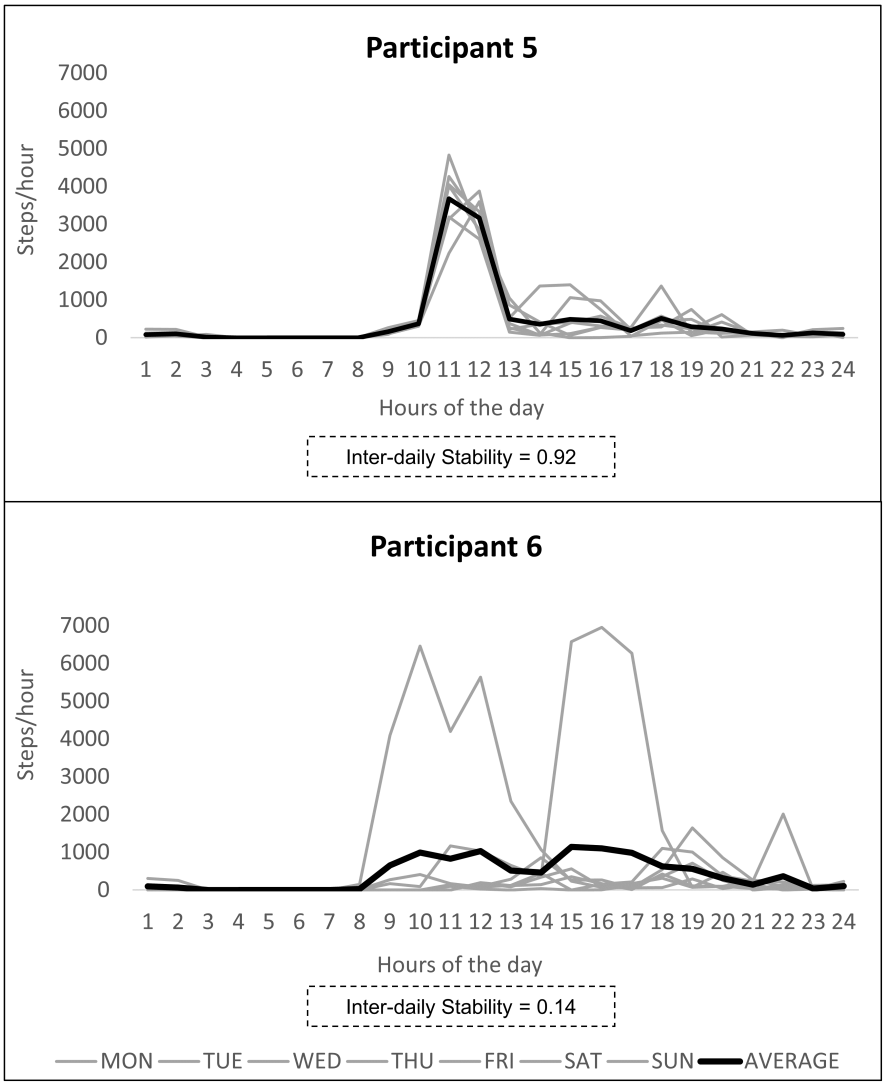

FIGURE 2 Graphical representation of real data from The Maastricht Study illustrating two participants individual day PA profile (gray lines) superimposed on their average daily PA profile (black line) showing the difference between a high and low inter-daily stability. Participant 5 has a very high inter-daily stability at 0.92 clearly indicated by how well the gray lines reflect the average black line showing that this participant does exactly the same amount of stepping activity at exactly the same times every day of the week. Participant 6 on the other hand has a low inter-daily stability at 0.14 due to obtaining a very different amount of stepping activity at different times on different days of the week; this participant shows little to no routine of activity.

1. Arterial stiffness was evaluated using carotid–femoral pulse wave velocity (cfPWV), determined according to recent guidelines[18] with the use of applanation tonometry (SphygmoCor; Atcor Medical, Sydney, Australia), as described previously.[6,15] A lower value indicates a better vascular health.
2. Skin microvascular function was evaluated using heat-induced skin hyperemia, measured with a laser Doppler system (Periflux 5000, Perimed), as the percent increase in skin blood flow on the dorsal side of the wrist of the left hand in response to local heating to 44°C as described previously.[8,17] A higher value indicates a better microvascular health.
3. Retinal vessel reactivity was evaluated by retinal dynamic vessel analysis as the arteriolar and venular dilation in response to flicker light stimulation using the Dynamic Vessel Analyzer (DVA, IMEDOS, Jena, Germany). The integrated DVA software (version 4.51, Imedos) automatically calculated baseline diameter, absolute dilation and percentage dilation, as previously described.[15,17] Due to significant associations between baseline retinal venule and arteriole diameter with total step count and time spent faster paced stepping ($p = 0.024$ to <0.001) it was deemed more appropriate to use the absolute total response to flicker light stimulation (ΔMU) rather than the percentage increase. A higher value indicates a better microvascular health in both the arteriole and venule.
4. Retinal vessel diameters were obtained using fundus photography and presented as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) in μm. Analysis was performed automatically with the RHINO software developed by the RetinaCheck Project group at the Eindhoven University of Technology (Eindhoven, the Netherlands).[17,19] A larger arteriole diameter indicates a better vascular health. In contrast, a narrower venule diameter indicates a better vascular health.

2.6 | Measurement of covariates

Full details on the measurement of covariates can be found in File S1 and are described briefly below. Demographic and lifestyle information was obtained via interview and questionnaires as follows: Age, sex (at birth), smoking behavior (never, former, and current), alcohol consumption (none, low, and high), energy intake (kcal/day), and diet quality (Dutch Healthy Diet Index[20]) by using a validated food frequency questionnaire.[21] Educational level, occupational status, equivalent income, and household income[22] were categorized into low, medium, and high. Dichotomous variables (yes/no) measured were mobility limitations,[23] presence of CVD,[24] presence of eye disease (retinopathy, glaucoma, and macular degeneration), and medications (including anti-hypertensives, lipid-modifying, and glucose-lowering medications).

Anthropometry measures included stature (m), mass (kg), waist circumference (cm), and body mass index (BMI, kg/m^2). Fasting blood samples were obtained for the measurement of glucose level (mmol/l), glycosylated hemoglobin (HbA1c), cholesterol (total, HDL, LDL, total:HDL ratio) and triglycerides. Two 24-h urine samples were collected for the calculation of estimated glomerular filtration rate (eGFR) and albuminuria. Blood pressure (including systolic, diastolic, and mean arterial pressure; SBP, DBP and MAP, respectively) (mmHg) and heart rate (bpm) was measured as both the average of three office measurements, and 24-h ambulatory blood pressure measurements.

T2DM status was determined using fasting glucose, and an oral glucose tolerance test (OGTT) was completed in all participants except those who used insulin or in those with a fasting glucose concentration >11 mmol/L. From this glucose metabolism status was calculated based on the World Health Organisation (WHO) 2006 criteria[25] and dichotomized into those with or without T2DM for main analyses, and additionally categorized into without T2DM, prediabetes or T2DM for sensitivity analyses.

2.7 | Statistical analysis

All analyses were completed using STATA v17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.) Multivariate regression was used to identify the association between average daily step count with vascular health outcomes; cfPWV, heat-induced skin hyperemia, retinal vessel reactivity, and retinal vessel diameter. Results are adjusted for major confounders, age and sex (including terms for age^2 due to the nonlinear association (vascular health drops more rapidly in old age than it does at younger ages[26]) and an age × sex interaction as vascular health drops more rapidly with age in males as it does in females[27]) and potential confounders; smoking status, alcohol intake, Dutch healthy diet index, education level, and moderators CVD and T2DM status. Additionally, interaction terms for CVD, T2DM and sex with average daily step count were included in a separate model to identify any interaction. Where there was a significant interaction, (p for interaction <0.05), results are presented separately (e.g., for those with and without T2DM). Each of the pattern metrics were then added individually to identify if there was a significant association beyond that of average daily step count. Multicollinearity in each model was checked using the variance inflation

factor (VIF). All VIFs in all models were <3, except for analysis of within-day variability with faster paced stepping in which the VIF ranged from 4.06 to 4.61.

All results presented are the fully adjusted model as described above. PA pattern metrics were alternatively calculated using the time spent faster paced stepping (min/day) instead of step count, and all analyses repeated. To adjust for multiple comparisons a significant association was taken as $p < 0.01$ (five analyses in each outcome).

Finally, a number of sensitivity analyses were completed using alternate classifications of glucose and diabetes status (3), possible mediators (2), and additional and alternate covariates (4). These are described in Tables S6–S9. For analyses of retinal vessel reactivity an additional sensitivity analysis was completed for the percent increase in retinal venule and arteriole diameters in response to flicker light stimulation, instead of the absolute change as reported in main results.

3 | RESULTS

Out of 9188 participants, 1266 were not given an accelerometer due to logistical reasons. A further 1492 participants recorded <6 days PA data, Figure 3. In comparison with those whom had valid accelerometry data for ≥6 days ($N = 6430$), those with without valid PA data were on average slightly younger, more likely to be male, had a higher BMI, waist circumference, were more likely to be categorized as obese, were less likely to smoke or drink alcohol, were more likely to have mobility limitations, T2DM, high fasting glucose, and HbA1c, higher energy consumption, higher triglycerides, and total-cholesterol-to-HDL-ratio.

Of the 6430 participants with valid PA data, 1593 had 6 days, whereas 4837 had 7 days of valid PA data. Participants with valid accelerometer data were on average 60 years old, about half of them were male, 23% had T2DM, and 17% had a history of CVD. Participants achieved on average 9566 steps/day, or 118 min/day of PA, of which 40 min/day was faster paced stepping and 78 min/day slower paced stepping. Table 1 describes the demographic, lifestyle, PA exposure, and vascular health outcome variables in the 6430 participants with valid accelerometer data. Tables S2 and S3 show multivariable regression models between pattern metrics of step count and time spent faster paced stepping and covariates to identify how the pattern metrics vary across demographic and lifestyle characteristics. In particular, a lower variability and a

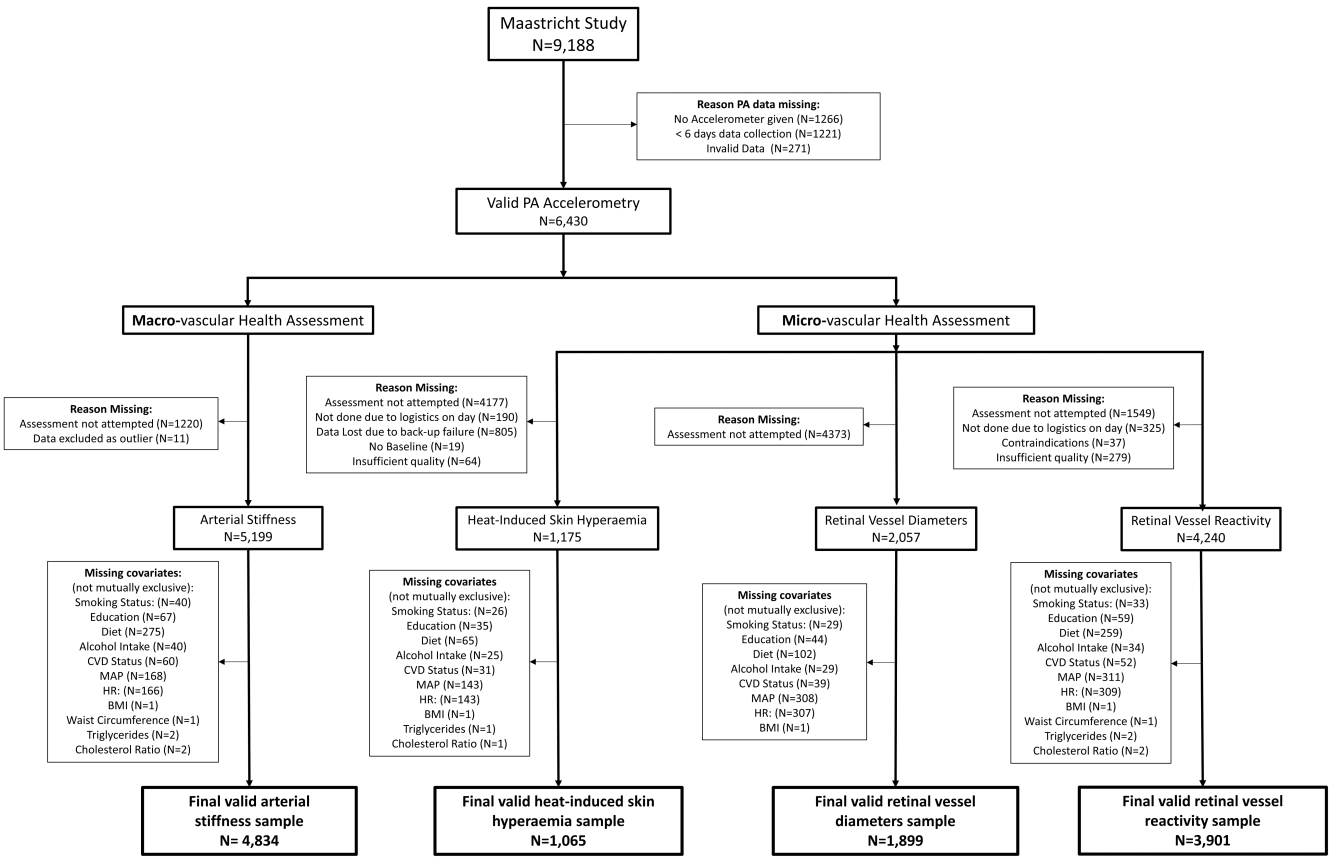

FIGURE 3 Flow diagram of missing data and final valid sample sizes within The Maastricht Study. BMI, body mass index; CVD, cardiovascular disease; HR, heart rate; MAP, mean arterial pressure; *N*, sample size; PA, physical activity.

higher inter-daily stability in PA accumulation was seen in older participants, females, smokers, those with a poorer diet, lower education, and in those with CVD or T2DM. Interestingly, the opposite was seen for those who consume a larger amount of alcohol. Differences were more pronounced when calculating pattern metrics using time spent faster paced stepping (Table S3).

Tables S4a and S4b presents inter-correlations between different pattern metrics and average step count (S4a) and time spent faster paced stepping (S4b). As the between- and within-day variability are calculated from SD of differences in step count/faster paced stepping, these two pattern metrics have higher correlations with average PA. In particular within-day variability is highly correlated with average step count ($r=0.80$) and faster paced stepping ($r=0.86$).

3.1 | Associations between stepping pattern metrics and indices of vascular health

3.1.1 | Arterial stiffness

A significant interaction was identified for T2DM status ($P_{interaction}=0.001$) and thus stratified results are presented. Higher average daily step count was associated with lower (i.e., better) cfPWV in those with and without T2DM, and the association was stronger in those with as compared to those without T2DM (Without T2DM; Std $\beta=-0.07$, $\Delta R^2=0.004$, $p<0.001$. With T2DM; Std $\beta=-0.12$, $\Delta R^2=0.012$, $p<0.001$). After adjustment for average daily step count, a higher inter-daily stability (i.e., similar amount and timing of PA on each day of the week) was associated with a higher cfPWV in those without T2DM (Std $\beta=0.04$, $\Delta R^2=0.002$, $p=0.007$), but there was no association in those with T2DM, Figure 4. This association between inter-daily stability and cfPWV remained in some, but not all sensitivity analyses, although $p<0.06$ for all (Table S6). There was no association with any other pattern metric in the main analytical model ($p=0.148$–0.970), Figure 4. In sensitivity analyses (Table S6), a higher between-day variability was associated with a lower cfPWV in those with prediabetes.

A longer time spent faster paced stepping was associated with lower (i.e., better) cfPWV in those with and without T2DM, and the association was stronger in those with T2DM (Without T2DM; Std $\beta=-0.05$, $\Delta R^2=0.003$, $p=0.001$. With T2DM; Std $\beta=-0.10$, $\Delta R^2=0.011$, $p=0.001$). A longer time spent slower paced stepping was additionally associated with lower cfPWV in those without T2DM (Std $\beta=-0.04$, $\Delta R^2=0.001$, $p=0.009$). There was no association with any pattern metric when calculated from the time spent faster paced stepping ($p=0.049$–0.909), Figure 5.

3.2 | Skin microvascular function

A significant interaction was identified for T2DM status ($P_{interaction}<0.001$) and thus results are presented separately. Higher average daily step count was associated with a higher (i.e., better) heat-induced skin hyperemia in those with T2DM (Std $\beta=0.27$, $\Delta R^2=0.060$, $p<0.001$). This positive association remained in all sensitivity analyses (Table S7). There was no association beyond that of average daily step count in participants with or without T2DM for any of the step count pattern metrics in main analyses ($p=0.117$–0.801), Figure 4, or in any sensitivity analyses (Table S7).

A higher time spent faster paced stepping was associated with a higher (i.e., better) heat-induced skin hyperemia in participants with T2DM (Std $\beta=0.25$, $\Delta R^2=0.061$, $p<0.001$), Figure 5 and Table S5. After adjustment for time spent faster paced stepping, a higher within-day variability was associated with a lower (i.e., worse) heat-induced skin hyperemia in those with T2DM (Std $\beta=-0.31$, $\Delta R^2=0.020$, $p=0.008$), Figure 5.

3.3 | Retinal vessel reactivity

In analyses of retinal vessel reactivity, no interaction effects with potential moderators were found ($P_{interaction}>0.05$); thus results are presented for the whole cohort. There was no association between average daily step count, or time spent faster paced stepping with either venular or arteriolar reactivity, Figure 6. After adjustment for multiple testing there was no association for any of the pattern metrics with arteriole reactivity ($p=0.011$–0.908) or venule reactivity ($p=0.282$–0.940), Figure 6. Additionally, there was no association between any pattern metrics calculated from time spent faster paced stepping ($p=0.414$–0.973), Figure 7.

In sensitivity analyses, when glucose metabolism status was alternatively defined using a threshold of above or below 6.5% HbA1c, a higher proportion of PA completed in the most active consecutive 2 days was associated with a lower (worse) arteriole reactivity (Std $\beta=-0.04$, $p=0.008$), Table S8. This pattern metric was borderline significance after adjustment for multiple testing ($p=0.011$) in the main analytical model. An additional sensitivity analysis was completed for retinal vessel reactivity whereby the percent increase in diameter in response to flicker light stimulation was used as the outcome of interest instead

TABLE 1 Participant characteristics, for participants with valid accelerometry data (24 h for >6 days). Left: demographic characteristics. Middle: PA exposure variables and pattern metrics. Right: vascular health outcome variables.

Demographic and lifestyle characteristics			PA exposure variables and pattern metrics		Vascular health outcome variables		
	N	Mean ± SD		Mean ± SD		*N*	Mean ± SD
Age (Years)	6430	60.2 ± 8.6					
Sex (% Male)	6430	49.2	**Average daily PA**		**Arterial stiffness**		
BMI (kg/m^2)	6429	26.7 ± 4.3	Time spent stepping (min/day)	118.3 ± 39.3	cfPWV (m/s)	5199	8.99 ± 2.14
Waist circumference (cm)	6429	94.5 ± 13.3	Step count (steps/day)	9566 ± 3622			
24 hr SBP (mmHg)	5667	118.8 ± 11.6	Faster paced stepping (min/day)	39.9 ± 22.7	**Skin microvascular function**		
24 hr DBP (mmHg)	5667	72.8 ± 7.2	Slower paced stepping (min/day)	78.3 ± 26.5	Baseline skin blood flow (PU)	1175	11.1 ± 6.5
MAP (mmHg)	5320	96.7 ± 10.6	Sedentary time (min/day)	560.5 ± 98.8	Heat-induced skin hyperemia (%)	1175	1125.2 ± 765.4
HR (bpm)	5322	61.7 ± 9.3	Waking time (hours/day)	941.0 ± 53.1	Heat-induced skin hyperemia (PU)	1175	112.4 ± 57.1
BMI Category (%)	6429						
Normal weight		37.8	**Step count PA pattern metrics**		**Retinal vessel reactivity**		
Overweight		42.6	Most active 2 days (consecutive %)	37.9 ± 5.1	Baseline arteriolar diameter (MU)	4241	115.4 ± 15.7
Obese		19.6	Between-day variability (BDV)	5137.1 ± 3064.3	Arteriolar response to flicker light (%)	4240	3.02 ± 3.04
Education (%)	6339		Within-day variability (WDV)	701.7 ± 293.9	Arteriolar response to flicker light (MU)	4240	4.36 ± 3.83
Low		33.8	Intra-daily variability (IV)	1.2 ± 0.3			
Medium		26.9	Inter-daily stability (IS)	0.4 ± 0.1	Baseline venular diameter (MU)	4340	147.2 ± 21.0
High		39.3			Venular response to flicker light (%)	4339	3.80 ± 2.38
Occupational status (%)	2504				Venular response to flicker light (MU)	4339	7.47 ± 4.21
Low		34.6	**Faster-paced stepping PA pattern metrics**				
Intermediate		32.5	Most active 2 days (consecutive %)	41.8 ± 7.7	**Retinal vessel diameters**		
High		32.9	Between-day variability (BDV)	30.1 ± 22.9	Venular diameter (μm)	2057	214.3 ± 31.1
Smoking status (%)	6377		Within-day variability (WDV)	4.1 ± 2.4	Arteriolar diameter (μm)	2057	141.9 ± 20.3
Never		38.8	Intra-daily variability (IV)	1.3 ± 0.3			
Former		49.2	Inter-daily stability (IS)	0.3 ± 0.1			
Current		12.0					
Alcohol consumption (%)	6376						

(Continues)

TABLE 1 (Continued)

Demographic and lifestyle characteristics			PA exposure variables and pattern metrics	Vascular health outcome variables	
	N	Mean ± SD	Mean ± SD	*N*	Mean ± SD
None		17.8			
Low		59.8			
High		22.4			
Mobility limitations (% yes)	6339	19.9			
Diabetes status (% yes)	6430	21.3			
Medication for T2DM (%)	6422	16.0			
Fasting glucose (mmol)	6429	5.8 ± 1.5			
HbA1c (mmol/mol)	6423	38.9 ± 9.1			
Dutch Health Diet Index	5987	84.6 ± 15.1			
Energy intake (kcal)	5987	2106 ± 597			
History of CVD (%)	6352	16.7			
Hypertension (%)	6418	53.2			
Antihypertensive use (%)	6422	36.8			
Triglycerides (mmol/l)	6427	1.39 ± 0.87			
Cholesterol-to-HDL ratio	6427	3.6 ± 1.2			
Lipid-lowering medication (%)	6422	29.7			
GFR	2237	87.7 ± 14.7			

Abbreviations: BMI, body mass index; cfPWV, carotid–femoral pulse wave velocity; CVD, cardiovascular disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; HR, heart rate; MAP, mean arterial pressure; *N*, sample size; PA, physical activity; SBP, systolic blood pressure; SD, standard deviation; T2DM, Type 2 diabetes mellitus.

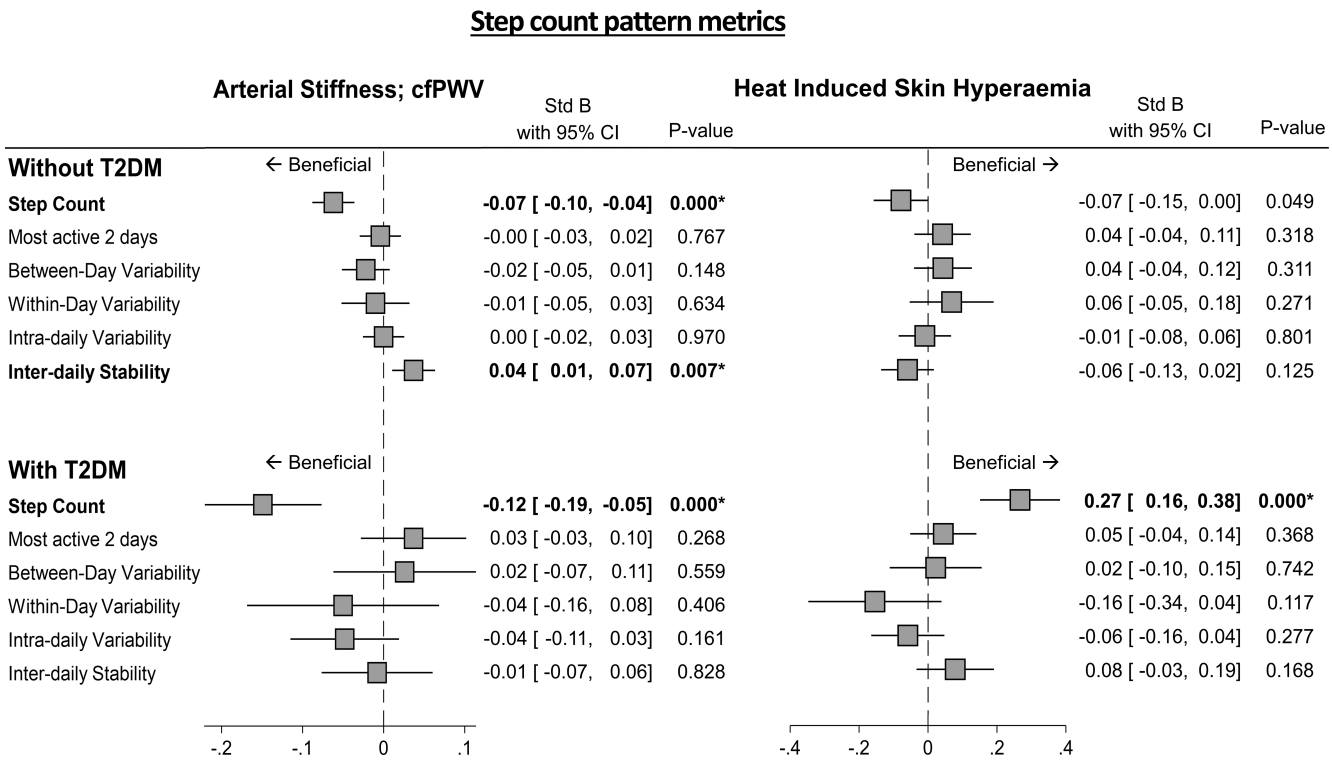

FIGURE 4 Forest plots displaying the standardized beta (Std β) (gray squares) and standardized 95% confidence intervals (black lines) for average daily step count and each of the step count pattern metrics including for cfPWV (left) and heat-induced skin hyperemia (right). To adjust for multiple testing a p-value <0.01 was interpreted as significant, indicated by bold text*.

of the absolute change in diameter. Associations did not change (Table S8).

3.4 | Retinal vessel diameters

In analyses of retinal vessel diameters, no interaction effects with potential moderators were found ($P_{interaction}$ >0.05) thus results are presented for the whole cohort. There was no association between average daily step count or time spent faster paced stepping with either arteriolar or venular diameters (p = 0.137–0.984), Figures 6 and 7. Addition of pattern metrics did not change the model in the arteriole. In the venule, a higher inter-daily stability was associated with a higher (i.e., worse) venule diameter (inter-daily stability Std. β = 0.07, ΔR^2 = 0.005, p = 0.002). This association remained similar in most, but not all sensitivity analyses (Table S9).

When calculating pattern metrics using time spent faster paced stepping a higher inter-daily stability was associated with a higher (i.e., worse) venule diameter after adjustment for time spent faster and slower paced stepping (inter-daily stability std β = 0.07, ΔR^2 = 0.004, p = 0.007), Figure 7.

4 | DISCUSSION

This cross-sectional analysis aimed to investigate the association between the pattern of physical activity accumulation on arterial stiffness and multiple measures of microvascular health in middle-aged and older adults. The main findings are as follows: First, the association between average PA volume and both cfPWV and heat-induced skin hyperemia were stronger in those with T2DM compared to without. Second, after adjustment for PA volume, a higher inter-daily stability (i.e., similar amount and timing of PA on each day of the week) was associated with a higher (worse) cfPWV in people without T2DM and with retinal venular diameter in the whole cohort. Third, although heat-induced skin hyperemia was unrelated to step count pattern metrics, a higher within-day variability of time spent faster paced stepping was associated with a lower (worse) heat-induced skin hyperemia in those with T2DM. These results suggest that over and above PA volume, the pattern in which PA is accumulated may impact vascular health and that these associations may differ depending on PA intensity and metabolic health.

The apparent benefit of a less stable PA profile over the course of a week (lower inter-daily stability) could

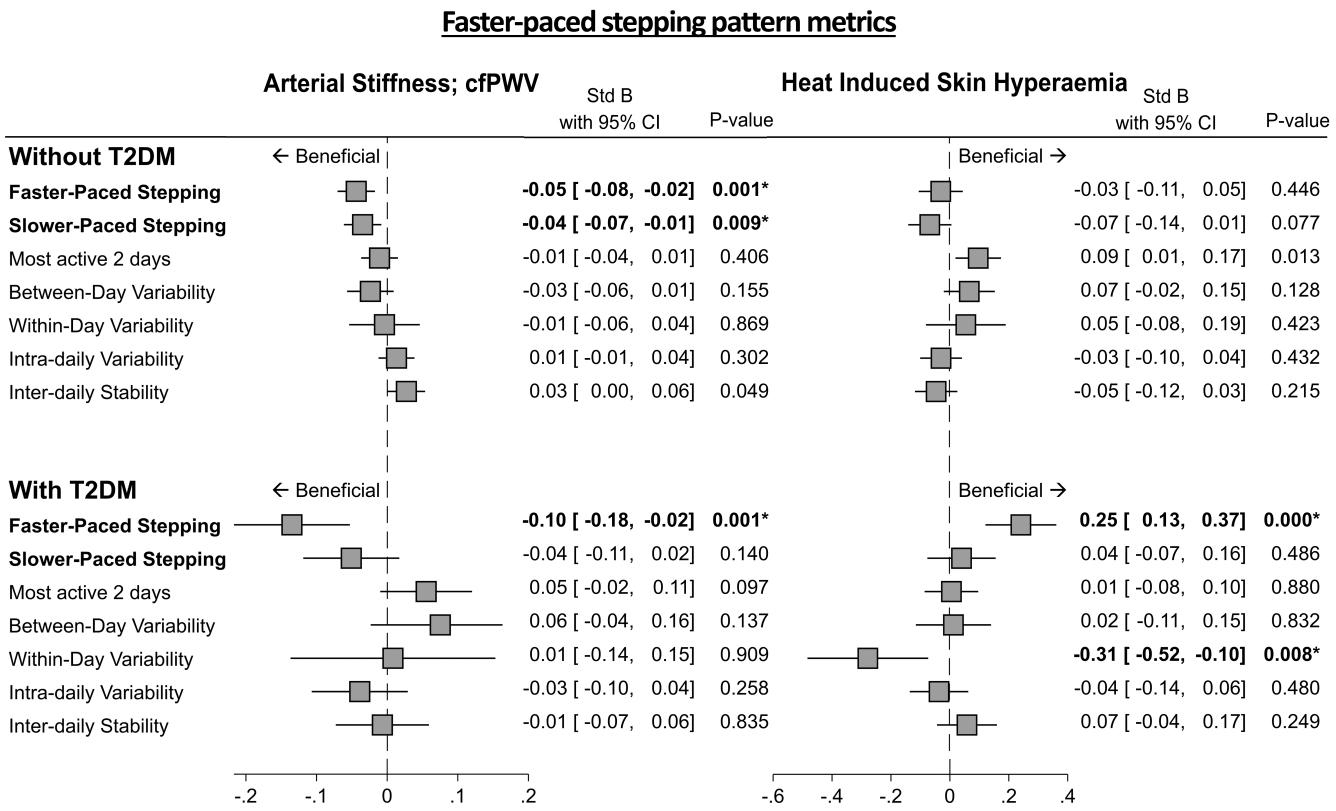

FIGURE 5 Forest plots displaying the standardized beta (Std β) (gray squares) and standardized 95% confidence intervals (black lines) for average daily time spent faster and slower paced stepping and each of the faster paced stepping pattern metrics including for cfPWV (left) and heat-induced skin hyperemia (right). To adjust for multiple testing a p-value <0.01 was interpreted as significant, indicated by bold text*.

be the presence of structured exercise sessions. Although the total volume of PA was adjusted for in all analytical models, a less stable profile of PA by definition means that more PA is accumulated on some hours/days than others, and the bigger the difference between hours/days the less stable the profile. During exercise, a large volume of PA is accumulated in a short period of time resulting in a greater challenge to the vascular system, which, over time is likely to elicit changes to the structure of the vascular system.[28] In contrast, those with a more stable daily routine of PA (with the same overall volume) will likely accumulate that PA in more frequent, shorter and less intense doses on each day of the week. Thus, more frequent, yet smaller challenges to the vascular system, while it may be enough to influence acute vascular function (measured by flow-mediated dilation),[4] may not be a large enough stimulus to elicit chronic adaptations to the structure of the vascular system in the form of cfPWV and retinal venular diameter.

Another possible explanation for the poorer vascular health observed in those with more stable activity profiles is the accumulation of occupational PA. Those who complete the majority of PA as part of their regular work may have a higher stability across days, as this PA will likely be completed at the same times on each workday. While still controversial, there is some evidence to suggest that occupational activity may not confer the same benefits as leisure time activity due to the nature of the specific activities which tend to be of a lower intensity, repetitive, and without sufficient recovery time, and may also have higher exposure to environmental hazards and stress.[29]

In the retinal microvasculature there was no association with average step count or time spent faster- paced stepping, whereas there was a strong association between inter-daily stability and retinal venule diameter. This has not been reported before and suggests in this cohort it may be the pattern, not the amount of PA that associate with vascular health in the retina. However, these findings contrast previous research which suggests a larger volume of PA, as well as exercise intervention studies improve retinal vessel diameters.[30,31]

In analysis of the heat-induced skin hyperemia there was no association between any PA pattern metrics when using step count as the variable of determination, but there was a strong negative association with within-day variability in those with T2DM when using the time spent faster paced stepping to calculate the pattern metrics. This

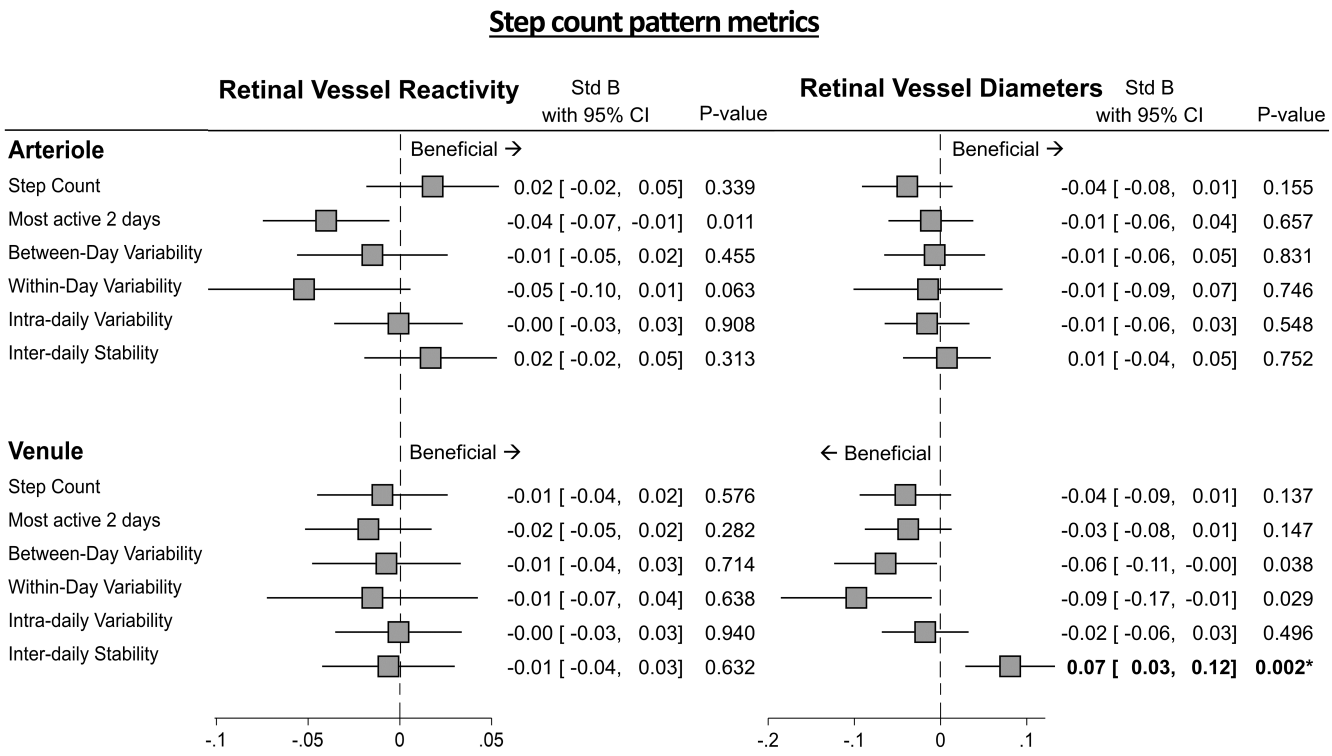

FIGURE 6 Forest plots displaying the standardized beta (Std β) (gray squares) and standardized 95% confidence intervals (black lines) for average daily step count and each of the step count pattern metrics for retinal vessel reactivity (left) and retinal vessel diameters (right). To adjust for multiple testing a p-value <0.01 was interpreted as significant, indicated by bold text*.

suggests that it is the pattern of higher intensity stepping activity only that influences skin microvascular function in those with T2DM, rather than the pattern of stepping at all intensities. This may be due to the thermoregulation of the skin microvasculature.[32]

While further research is required to fully understand these associations, we have shown that it is not necessarily the amount of PA alone that benefits vascular health, but rather the pattern of PA accumulation may also have an impact. In particular, accumulating weekly PA with a lower inter-daily stability (i.e., irregular amounts of PA across the day/week) is beneficially associated with vascular health in those without T2DM, whereas accumulating faster paced stepping with a lower variability (i.e., higher intensity PA spread more evenly throughout the day) is beneficially associated with skin microvascular health in those with T2DM. This is important because it suggests some interplay between the physiological mechanisms underpinning the beneficial effect of PA on vascular health, and the underlying physiology in the pathology of T2DM.

Given the cross-sectional and exploratory nature of the present analysis, specific recommendations on how people should move differently to improve their vascular health is currently premature. However, these results do suggest for the first time that the impact of PA on vascular health moves beyond the simple notion that "more is better" and future guidance could additionally begin to incorporate how to "move differently."

5 | STRENGTHS AND LIMITATIONS

This study is the first to evaluate the associations of differences in PA patterning, beyond that of total PA volume, with multiple measures of vascular health. A strength of this work is the adjustment for total volume of PA in all analytical models as well as the careful selection of covariates, with comprehensive sensitivity analyses. This allowed us to draw firm conclusions on the independent contribution of PA pattern (beyond that of average volume of PA) on indices of vascular health. However, due to the cross-sectional nature of data collection, the direction of causality in the current analysis cannot be determined and the possibility of unmeasured or residual confounding remains. We limited the analyses to those with hour-by-hour 24 hr PA data for ≥6 days, which permitted novel analyses into PA pattern without substantially limiting the analytical sample size, and without need for data imputation. However, we acknowledge that, like many studies employing single short accelerometer wear periods, the behavior captured may not always represent habitual behavior. Additionally, summarizing the data into

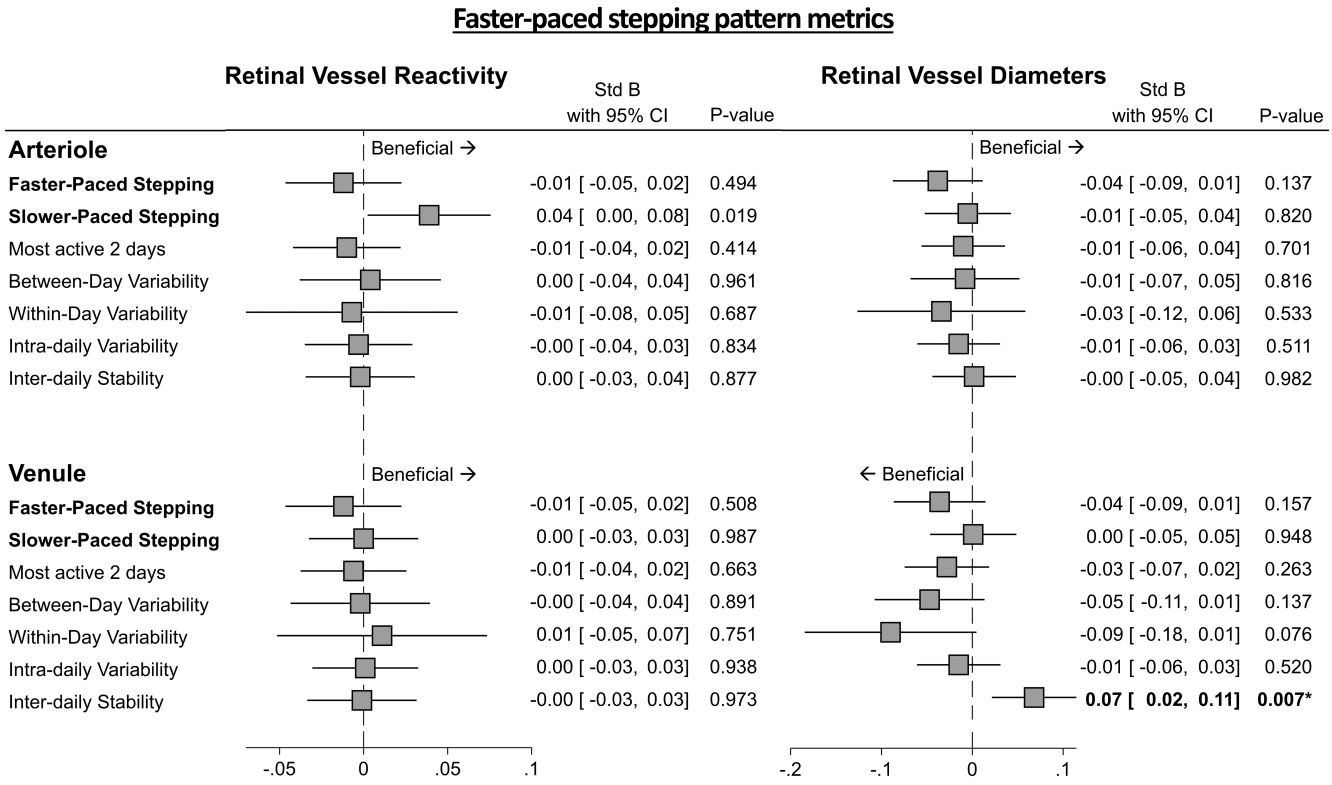

FIGURE 7 Forest plots displaying the standardized beta (Std β) (gray squares) and standardized 95% confidence intervals (black lines) for average daily time spent faster and slower paced stepping and each of the faster paced stepping pattern metrics for retinal vessel reactivity (left) and retinal vessel diameters (right). To adjust for multiple testing a p-value <0.01 was interpreted as significant, indicated by bold text*.

hour-by-hour time windows may mask important differences in how PA events occur within each hour. With advancing accelerometer analysis techniques it is possible for future work to utilize event-based PA data where each PA event is described separately (type, duration, and intensity) rather than aggregated into hourly summary measurements.[33,34]

The activPAL accelerometer collects and summarizes PA data using step counts which are derived from raw acceleration values. Stepping is the most commonly performed habitual PA, is easy to understand and implement on a population level which facilitates interpretation of these results to the general population. The activPAL has an excellent analytical validity in a laboratory setting in comparison to the gold-standard direct observation with an error rate of <1% for detection of steps between 0.67 and 1.56 m/s (equivalent to step rates of approximately between 75 and 120 steps/min).[10] However, it is limited in its ability to accurately detect stepping activity at both extremes of low[35–37] and higher step rates.[38] Future research could consider combining the device-based measurement of habitual PA with contemporaneous contextual information through self-report in order to identify different modalities of habitual PA that accumulate in different patterns. The addition of this information in the present analysis would have been useful to further understand whether the detrimental impact of a high inter-daily stability is in fact explained by a lack of very large bouts of physical exercise sessions, or alternatively driven by a higher level of occupational activity.

5.1 | Perspective

This analysis has provided the first evidence that different patterns in which stepping activity is accumulated, both across days of the week and across hours of the day, has a differential association with vascular health, beyond that of PA volume. Additionally, we have shown that these associations differ depending on T2DM status. In particular, in those without T2DM, accumulating a given volume of PA with lower inter-daily stability (possibly indicative of higher doses of PA on a smaller number of days) could potentially confer the greatest benefit to vascular health. In contrast, in those with T2DM, while the total volume of PA remains most important overall, faster paced stepping with a lower variability (spread more evenly throughout hours of the day) may be more beneficial for skin microvascular health. While specific recommendations on how to move differently are at this stage premature, this may

have important implications for those at risk of vascular disease if these findings are confirmed in future prospective studies. We strongly recommend future research to consider the pattern of PA accumulation, and move away from simple daily aggregate measures of volume of PA.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data of this study derived from The Maastricht Study, but restrictions apply to the availability of these data, which were used under license for the current study. Data are, however, available from the authors upon reasonable request and with permission of The Maastricht Study management team.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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