Original research

Standing is associated with insulin sensitivity in adults with metabolic syndrome

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ABSTRACT

Objectives: To determine how components of accelerometer-measured sedentary behavior (SB) and physical activity (PA), and fitness are associated with insulin sensitivity in adults with metabolic syndrome.
Design: Cross-sectional.
Methods: Target population was middle-aged (40–65 years) sedentary adults with metabolic syndrome. SB, breaks in SB, standing, and PA were measured for four weeks with hip-worn accelerometers. VO_2max (ml/min/kg) was measured with maximal cycle ergometry. Insulin sensitivity was determined by hyperinsulinaemic-euglycaemic clamp (M-value) and fasting blood sampling (HOMA-IR, insulin). Multivariable regression was used for analyses.
Results: Sixty-four participants (37 women; 58.3 [SD 6.8] years) were included. Participants spent 10.0 (1.0) h sedentary, 1.8 (0.6) h standing, and 2.7 (0.6) h in PA and took 5149 (1825) steps and 29 (8) breaks daily. In sex-, age- and accelerometer wear time-adjusted model SB, standing, steps and VO_2max were associated with M-value ($\beta = -0.384$; $\beta = 0.400$; $\beta = 0.350$; $\beta = 0.609$, respectively), HOMA-IR ($\beta = 0.420$; $\beta = -0.548$; $\beta = -0.252$; $\beta = -0.449$), and insulin ($\beta = 0.433$; $\beta = -0.541$; $\beta = -0.252$; $\beta = -0.453$); all *p*-values < 0.05. Breaks associated only with M-value ($\beta = 0.277$). When further adjusted for body fat %, only standing remained significantly associated with HOMA-IR ($\beta = -0.381$) and insulin ($\beta = -0.366$); significance was maintained even when further adjusted for SB, PA and fitness. Light and moderate-to-vigorous PA were not associated with insulin sensitivity.
Conclusions: Standing is associated with insulin sensitivity markers. The association with HOMA-IR and insulin is independent of adiposity, PA, SB and fitness. Further studies are warranted, but these findings encourage replacing sitting with standing for potential improvements in insulin sensitivity in adults at increased type 2 diabetes risk.

Practical implications

- Standing is associated with insulin sensitivity in inactive sedentary adults at increased risk of developing type 2 diabetes.
- Adiposity appears to partially mediate the relationship between sedentary behavior, fitness and insulin sensitivity, further highlighting the importance of healthy body composition for metabolic health.
- These findings can provide additional insights to aid in development of interventions, policies and guidelines for type 2 diabetes prevention in inactive high-risk populations.

1. Introduction

Although health benefits of regular physical activity (PA) are well-known, physical inactivity is common globally.[1] Sedentary behavior (SB) has been identified as an important health risk, particularly for type 2 diabetes (T2D).[2,3] Low cardiorespiratory fitness is also a risk factor for insulin resistance[4] (predictor of T2D), and low fitness and high SB together increase the odds of metabolic syndrome (MetS) and T2D.[5]

Accelerometer-measured PA has been consistently reported as major determinant of insulin sensitivity,[6,7] whereas the role of SB and patterns of SB (i.e., breaks in sitting) is more unclear.[8–10] Furthermore, studies investigating associations between standing and insulin sensitivity are lacking. Only few studies have accounted for fitness, and most have used only surrogate markers of insulin resistance (e.g., homeostatic model of insulin resistance [HOMA-IR], fasting insulin).

To our knowledge, only one study has investigated associations of accelerometer-measured SB and PA with insulin sensitivity assessed with the gold standard hyperinsulinaemic-euglycaemic clamp,[6] whereas associations between breaks and clamp-measured insulin sensitivity have not been studied. Additionally, associations of SB, PA and fitness with insulin sensitivity have not been studied in sedentary adults with MetS. As SB is associated with increased cardiometabolic risk and MetS strongly predicts T2D, understanding the relationship between SB and PA habits and insulin sensitivity in this population is important from T2D prevention perspective. Therefore, we investigated the associations of accelerometer-measured SB, breaks, standing, and PA, and fitness with insulin sensitivity – measured by hyperinsulinaemic-euglycaemic clamp and surrogate measures – in inactive sedentary adults with MetS.

2. Methods

Data consists of baseline data of an intervention (Clinicaltrials.gov NCT03101228) collected 2017–2019 at Turku PET Centre (Turku, Finland). A four-week accelerometer-measurement was carried out during intervention screening phase, after which hyperinsulinaemic-euglycaemic clamp, maximal cycle ergometry, fasting blood sampling and measurements of blood pressure (BP) and body composition were performed to determine baseline values. All participants gave written informed consent. The study was approved by the Ethics Committee of the Hospital District of Southwest Finland (16/1810/2017), and good clinical practice and Declaration of Helsinki were followed.

Participants were recruited from the local community by newspaper advertisements. The inclusion criteria were age 40–65 years; physical inactivity (< 120 min/week of self-reported moderate-to-vigorous PA [MVPA]); accelerometer-measured sitting time ≥ 10 h/day or 60 % of accelerometer wear time/day; BMI 25–40 kg/m^2; BP < 160/100 mm Hg; fasting glucose < 7.0 mmol/l, and fulfilment of MetS criteria including three of the following: waist circumference (WC) ≥ 94 cm for men/≥ 80 cm for women, triglycerides ≥ 1.7 mmol/l, HDL < 1.0 mmol/l for men/< 1.3 mmol/l for women, systolic BP ≥ 130 and/or diastolic BP ≥ 85 mm Hg, or fasting glucose > 5.6 mmol/l. The exclusion criteria were previous cardiac event; diagnosed diabetes; abundant alcohol consumption (according to national guidelines); use of narcotics, cigarette or snuff tobacco; depressive or bipolar disorder; and any chronic disease/condition that could endanger participant safety or study procedures, or interfere with interpretation of results.

SB, breaks in SB, standing, and PA were assessed by a tri-axial accelerometer (UKK AM30, UKK Institute, Tampere, Finland) with digital acceleration sensor (ADXL345, Analog Devices, Norwood, MA, USA) attached to a hip-mounted belt, as reported previously.[11] Accelerometers were worn during waking hours for four consecutive weeks (except during water-based activities). Wear time of 10–19 h/day and ≥ four days of measurement was considered valid. Data was analyzed in six-second epochs by validated mean amplitude deviation (MAD) method, and MAD values were converted to metabolic equivalents (METs).[12] SB

and standing were defined as ≤ 1.5 METs, light-intensity physical activity (LPA) as 1.5–2.9 METs, and MVPA as ≥ 3.0 METs. Moderate and vigorous activity are combined as MVPA, as the time spent in vigorous activity was negligible. In order to differentiate between SB and standing, body posture was assessed by validated angle for posture estimation (APE) method, which identifies postures with 90 % accuracy in free-living conditions.[13] SB was defined as APE ≥ 11.6° and standing as < 11.6°. Breaks were determined as SB periods with one-minute exponential moving average < 1.5 METs ending in vertical acceleration and subsequent standing posture or movement.[13] Number of steps/day was determined by an algorithm splitting acceleration into vertical and horizontal components. The algorithm requires walking speed of ~3 km/h to detect every step.[13] Periods with acceleration of each axis within 187.5 mg range for ≥ 30 min were considered non-wear time.

Whole-body insulin-stimulated glucose uptake (M-value) was measured with hyperinsulinaemic-euglycaemic clamp after fasting overnight. Insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) was infused at 40 mU/m^2 body surface area/min rate during the first 4 min, at 20 mU/m^2/min during minutes 4–7, and thereafter at 10 mU/m^2/min. Four minutes after starting insulin infusion, 20 % glucose infusion was started. The rate was adjusted according to plasma glucose concentration measured every 5–10 min. M-value (μmol/kg/min) was calculated in 20-min intervals from steady-state glucose values. Fasting insulin and HOMA-IR were determined as surrogate markers of insulin sensitivity as described below.

Venous blood samples were drawn after fasting ≥ 10 h and analyzed at the Turku University Hospital Laboratory. Plasma insulin was measured by electrochemiluminescence immunoassay (Cobas 8000 e801), plasma glucose by enzymatic reference method with hexokinase GLUC3, and cholesterol (total, LDL, HDL), triglycerides and non-esterified fatty acids (NEFA) by enzymatic colorimetric tests (Cobas 8000 c702). HbA$_{1c}$ was measured by turbidimetric inhibition immunoassay (Cobas 6000 c501); all analyzers by Roche Diagnostics GmbH, Mannheim, Germany. HOMA-IR was calculated with formula (insulin x glucose/22.5).

Fitness was assessed by maximal cycle ergometry (eBike EL Ergometer + CASE v6.7, GE Medical Systems Information Technologies, Inc. Milwaukee, WI, USA) with direct respiratory gas measurements (Vyntus CPX, CareFusion, Yorba Linda, CA, USA). Cycling pace was ~65 rpm, and intensity started at 25 W. Every 3 min the intensity increased by 25 W until volitional exhaustion, and perceived exertion on Borg scale and BP were measured. VO$_2$max was determined if ≥ 1 criterion was met: respiratory exchange ratio > 1.0, plateau in VO$_2$, or heart rate within ±10 bpm of age-predicted maximum. VO$_2$max was defined as the highest one-minute average in ml/min/kg. VO$_2$max per fat free mass (FFM) (ml/min/kg$_{FFM}$), and maximal load (Wmax) were also determined. Fitness data is available for 58 participants, as the test was stopped for other reasons (e.g., knee pain) for five participants before reaching volitional exhaustion and the abovementioned criteria, and results of one participant were lost due to technical difficulties.

Weight, body fat % and FFM were estimated with air displacement plethysmography (Bod Pod, COSMED USA, Inc., Concord, CA, USA) after fasting ≥ 4 h. Height was measured with stadiometer. BMI was calculated from weight and height (kg/m^2). WC was measured midway between the iliac crest and the lowest rib. BP was measured by a digital monitor (Apteq AE701f, Rossmax International LtD, Taipei, Taiwan) after ≥ 10 min of sitting.

Descriptive statistics (means [SD]) were calculated, and differences between sexes were tested with unpaired *t*-test. Normal distribution was evaluated visually and by Shapiro-Wilk test, and log10 transformation was performed as needed. Associations were examined with Pearson partial correlation analysis and multivariable linear regression. Regression model always included one insulin sensitivity marker as dependent, and one activity/fitness parameter as independent variable. Model 1 was adjusted for sex, age, and accelerometer wear time, and Model 2 additionally for body fat %. To further analyze independence

of associations between standing and insulin sensitivity, SB, total PA and VO_2max (ml/min/kg) were included in Model 3. Variance inflation factors < 5 were considered not to have multicollinearity issues. Missing data was handled by pairwise deletion. Statistical significance was set at $p < 0.05$ (two-tailed). For illustrative purposes data are stratified by tertiles of standing time and log10 transformed insulin sensitivity markers are presented in original scale. Analyses were performed with IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA) and JMP Pro 15.1.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

Mean age was 58.3 (SD 6.8) years and BMI 31.6 (4.3) kg/m². Accelerometers were worn for 26 (4) days, and 14.54 (0.97) h/day. Participants spent 10.0 (1.0) h sedentary, 1.8 (0.6) h standing, and 2.7 (0.6) h in PA, and took 5149 (1825) steps and 29 (8) breaks daily (Table A.1 in supplementary file online).

Correlation analyses between adiposity, lipid profile, and insulin sensitivity showed that weight, BMI, body fat % and WC correlated with M-value ($r = -0.54; -0.59; -0.61;$ and -0.67, respectively), HOMA-IR ($r = 0.59; 0.62; 0.61; 0.59$), and fasting insulin ($r = 0.58; 0.61; 0.62; 0.58$). HDL also correlated with M-value ($r = 0.26$), HOMA-IR ($r = -0.27$) and insulin ($r = -0.30$), while triglycerides correlated with M-value ($r = -0.31$) and insulin ($r = 0.26$). NEFA correlated only with M-value ($r = -0.38$); all p-values < 0.05 (Table A.2).

In correlation analyses between activity/fitness parameters and adiposity, SB, MVPA, steps and VO_2max (ml/min/kg) correlated with weight ($r = 0.40; -0.42; -0.51;$ and -0.61, respectively), BMI ($r = 0.32; -0.36; -0.45; -0.64$), body fat % ($r = 0.33; -0.35; -0.43; -0.64$), and WC ($r = 0.35; -0.42; -0.49; -0.61$). Breaks correlated with weight ($r = -0.30$), BMI ($r = -0.34$) and WC ($r = -0.34$), while standing correlated with body fat % ($r = -0.31$). Total PA

correlated with weight ($r = -0.37$) and WC ($r = -0.29$); all p-values < 0.05 (Table A.3).

Multivariable regression model was used to examine associations between activity/fitness parameters and insulin sensitivity. In sex-, age- and wear time-adjusted model SB was detrimentally, and standing, steps, and VO_2max (ml/min/kg) beneficially associated with M-value, HOMA-IR and insulin (Table 1). Breaks associated only with M-value. Neither LPA, MVPA nor total PA was associated with any insulin sensitivity markers. All associations between fitness and insulin sensitivity turned non-significant when fitness was expressed as VO_2max ($ml/min/kg_{FFM}$) (Table 1).

Adding body fat % to the model turned all associations with M-value non-significant (Table 1). All associations with HOMA-IR and insulin also turned non-significant, except for standing. The association between standing and M-value was near-significant ($p = 0.08$) also, and remained significant when adjusted for BMI instead of body fat % (data not shown). The associations of standing with HOMA-IR and insulin remained significant when SB, total PA and VO_2max (ml/min/kg) were entered into the model one at a time along with sex, age, wear time and body fat %, as well as when SB, PA and fitness were all included in the same model (Table 2). When adjusted for sex, age, body fat %, SB, PA, and fitness, also the association between standing and M-value was near-significant ($p = 0.07$). Fig. 1 shows the unadjusted associations of standing with M-value and HOMA-IR. Dose-response associations were observed between tertiles of standing time (< 1.5, 1.5–2.0, and > 2.0 h/day) and both markers.

None of the SB, PA or fitness variables were associated with fasting glucose or HbA$_{1c}$ (Table A.4). All analyses were also run with lean mass M-value ($\mu mol/kg_{FFM}/min$) as the dependent variable instead of M-value ($\mu mol/kg/min$), but the results were unchanged (data not shown). Additional analyses including the interaction between sex and each SB/PA variable in Model 1 were run, and stronger association of standing with HOMA-IR and insulin in men was the only statistically

Table 1
Associations between activity/fitness parameters and insulin sensitivity estimated by multivariable linear regression model.

| | M-value (μmol/kg/min) | | | | HOMA-IR[a] | | | | Fasting insulin[a] (pmol/l) | | | |
| | Model 1 | | Model 2 | | Model 1 | | Model 2 | | Model 1 | | Model 2 | |
	β	p	β	p	β	p	β	p	β	p	β	p
Sedentary, h/day	−0.384 (−0.671, −0.099)	**0.01**	−0.171 (−0.424, 0.082)	0.18	0.420 (0.141, 0.700)	**0.004**	0.214 (−0.034, 0.463)	0.09	0.433 (0.152, 0.715)	**0.003**	0.221 (−0.027, 0.47)	0.08
Standing, h/day	0.400 (0.134, 0.667)	**0.004**	0.212 (−0.022, 0.447)	0.08	−0.548 (−0.792, −0.304)	**<0.001**	−0.381 (−0.598, −0.165)	**<0.001**	−0.541 (−0.789, −0.292)	**<0.001**	−0.366 (−0.585, −0.148)	**0.001**
LPA, h/day	0.060 (−0.215, 0.335)	0.67	0.052 (−0.168, 0.272)	0.64	−0.001 (−0.274, 0.271)	0.99	0.010 (−0.209, 0.228)	0.93	−0.031 (−0.307, 0.244)	0.82	−0.020 (−0.238, 0.199)	0.86
MVPA, h/day	0.219 (−0.043, 0.482)	0.10	−0.002 (−0.232, 0.228)	0.99	−0.142 (−0.405, 0.120)	0.26	0.084 (−0.142, 0.310)	0.46	−0.147 (−0.412, 0.118)	0.27	0.086 (−0.140, 0.312)	0.45
Total PA, h/day	0.157 (−0.110, 0.425)	0.24	0.036 (−0.184, 0.257)	0.74	−0.077 (−0.342, 0.189)	0.57	0.048 (−0.170, 0.265)	0.66	−0.099 (−0.367, 0.169)	0.46	0.028 (−0.19, 0.245)	0.80
Breaks in sedentary time/day	0.277 (0.015, 0.539)	**0.04**	0.125 (−0.097, 0.348)	0.26	−0.206 (−0.471, 0.056)	0.12	−0.048 (−0.271, 0.175)	0.67	−0.217 (−0.484, 0.049)	0.11	−0.054 (−0.277, 0.170)	0.63
Steps/day	0.350 (0.105, 0.594)	**0.01**	0.107 (−0.121, 0.337)	0.35	−0.252 (−0.501, −0.003)	**0.047**	0.005 (−0.224, 0.234)	0.96	−0.252 (−0.504, −0.001)	**0.049**	0.014 (−0.214, 0.243)	0.90
VO₂max, ml/min/kg[b]	0.609 (0.351, 0.866)	**<0.001**	0.281 (−0.015, 0.577)	0.06	−0.449 (−0.720, −0.178)	**0.002**	−0.050 (−0.349, 0.249)	0.74	−0.453 (−0.727, −0.179)	**0.002**	−0.031 (−0.327, 0.265)	0.83
VO₂max, ml/min/kg$_{FFM}$[b]	0.238 (−0.047, 0.524)	0.10	0.217 (−0.006, 0.439)	0.06	−0.055 (−0.341, 0.230)	0.70	−0.033 (−0.259, 0.192)	0.77	−0.051 (−0.339, 0.237)	0.72	−0.028 (−0.252, 0.195)	0.80
Wmax[b]	0.338 (−0.012, 0.689)	0.06	0.099 (−0.199, 0.398)	0.51	−0.042 (−0.396, 0.312)	0.81	0.218 (−0.068, 0.504)	0.13	−0.048 (−0.406, 0.310)	0.79	0.222 (−0.061, 0.505)	0.12

HOMA-IR = homeostatic model of insulin resistance; LPA = light-intensity physical activity; MVPA = moderate-to-vigorous physical activity; PA = physical activity; FFM = fat free mass.
Values expressed as standardized β coefficients (95% CI); all values on log scale. Bold p-values indicate statistical significance (p < 0.05).
Model 1 adjusted for sex, age, and accelerometer wear time.
Model 2 adjusted for sex, age, body fat %, and accelerometer wear time.
[a] Data available for 63 participants.
[b] Data available for 58 participants.

Table 2
Associations of standing with insulin sensitivity markers when adjusted for SB, total PA, and fitness.

	M-value (µmol/kg/min)		HOMA-IR[a]		Fasting insulin[a] (pmol/l)	
	β	p	β	p	β	p
Standing (h/day) adjusted for:						
Sex, age, body fat %, wear time, SB (h/day)	0.195 (−0.136, 0.525)	0.24	− 0.474 (−0.780, −0.170)	**0.003**	− 0.437 (−0.745, −0.128)	**0.006**
Sex, age, body fat %, wear time, total PA (h/day)	0.210 (−0.028, 0.449)	0.08	− 0.392 (−0.610, −0.173)	**<0.001**	− 0.374 (−0.595, −0.154)	**0.001**
Sex, age, body fat %, wear time, VO$_2$max (ml/min/kg)[b]	0.215 (−0.031, 0.462)	0.09	− 0.352 (−0.590, −0.115)	**0.005**	− 0.325 (−0.563, −0.087)	**0.009**
Sex, age, body fat %, SB (h/day), total PA (h/day), VO$_2$max (ml/min/kg)[b]	0.266 (−0.018, 0.550)	0.07	− 0.345 (−0.615, −0.074)	**0.01**	− 0.293 (−0.565, −0.022)	**0.04**

HOMA-IR = homeostatic model of insulin resistance; SB = sedentary behavior; PA = physical activity.
Values expressed as standardized β coefficients (95% CI); all values on log scale. Bold p-values indicate statistical significance (p < 0.05).
[a] Data available for 63 participants.
[b] Data available for 58 participants.

significant difference (men: β = −0.753 and − 0.763, respectively; women: β = −0.401 and − 0.377; *p*-values < 0.05).

4. Discussion

Our results indicate that standing is favorably associated with whole-body insulin sensitivity (M-value), HOMA-IR and fasting insulin. The association with HOMA-IR and insulin remains significant even when adjusted for adiposity, SB, PA, and fitness. Thus it seems that the more time is spent standing, the better insulin sensitivity is in sedentary adults at increased T2D risk. Adjustment for adiposity eliminated associations of SB and fitness with insulin sensitivity, suggesting that adiposity likely mediates these associations. This further highlights the importance of healthy body composition for metabolic health in individuals with MetS.

Novel finding was the positive association between standing and insulin sensitivity. This is likely explained by muscle contraction required for standing, which promotes translocation of GLUT4 and thus increases glucose uptake and insulin sensitivity.[14] We observed an association – although eliminated by adjustment for BMI – between standing and HOMA-IR also in our study of 144 overweight/obese adults with and without MetS.[11] In contrast, Biddle et al.[15] found no association between standing and insulin sensitivity in compositional data analysis in individuals at T2D risk. To our knowledge, no other studies have investigated associations between standing and insulin sensitivity. However, isotemporal substitution modeling and experimental evidence suggests that insulin metabolism in high-risk individuals may be improved by replacing sitting with standing[16] or interrupting sitting with standing

bouts.[17] In healthy populations, however, interrupting or replacing sitting with standing have not affected insulin action.[18,19] It may be that increased standing and frequent standing breaks could benefit sedentary populations with metabolic dysfunctions, whereas in general population standing might not be enough to improve insulin metabolism. However, in healthy adults standing has an inverse dose-response association with mortality,[20] and increased energy expenditure in comparison to sitting.[21] Therefore, standing is proposed as a feasible and generally safe alternative to sitting, and our results extend the evidence base.

This is the first study investigating associations between accelerometer-measured breaks in SB and insulin sensitivity measured by hyperinsulinaemic-euglycaemic clamp. Previous evidence from studies using surrogate markers is inconsistent.[8,10,22] We found that breaks were associated with clamp-measured whole-body insulin sensitivity but not surrogates, when adjusted for sex, age and accelerometer wear time. Breaking up sitting requires contraction of muscles which contribute largely to whole-body glucose disposal, whereas surrogate measures primarily reflect hepatic insulin sensitivity,[23] possibly explaining the association only with M-value. However, adjustment for adiposity eliminated the association.

The offsetting effect of adiposity on the associations of breaks, SB and fitness with insulin sensitivity suggests that adiposity mediates to some extent these associations, which is biologically plausible. Obesity increases plasma concentration of NEFA, which then increases glucose and triglyceride production and other lipid/lipoprotein abnormalities, and reduces insulin-mediated glucose uptake and glycogen synthesis; all of which contribute to development of insulin resistance.[24] Our
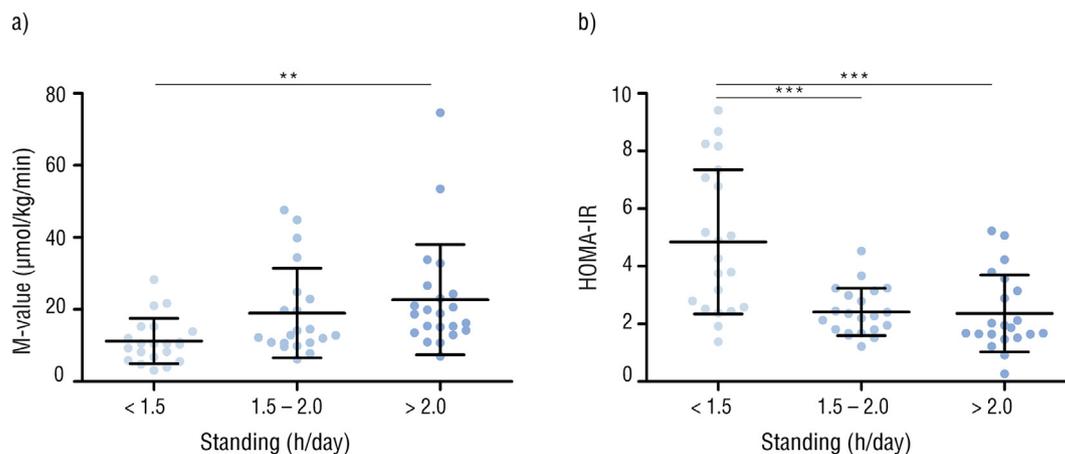

Fig. 1. Dose-response association between standing time and insulin sensitivity.
Insulin sensitivity presented as a) M-value and b) HOMA-IR stratified by tertiles of accelerometer-measured standing time (h/day; means [SD]) in sedentary adults with metabolic syndrome (*n* = 64). HOMA-IR = homeostatic model assessment of insulin resistance. ** = *p* < 0.01, *** = *p* < 0.001.

finding of body fat % and WC, as well as NEFA, triglycerides and HDL, correlating with M-value supports this mechanism. It is supported also by previous studies in which adiposity explained 65 % of the variance in clamp-measured insulin sensitivity,[25] and mediated the associations of MVPA and fitness with insulin sensitivity.[26,27] Additionally, we observed inverse correlations between adiposity measures, PA and fitness, and adjustment for adiposity turned the association between fitness and insulin sensitivity non-significant. These data together suggest that PA and fitness likely affect insulin sensitivity through effects leading to improved body composition. It is worth noting, however, that health status may also influence activity habits; e.g., it is possible that obesity leads to increased SB and not the other way around.[28]

Our finding that SB is not associated with insulin sensitivity when adjusted for adiposity is in line with results in overweight/obese adults with newly diagnosed T2D,[10] whereas in adults with family history of T2D SB was not associated with insulin sensitivity independent of adiposity.[7] We also found that neither LPA, MVPA nor total PA was associated with insulin sensitivity. Similar results have been reported previously regarding LPA,[7,29] although in our recent study including overweight/obese adults with and without MetS, LPA was associated with HOMA-IR.[11] Also contrary to our current findings, other studies show an association between MVPA and insulin sensitivity.[9,29] Findings regarding total PA, however, are more conflicting.[7,11,29] It is possible that the amount of PA in this specific, homogenous population – highly sedentary adults with MetS – is not enough and has too little variation to detect significant associations, or the association is blunted by adiposity and other risk factors.

Similar to previous studies in healthy and high-risk populations,[8,29,30] neither fasting glucose nor HbA$_{1c}$ was associated with SB/PA variables. Discrepancies between our study and others could be due to differences in populations and methodology. Our participants were sedentary adults with MetS, and thus at great likelihood of developing T2D. Although most abovementioned studies were also conducted in high-risk populations, participants were slightly healthier overall, and without as many metabolic abnormalities as in our study. Others have not specifically recruited sedentary/inactive participants, which has possibly led to greater PA variation and thus enabled detection of significant associations between PA and insulin sensitivity. Other reasons for discrepancies could be methodological issues relating to e.g., assessments of PA and insulin sensitivity. Previous accelerometer-assessments have generally lasted only ≤ 7 days, and insulin sensitivity assessment methods have varied. Discrepancies could also be affected by variation in confounding factors and analysis methods.

A major strength of our study is the insulin sensitivity assessment with hyperinsulinaemic-euglycaemic clamp in addition to surrogate markers. M-value directly measures the amount of metabolized glucose under steady-state conditions, thus providing a reliable measure of whole-body insulin sensitivity. It is a costly and labor-intensive method however, which is why surrogates are often used. Another strength is the accelerometer-measurement of SB and PA with validated analysis algorithms for both the intensity of PA and body posture. Additionally, the 4-week measurement in our study likely represents the participants' long-term behaviors more truthfully than the ≤ 7- day measurement period used in most accelerometer studies.

Our study also has limitations. The cross-sectional setting limits interpretation of causality, and although accelerometers provide more accurate measures of SB and PA than self-report and insights into patterns of SB (i.e., breaks), they cannot account for all activities (e.g., water-based activities). Furthermore, the step detection algorithm may not be able to count all sporadic steps taken at slow speeds; however, they still accumulate LPA. Our results might be generalizable only to inactive, sedentary, white populations with MetS, but given the prevalence of sedentary lifestyles and overweight/obesity, these results are likely generalizable to a larger proportion of Western populations. The considerable number of outcomes and analyses is also a potential limitation, although the main results remain even with lower statistical

significance level ($p \leq 0.01$). The relatively small sample size may also have limited detection of some associations. Thus further studies with larger samples are needed to confirm our findings, and to study causal relations between standing, as well as other SB/PA habits, and insulin sensitivity.

5. Conclusion

Our results indicate that standing is associated with insulin sensitivity. Furthermore, the association of standing with HOMA-IR and fasting insulin is independent of adiposity, SB, PA, and fitness. This suggests that simply standing more might be a potential way to improve insulin sensitivity in sedentary high-risk populations, warranting further (intervention) studies. Adiposity likely mediates the associations of SB and fitness with insulin sensitivity, which further highlights the importance of healthy body composition to prevent development of T2D in individuals at increased risk.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Declaration of interest statement

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

Confirmation of ethical compliance

All participants gave written informed consent. The study was approved by the Ethics Committee of the Hospital District of Southwest Finland (16/1810/2017), and good clinical practice and Declaration of Helsinki were followed.

Appendix A. Supplementary data

Supplementary data to this article (Tables A.1-A.4) can be found online at https://doi.org/10.1016/j.jsams.2021.08.009.

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