Physical Activity Is Associated With Glucose Tolerance Independent of Microvascular Function: The Maastricht Study

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Context and Objective: Moderate-to-vigorous physical activity (MVPA) and physical fitness (PF) are positively associated with glucose tolerance. Such associations may be partly conditioned by microvascular function, which is a common correlate to MVPA, PF, and glucose tolerance. To test this hypothesis, the present study sought to investigate independent associations of MVPA and PF with glucose tolerance and to what extent these associations are mediated by microvascular function.

Design, Setting, Participants, and Outcome Measures: Data from The Maastricht Study were used (n = 512 for MVPA and n = 488 for PF analyses; mean age, 59 [SD = 9] y, 52 % men). Glucose tolerance was assessed by 2-hour postload plasma glucose levels (2hPG). The total number of weekly hours of MVPA was estimated with the Community Healthy Activities Model Program for Seniors questionnaire. Walking speed during the 6-minute walk test was used to evaluate PF. Microvascular function was determined by postocclusive capillary recruitment and flowmotion with capillaroscopy and laser Doppler flowmetry in skin microcirculation.

Results: In univariate analyses, MVPA, PF, and microvascular function variables were associated with 2hPG. MVPA (n = 512, $\beta = -0.056$, $P = .019$) and PF (n = 488, $\beta = -0.368$, $P = .006$) remained associated with 2hPG after adjustment for established cardio-metabolic risk factors and history of cardiovascular disease; addition of microvascular function variables as potential mediators did not materially change the associations of MVPA ($\beta = -0.054$, $P = .024$) and PF ($\beta = -0.364$, $P = .006$) with 2hPG. No mediation effects of microvascular function variables were detected.

Conclusions: MVPA and PF were independently associated with 2hPG, irrespective of established risk factors and generalized microvascular function. The possibility that specific microvascular functions, eg, insulin-mediated vasodilation, influence the association of MVPA and PF with 2hPG needs further investigation. **(*J Clin Endocrinol Metab* 101: 3324–3332, 2016)**

Exercise prescription is considered a central strategy aimed to improve glucose tolerance (1). The association of moderate-to-vigorous physical activity (MVPA) and cardiorespiratory fitness (henceforward physical fitness [PF]) with glucose tolerance is well established but remains incompletely explained (2–4). Theoretically, any factor influencing glucose absorption, production, transport, and/or utilization may contribute to glucose tolerance, as assessed by the standard 2-hour oral glucose tolerance test (OGTT) (5).

Sound evidence demonstrates that higher levels of MVPA, and thereby PF, are associated with myocyte phenotypic modifications including increases in insulin sensitivity, glucose transporters as well as enzymes responsible for the phosphorylation, storage, and oxidation of glucose (6–8), all of which potentially improve glucose

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Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; LDF, laser Doppler flowmetry; MVPA, moderate-to-vigorous physical activity; OGTT, oral glucose tolerance test; PF, physical fitness; 2hPG, 2-hour postload plasma glucose levels; POCR, postocclusive capillary recruitment; PSD, power spectral density; SBP, systolic blood pressure; SFM, skin flowmotion; T2DM, type 2 diabetes mellitus.

tolerance. Additionally, regular exercise may facilitate nutrient and hormone delivery to insulin-dependent tissues through adaptations in microvascular function (9, 10). These include enhanced endothelium dependent and independent dilator responses (11) as well as increased microvascular vasomotion, ie, the spontaneous changes in arteriolar diameter that regulate blood flow distribution (12). In support of the "vascular hypothesis," changes in microvascular function and glucose homeostasis with exercise training commonly run in parallel to each other (13, 14). Conversely, short-term physical inactivity leads to microvascular dysfunction and decreased glucose tolerance (15). Thus, the relationship between MVPA, PF, and glucose tolerance could be determined, at least in part, by concurrent changes in microvascular function. However, this has not yet been tested.

The aim of the present study was to investigate the associations of MVPA and PF with glucose tolerance and whether microvascular function may, to a degree, explain these associations in a population-based setting. We hypothesized that MVPA and PF are positively associated with glucose tolerance and the strength of the associations are partly explained by microvascular function.

Materials and Methods

Study population

In this study, we used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously (16). In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus (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 for reasons of efficiency. The present report includes cross-sectional data from the first 866 participants, who completed the baseline survey between November 2010 and March 2012. 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, on the basis of the Health Council's opinion (permit 131088-105234-PG). All participants gave written informed consent. For the present analyses, participants were selected in whom data were available on 2-hour postload plasma glucose levels (2hPG), microvascular function, covariates (see below), and MVPA (n = 512) or PF (n = 488) (Figure 1).

Glucose tolerance

The study participants, except those who used insulin (n = 56), underwent a standardized 2-h 75-g OGTT according to WHO guidelines (5). For safety reasons, the OGTT was not conducted in individuals with a fasting glucose level above 11.0 mmol/L, as determined by a finger prick. Although glucose tolerance can be defined by fasting glucose levels, these are primarily affected by β-cell function (17), which is not considered a key physiological parameter to the purpose of this study. The 2hPG was used as the outcome measure and was assessed with a standard enzymatic hexokinase reference method.

Moderate-to-vigorous PA

MVPA was determined through 28 items from the modified Community Healthy Activities Model Program for Seniors questionnaire (18), which evaluates the frequency of an activity (times per week) and its duration (hours per week). Activities that were recorded included: walking leisurely/fast/briskly, cycling leisurely/fast/briskly, light/heavy gardening, light/heavy housekeeping, jogging/running, swimming, tennis/badminton/table tennis, team sport indoors/outdoors, light exercise to maintain a physical condition (eg, stretching/flexibility), and heavy aerobic/strength exercise. Metabolic equivalents were estimated for each activity on the basis of the PA compendium by Ainsworth et al (19). Only activities with at least 3 metabolic equivalents were considered as requiring moderate or vigorous intensity for all participants; fast walking, fast cycling, heavy gardening, heavy household work, jogging/running, swimming, tennis, team sport, and heavy aerobic/strength exercise (20). The total number of weekly MVPA hours was used as a study variable.

Physical fitness

The walking speed during the fast 6-minute walk test was taken as a proxy for PF. Participants were excluded if any of these conditions were reported: ambulatory aid, myocardial infarction, angioplasty, heart surgery, angina pectoris/chest pain, shortness of breath, or fainting. The included participants were instructed to walk from one end to the other of a 20-m hallway as fast as possible while attempting to cover as much ground as possible in the allotted 6 minutes. Technicians encouraged the participants with the standardized statements "you're doing well" or "keep up the good work" but were asked not to use other phrases. Participants were allowed to stop and rest during the test but were instructed to resume walking as soon as they felt able to do so.

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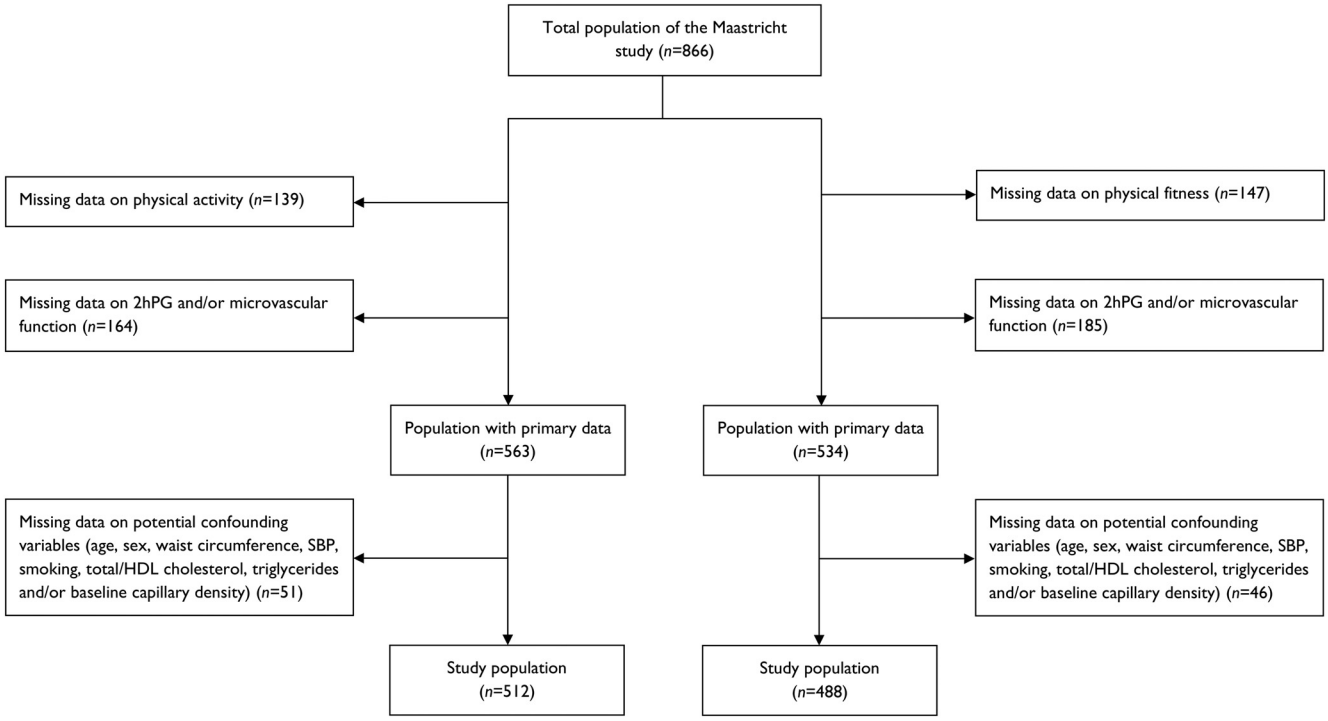

Figure 1. Flow diagram.

Microvascular function

All vascular measurements were performed by trained technicians unaware of the participant clinical status, in quiet, temperature-controlled room (24°C) after 10 minutes of acclimatization with the participants in supine position, as previously described (21, 22). Participants were allowed to have a light meal (breakfast and/or lunch) and were asked to refrain from smoking and caffeine intake 3 hours before the beginning of the measurements.

Postocclusive capillary recruitment (POCR) during reactive hyperemia was determined in the dorsal skin of the distal phalanges of the third and fourth finger of the right hand, using a digital video microscope (Capiscope; KK Technology) (21). Reactive hyperemia was induced by inflating a miniature cuff encircling the base of the finger inflated to suprasystolic pressure (260 mm Hg) for 4 minutes. Capillaries were visualized 4.5 mm proximal to the terminal row of capillaries in the middle of the nailfold, where capillaries run perpendicularly to the skin, with a region of interest of 1 mm^2. Capillary density was defined as the number of erythrocyte-perfused capillaries per mm^2 of skin counted during 15 seconds. The number of continuously perfused capillaries was counted using a semiautomatic procedure (CapiAna) and running movie files (21). POCR was calculated by subtracting the baseline capillary density from the peak capillary density during reactive hyperemia. In addition, the maximal number of capillaries during venous occlusion (digital cuff inflated to 60 mm Hg for 120 s) was determined (23). The intra- and interobserver coefficient of variations of CapiAna were 2.5% and 5.6%, respectively.

Skin flowmotion (SFM) was assessed by means of a laser Doppler system (Periflux 5000; Perimed) equipped with a thermostatic laser Doppler probe (PF 457; Perimed) at the dorsal side of the left wrist. Skin temperature was monitored continuously and maintained at a minimum of 30°C. The laser Doppler flowmetry (LDF) output was recorded for 25 minutes at a sample rate of 32 Hz. Fast Fourier transform algorithm was performed using Perisoft dedicated software (PSW version 2.50) to measure the power density of the LDF oscillation. The frequency spectrum between 0.01 and 1.6 Hz was divided into 5 SFM components: 1) endothelial, 0.01–0.02 Hz; 2) neurogenic, 0.02–0.06 Hz; 3) myogenic, 0.06–0.15 Hz; 4) respiratory, 0.15–0.40 Hz; and 5) heartbeat, 0.40–1.60 Hz (24). Each LDF frequency component was first quantified in raw power spectral density (PSD) units. PSD data are influenced by the variation in the LDF signal strength between experiments (24). Therefore, the PSD value of each frequency component was normalized, defined as the percentage ratio between the PSD value of that frequency subinterval and the PSD value of the total spectrum from 0.01 to 1.60 Hz (obtained by the sum of the PSD value of each frequency subinterval) (25). Finally, a composite microvascular SFM was calculated as the sum of endothelial, neurogenic, and myogenic normalized (%) components.

Covariates

Covariates included age, sex, waist circumference, systolic blood pressure (SBP) (Omron 705IT), smoking, total to high-density lipoprotein (HDL) cholesterol ratio (Beckman Synchron LX20; Beckman Coulter, Inc), triglycerides (Beckman Synchron LX20; Beckman Coulter, Inc), baseline capillary density (CapiAna), hypertension (as defined by average SBP $\geq$ 140 mm Hg, diastolic BP $\geq$ 90 mm Hg, and/or use of antihypertensive medication), medication for hypertension, medication for hyperlipidemia, and history of cardiovascular disease (CVD) according to a modified version of the Rose questionnaire (16, 26).

Statistical analysis

All statistical analyses were performed using IBM SPSS version 20 software package. Because of nonnormal distribution, all variables were logarithmically transformed to approximate nor-

mality before parametric testing. Differences in clinical status between excluded and included individuals were assessed in order to explore the presence of selection bias. Likewise, differences in clinical status, microvascular function, MVPA and PF among tertiles of 2hPG were tested with ANOVA or the χ^2 test as appropriate. Univariate associations of 2hPG with and among covariates, microvascular function, MVPA and PF were determined by means of Pearson's correlation coefficients (r). Multiple linear regression models were used to examine the independent association of MVPA and PF with 2hPG (Model 1). Microvascular function variables such as POCR and composite SFM were added as potential mediators to the regression models 2 and 3, respectively, using methods developed by Preacher and Hayes (27). Mediation occurs when a determinant affects a dependent variable indirectly through at least one interceding variable, or mediator. In model 4, both POCR and composite SFM were added as potential mediators in a simultaneous manner. All models were adjusted for the following covariates: age, sex, waist circumference, SBP, pack years of smoking, total to HDL cholesterol ratio, triglycerides, baseline capillary density, presence of hypertension, medication for hypertension, medication for hyperlipidemia, and history of CVD. Covariates may correlate to the variables under investigation and thereby affect (confound) their associations. The Sobel test was conducted to determine the significance of any potential mediation effect. In addition, we used interaction terms to explore whether any association differed according to sex, presence of hypertension or previous CVD. A 2-tailed $P < .05$ (0.10 for interactions) was considered significant.

Results

Clinical variables, microvascular function, MVPA, and PF

Table 1 presents the characteristics of the study population across tertiles of 2hPG in the participants included in the MVPA analysis (n = 512). Equivalent data were observed in the PF analysis (data not shown). The study population was comprised of 245 (52.1%) males, 110 individuals (21.5%) with T2DM, and 266 individuals (52.0%) with hypertension. All clinical variables evaluated, except for baseline capillary density, were different across tertiles of 2hPG ($P < .05$). As regards microvascular function, all variables, except for respiratory SFM, were different across tertiles of 2hPG ($P < .05$). With respect to MVPA and PF, both were different across tertiles of 2hPG ($P < .05$).

Univariate analyses

The univariate linear correlation of covariates, microvascular function, and MVPA/PF with 2hPG is illustrated in Tables 2 and 3. All potential confounders (except for total to HDL cholesterol ratio and baseline capillary density) were positively correlated to 2hPG, whereas microvascular function variables and MVPA were negatively correlated to 2hPG (Table 2). With respect to the PF analysis (Table 3), all covariates (except for baseline capillary density) were positively correlated to 2hPG, whereas microvascular function variables and PF were negatively correlated to 2hPG.

As for univariate linear correlations of MVPA/PF with microvascular function measures, MVPA was not correlated to microvascular function ($P \geq .1$), except if considering females alone (n = 245), in whom MVPA was positively correlated to endothelial SFM ($r = 0.17$, $P = .007$), neurogenic SFM ($r = 0.15$, $P = .021$), and composite microvascular SFM ($r = 0.16$, $P = .014$). In turn, PF was positively correlated to endothelial SFM ($r = 0.12$, $P = .010$), neurogenic SFM ($r = 0.15$, $P < .001$), myogenic SFM ($r = 0.18$, $P < .001$), and composite microvascular SFM ($r = 0.18$, $P < .001$) in the study population but was not correlated to POCR unless considering males alone (n = 255) ($r = 0.15$, $P = .014$).

Independent associations

Tables 4 (MVPA analysis) and 5 (PF analysis) show multiple regression models regarding the independent association of MVPA/PF with 2hPG. All regression models were adjusted for age, sex, waist circumference, SBP, pack years of smoking, total to HDL cholesterol ratio, triglycerides, baseline capillary density, presence of hypertension, medication for hypertension, medication for hyperlipidemia, and history of CVD. MVPA was associated with 2hPG ($\beta = -0.056$, $P = .019$) (Table 4, model 1). The addition of microvascular function variables as potential mediators did not materially change this association ($\beta = -0.054$, $P = .024$) (Table 4, model 4). Likewise, PF was associated with 2hPG ($\beta = -0.368$, $P = .006$) (Table 5, model 1). The addition of microvascular function variables as potential mediators did not materially change this association ($\beta = -0.364$, $P = .006$) (Table 5, model 4). There were no mediation effects of microvascular function variables in any model (P for mediation $\geq .387$).

Additional analyses

Clinical variables such as age, waist circumference, body mass index, SBP, fasting glucose, pack/years of smoking, presence of hypertension, medication for hypertension and history of CVD presented higher values, whereas HDL cholesterol and low-density lipoprotein cholesterol were lower, in the excluded (n = 354 and n = 378 in the MVPA and PF analyses, respectively) compared with the included participants ($P < .05$).

There was no interaction for the association of MVPA and PF with 2hPG according to sex, presence of hypertension or previous CVD, in unadjusted analyses and after adjustment for potential covariates and microvascular function variables. In addition, analyses with baseline cap-

Table 1. Clinical Variables, Microvascular Function, MVPA, and PF Across Tertiles of 2hPG[a]

	Study Population (n = 512)			
	T1 (2.5–5.4 mmol/L) (n = 171)	T2 (5.5–7.6 mmol/L) (n = 171)	T3 (7.7–25.2 mmol/L) (n = 170)	*P*
Clinical variables				
Age (y)	55.5 ± 8.2	58.4 ± 8.2	62.4 ± 7.6	<.001
Sex (male)	78 (45.6)	83 (48.5)	106 (62.6)	.004
BMI (kg/m^2)	25.6 ± 4.1	26.6 ± 3.9	28.9 ± 4.7	<.001
Waist circumference (cm)	91.2 ± 12.5	94.8 ± 11.6	102.1 ± 12.8	<.001
Male (cm)	97.6 ± 10.2	99.6 ± 9.5	104.4 ± 11.1	<.001
Female (cm)	85.9 ± 11.8	90.1 ± 11.5	98.2 ± 14.5	<.001
Heart rate (bpm)	65.4 ± 9.3	66.7 ± 10.1	71.5 ± 12.5	<.001
SBP (mm Hg)	128.7 ± 16.7	136.3 ± 18.2	143.7 ± 18.4	<.001
DBP (mm Hg)	74.3 ± 10.0	77.2 ± 10.4	79.5 ± 9.8	<.001
Baseline capillary density (cap/mm^2)	74.2 ± 17.8	73.6 ± 17.0	73.5 ± 18.1	.815
Fasting glucose (mmol/L)	5.2 ± 0.6	5.5 ± 1.1	6.7 ± 1.1	<.001
Total cholesterol (mmol/L)	5.5 ± 1.0	5.6 ± 1.1	5.1 ± 1.3	<.001
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	1.2 ± 0.4	<.001
LDL cholesterol (mmol/L)	3.5 ± 0.8	3.6 ± 1.0	3.0 ± 1.1	<.001
Total to HDL cholesterol ratio	4.0 ± 1.1	4.3 ± 1.4	4.4 ± 1.3	.015
Triglycerides (mmol/L)	1.1 ± 0.6	1.3 ± 0.8	2.0 ± 1.4	<.001
T2DM	2 (1.2)	3 (1.8)	105 (61.4)	<.001
Hypertension	58 (33.9)	77 (45.0)	131 (76.6)	<.001
History of CVD	17 (9.9)	17 (9.9)	36 (21.1)	.002
DM medication	0 (0)	3 (1.8)	72 (42.1)	<.001
Hypertension medication	37 (21.6)	36 (21.1)	99 (57.9)	<.001
Smoking (pack/years)	11.2 ± 14.3	11.1 ± 16.8	17.2 ± 25.1	.004
Microvascular function				
POCR (cap/mm^2)	30.4 ± 15.0	33.1 ± 15.2	28.1 ± 15.4	.011
Endothelial SFM (%)	6.9 ± 3.2	6.7 ± 3.0	6.1 ± 2.8	.035
Neurogenic SFM (%)	10.8 ± 4.2	10.2 ± 3.8	9.6 ± 3.6	.014
Myogenic SFM (%)	10.8 ± 3.6	10.6 ± 4.0	9.6 ± 3.6	.005
Composite microvascular SFM (%)[b]	28.6 ± 8.9	27.5 ± 8.4	25.2 ± 8.2	.001
Respiratory SFM (%)	15.0 ± 4.9	16.1 ± 5.8	15.9 ± 4.7	.140
Cardiac SFM (%)	56.4 ± 9.1	56.4 ± 9.2	58.9 ± 8.9	.018
PA/PF				
MVPA (h/wk)	6.4 ± 4.6	5.8 ± 4.2	4.9 ± 4.1	.006
Walking speed (km/h)	5.3 ± 2.2	5.1 ± 2.1	4.4 ± 2.3	<.001

Data are presented as mean ± SD or n (%) of the total group according to tertiles (T1–T3) of 2hPG. BMI, body mass index; DBP, diastolic BP; LDL, low-density lipoprotein; WHR, waist to hip ratio.

[a] Individuals presenting with 2hPG, microvascular function, covariates (detailed in Materials and Methods), and MVPA data. Similar results were obtained in individuals presenting with 2hPG, microvascular function, covariates, and walking speed data.

[b] Composite microvascular SFM was calculated as the sum of endothelial, neurogenic, and myogenic normalized (%) components.

illary density, % (instead of δ) PORH, or maximal capillary density during venous occlusion (% or δ) as potential mediators gave similar results.

Discussion

In this population-based study including middle-aged and older individuals, we investigated the associations of MVPA and PF with glucose tolerance and the potential mediating role of microvascular function in these associations. The key findings were: 1) MVPA and PF, as determined by walking speed from a 6-minute walk test, were positively associated with glucose tolerance after adjustment for established cardio-metabolic risk factors, hypertension and history of CVD; and 2) microvascular function did not mediate these associations.

Large population studies have consistently observed a positive relationship between PA and glucose tolerance (2–4, 28). The causal nature of that association is denoted by intervention studies in which exercise is the manipulated variable with parameters of glycemic control as outcome variables (29). Whether the effect of regular exercise on glucose tolerance is explained, in part, by concomitant changes in microvascular function has been proposed on the basis of parallel fluctuations of microvascular dilator reactivity and estimates of glucose homeostasis after exercise training/physical inactivity (13–15), concurring with the concept of "vascular conditioning" (10). In this

Table 2. Correlations of Covariates, Microvascular Function, and MVPA With 2hPG in Univariate Analyses

	Study Population (n = 512)	
	r	*P*
Covariates		
Age (y)	0.29	<.001
Sex (male)	0.16	<.001
Waist circumference (cm)	0.33	<.001
SBP (mm Hg)	0.29	<.001
Smoking (pack/years)	0.12	.007
Total to HDL cholesterol ratio	0.09	.052
Triglycerides (mmol/L)	0.32	<.001
Baseline capillary density (cap/mm^2)	0.03	.522
Microvascular function		
POCR (cap/mm^2)	−0.15	<.001
Endothelial SFM (%)	−0.14	.001
Neurogenic SFM (%)	−0.12	.005
Myogenic SFM (%)	−0.15	<.001
Composite microvascular SFM (%)	−0.17	<.001
PA		
MVPA (h/wk)	−0.17	<.001

regard, there are a plethora of local and systemic effects of physical (in)activity including changes in vascular shear stress, cyclic strain, and circulating factors, potentially contributing to adaptations in microvascular dilator/constrictor functions and thereby tissue blood flow regulation (30–32). However, after adjusting for a comprehensive set of confounding variables, the present study indicates that microvascular function, as determined by POCR and SFM, did not influence the association between MVPA and 2hPG. Although speculative, microvascular function may therefore not be the limiting factor for the improvement in glucose tolerance with MVPA. Moreover, such improvement does not seem to be attributed to any change in endogenous glucose production (33, 34). Other pathways through which MVPA may affect glucose tolerance might involve myocyte adaptations (see reviews in Ref. 35). These include, but are not limited to, increased expression and/or activity of key proteins involved in the regulation of insulin signal transduction, glucose uptake and metabolism in skeletal muscle such as Akt, glucose transporter 4 and AMP-activated protein kinase (36); indeed, they constitute potential therapeutic targets mimicking the beneficial effect of exercise on glucose tolerance (37).

Table 3. Correlations of Covariates, Microvascular Function, and PF With 2hPG in Univariate Analyses

	Study Population (n = 488)	
	r	*P*
Covariates		
Age (y)	0.31	<.001
Sex (male)	0.14	.03
Waist circumference (cm)	0.32	<.001
SBP (mm Hg)	0.30	<.001
Smoking (pack/years)	0.13	.004
Total to HDL cholesterol ratio	0.09	.037
Triglycerides (mmol/L)	0.34	<.001
Baseline capillary density (cap/mm^2)	0.03	.485
Microvascular function		
POCR (cap/mm^2)	−0.17	<.001
Endothelial SFM (%)	−0.14	.003
Neurogenic SFM (%)	−0.12	.008
Myogenic SFM (%)	−0.13	.005
Composite microvascular SFM (%)	−0.15	.001
PF		
Walking speed (km/h)	−0.31	<.001

According to our initial premise, PF was independently associated with 2hPG. Likewise, previous reports have found an independent predictive value of PF, as determined by peak oxygen uptake, for glucose tolerance and related markers (3, 38, 39), although this is not a universal finding (40). Importantly, glucose tolerance is inversely associated with sedentary time (41), which in turn may be, to a certain degree, dissociated from PF. On the other hand, glucose tolerance (42) as well as PF (43) are functions of the intensity of exercise training/PA. In this line, we found a closer association of PF, as compared with MVPA, with 2hPG. Contrary to our hypothesis, however, microvascular function did not intercede in the association between PF and 2hPG. This contradicts the prevailing notion supporting the enhanced microvascular function observed in individuals with higher PF levels as a central link between PF and glucose tolerance (9, 44). Collectively, our study suggests that the impact of MVPA and PF on glucose tolerance is not determined by microcirculatory mechanisms regulating nutrient and hormone delivery, despite the established beneficial effect of any improvement in microvascular function on cardiovascular health (45).

Of note, microvascular function was evaluated here with vasodilator stimuli thought to be, at least in part, endothelium dependent (POCR) (46, 47) as well as by periodical blood flow oscillations (SFM) of microvascular origin. These comprise common mechanisms of vasodilation and blood flow distribution in the microcirculation, modifiable with regular exercise (12, 24, 32, 46, 48, 49). Nevertheless, in the present study, the linear correlations of MVPA and PF, on the one hand, and POCR and SFM, on the other hand, were not utterly consistent and seemed to partially depend on sex. Thus, we cannot discard that a more specific measure of microvascular function (eg, insulin-mediated vasodilation/capillary recruitment) might have a stronger influence on the association of MVPA and PF with glucose tolerance. Nevertheless, insulin-mediated vasodilation, POCR, and glucose tolerance are blunted, whereas other endothelium-depen-

Table 4. Multiple Linear Regression Models Including POCR and Composite Microvascular SFM as Potential Mediators of the Association Between MVPA and 2hPG

Model	β (95% CI)	P	P for Mediation	R^2
1				
MVPA (h/wk)	−0.056 (−0.104, −0.009)	.019	—	0.355
2				
MVPA (h/wk)	−0.055 (−0.102, −0.008)	.023	—	0.359
POCR (cap/mm^2)	−0.047 (−0.105, 0.011)	.114	.445	
3				
MVPA (h/wk)	−0.056 (−0.103, −0.009)	.020	—	0.358
Composite microvascular SFM (%)	−0.083 (−0.191, 0.025)	.132	.809	
4				
MVPA (h/wk)	−0.054 (−0.101, −0.007)	.024	—	0.361
POCR (cap/mm^2)	−0.042 (−0.100, 0.017)	.161	.471	
Composite microvascular SFM (%)	−0.073 (−0.182, 0.036)	.188	.817	

All regression models were adjusted for age, sex, waist circumference, SBP, smoking, total to HDL cholesterol ratio, triglycerides, baseline capillary density, medication, presence of hypertension, and history of CVD. β, regression coefficient with 2hPG as outcome; CI, confidence interval.

dent dilator responses are preserved after short-term physical inactivity (15, 50). This suggests that only certain endothelial dilator pathways, at least some involved in the POCR response, are essential for our inquiry. Moreover, glucose and insulin delivery to the myocyte may be determined by the rhythmic change in tone of smooth muscle cells of terminal arterioles (25, 51). In this line, exercise training enhances microvascular SFM at rest and after acute exercise (12). Regardless, given the multifactorial and unknown vascular adaptations induced by regular exercise (30–32, 52), any choice of measures of microvascular function could have limited the scope of the present study. The possibility remains that the crucial microvascular parameter determining glucose tolerance involves a measure of constrictor function. In fact, glucose delivery to insulin-dependent tissues also depends on the (insulin-mediated) constriction of nonnutritive vascular routes, regulating the matching of blood flow to metabolism (10). In addition, it has been recently inferred that the improvement in glucose tolerance after exercise training is partly dependent on the increase in skeletal muscle capillarization (53). Further studies are warranted to determine the extent to which myocyte capillarization and microvascular constrictor function, which are increased by exercise training (53, 54), contribute to the exercise-induced improvement in glucose tolerance.

Additional limitations to this study primarily reside in the assessment of microvascular function in the skin and not in skeletal muscle, which would be the appropriate study tissue as regards glucose tolerance. Nonetheless, there is compelling evidence that skin microvascular responses mirror the functional state of the microcirculation in other tissues including skeletal muscle (55, 56). Furthermore, complete data were available in a subsample of the population that presented a healthier clinical profile compared with excluded individuals (due to missing data), which reduces the generalizability of the results. Ultimately, conclusions about causality cannot be drawn from

Table 5. Multiple Linear Regression Models Including POCR and Composite Microvascular SFM as Potential Mediators of the Association Between PF, as determined by Walking Speed, and 2hPG

Model	β (95% CI)	P	P for Mediation	R^2
1				
Walking speed (km/h)	−0.368 (−0.628, −0.107)	.006	—	0.332
2				
Walking speed (km/h)	−0.368 (−0.628, −0.107)	.006	—	0.337
POCR (cap/mm^2)	−0.061 (−0.125, 0.002)	.058	.387	
3				
Walking speed (km/h)	−0.368 (−0.628, −0.107)	.006	—	0.333
Composite microvascular SFM (%)	−0.044 (−0.160, 0.072)	.459	.618	
4				
Walking speed (km/h)	−0.364 (−0.625, −0.104)	.006	—	0.337
POCR (cap/mm^2)	−0.060 (−0.123, 0.004)	.066	.396	
Composite microvascular SFM (%)	−0.035 (−0.151, 0.081)	.558	.681	

All regression models were adjusted for age, sex, waist circumference, SBP, smoking, total to HDL cholesterol ratio, triglycerides, baseline capillary density, medication, presence of hypertension, and history of CVD. β, regression coefficient with 2hPG as outcome; CI, confidence interval.

cross-sectional analyses; longitudinal studies are therefore needed to confirm the findings.

In summary, this is the first cohort study demonstrating that MVPA and PF are inversely associated with 2hPG independent of general microvascular function and cardio-metabolic risk factors in middle-aged and older individuals. These findings suggest that higher levels of MVPA and PF are associated with improved glucose tolerance through physiological mechanisms other than enhanced substrate and hormone delivery. From a clinical perspective, microvascular function may not limit the beneficial effect of exercise on glucose tolerance. Further studies are needed to determine whether specific insulin-mediated microvascular function and/or the interplay between dilator-constrictor function may influence the association of MVPA and PF with glucose tolerance.

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