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Running title: Sedentary time, physical activity, and ADIPO-IR

Abstract

Introduction: This cross-sectional study examined associations of device-measured sedentary time and moderate-to-vigorous physical activity (MVPA) with adipose tissue insulin resistance in people with or at high-risk of type 2 diabetes (T2DM).

Methods: Data were combined from six previous experimental studies (within our group) involving patients with T2DM or primary risk factors (median (IQR) age 66.2 (66.0 – 70.8) years, body mass index (BMI) 31.1 (28.0 – 34.4) $kg.m^{-2}$, 62% male, n = 179). Adipose tissue insulin resistance was calculated as the product of fasted circulating insulin and non-esterified fatty acids (ADIPO-IR), while sedentary time and MVPA were determined from wrist-worn accelerometery. Generalised linear models examined associations of sedentary time and MVPA with ADIPO-IR with interaction terms added to explore the moderating influence of ethnicity (white European vs. south Asian), BMI, age, and sex.

Results: In finally-adjusted models, sedentary time was positively associated with ADIPO-IR, with every 30-min of sedentary time associated with a 1.80 (95% CI: 0.51 to 3.06; P = 0.006) unit higher ADIPO-IR. This relationship strengthened as BMI increased (β= 3.48 [95%CI=1.50 to 5.46], P=0.005 in the upper BMI tertile [≥ 33.2 $kg.m^{-2}$]). MVPA was unrelated to ADIPO-IR. These results were consistent in sensitivity analyses that excluded participants taking statins and/or metformin (n = 126) and when separated into the participants with T2DM (n = 32) and those at-high-risk (n = 147).

Conclusions: Sedentary time is positively related to adipose tissue insulin sensitivity in people with or at high-risk of T2DM. This relationship strengthens as BMI increases and may help explain established relationships between greater sedentary time, ectopic lipid, and hyperglycaemia.

Key words: Physical activity, insulin sensitivity, lipolysis, lipogenesis, obesity

Introduction

Insulin resistance is central to the pathophysiology of numerous obesity-related conditions, including type 2 diabetes (T2DM)(1) and non-alcoholic fatty liver disease (NAFLD)(2). The metabolic consequences of insulin resistance are tissue-specific, and while the metabolic sequel of insulin resistance in skeletal muscle and the liver are well defined, evidence relating to adipose tissue insulin resistance is less established. Adipose tissue insulin resistance typically develops with obesity, once adipocytes become enlarged and inflamed(3). A diminished anti-lipolytic response to insulin ensues, resulting in elevated circulating non-esterified fatty acids (NEFA) and exaggerated lipid deposition in the liver, pancreas, and skeletal muscle(4). These ectopic lipid deposits directly impair insulin signalling, glucose, and lipid metabolism(5).

The gold-standard technique for measuring adipose tissue insulin sensitivity in humans requires a multi-step hyperinsulinaemic-euglycaemic clamp with stable-isotope tracer (glycerol and/or palmitate)(6). However, issues relating to cost, practicality and technical expertise limit its utility. Alternatively, the adipose tissue insulin resistance index (ADIPO-IR), calculated as the product of fasting insulin and NEFA concentrations, provides a simple estimate of adipose tissue insulin resistance. While being a '*static'* metric, the ADIPO-IR has been validated against clamp-based assessments (7, 8), of adipose tissue insulin sensitivity, confirming the index adequately represents the dynamic interaction between insulin/hyperinsulinaemia, circulating NEFA and/or lipolysis. *In vitro* studies also show that the ADIPO-IR provides a valid representation of anti-lipolysis and lipogenesis (9).

In cross-sectional studies, ADIPO-IR is positively related to obesity(10–12) and indices of glycaemic control(13, 14), with evidence of dose-response. Positive associations of ADIPO-IR with biomarkers of chronic inflammation have also been observed(13). An inverse association has been identified between ADIPO-IR and pancreatic beta-cell function(15),

likely reflecting the lipotoxic effect of elevated circulating NEFA. Moreover, baseline ADIPO-IR was associated with a greater risk (odds ratio 1.59 per SD) of developing dysglycaemia (i.e. the onset of impaired fasting glucose, impaired glucose tolerance or T2DM) over nine years, independent of anthropometric and cardiometabolic biomarkers(16). Recently, ADIPO-IR was shown to predict the severity of liver fibrosis in patients with NAFLD and T2DM(17). These findings, along with others(18, 19), have prompted the suggestion that ADIPO-IR is a valid marker of adipose tissue dysfunction, which in itself is a more prognostic biomarker of metabolic health than adiposity *per se*.

The therapeutic potential of improving adipose tissue insulin sensitivity has been recognised(20), with the glucose-lowering effect of thiazolidinediones ascribed specifically to enhanced adipogenesis in more 'metabolically friendly' lipid depots(11). Our recent meta-analysis demonstrated that formal exercise training can improve adipose tissue insulin sensitivity, when measured via ADIPO-IR or clamp-based techniques(21). Importantly, however, this analysis focused solely on formal exercise training regimens within clinical trials. It did not examine incidental movement behaviours with more translational relevance. Despite established links between sedentary time and chronic inflammation(22) and dysregulated gluco-lipid metabolism(23), the relationship between habitual sedentary time and adipose tissue insulin sensitivity has received minimal attention.

Using a pooled data set of adults with or at high-risk of T2DM (derived from experimental trials in our group), this study examined associations between physical activity and sedentary time (device-measured) with adipose tissue insulin resistance. A secondary aim was to explore whether relevant demographic and biological factors mediated these associations. We hypothesised that sedentary time would be positively associated with ADIPO-IR, while physical activity would be inversely related. Furthermore, because aging, adipocyte size and

South Asian ethnicity have each been linked with greater adipose tissue insulin resistance(24–26), we hypothesised that associations of sedentary time and physical activity with ADIPO-IR would be stronger in individuals who were older, had higher body mass index (BMI) values, and were of South Asian ethnicity (compared to white European).

Methods

Ethical approval

This cross-sectional analysis pooled baseline data from previous experimental studies conducted within the National Institute for Health and Care Research (NIHR) Leicester Biomedical Research Centre (see Table 1, Supplementary Digital Content; Clinical Trials: NCT02453204, ISRCTN12337078, NCT03315988, NCT03482596, NCT04004273, NCT03549390). All studies obtained ethical approval from local National Health Service (NHS) research ethics committees (REC ref: 14-EM-1217; 15-EM-0259 17-WS-0184; 18-EM-0006; 18-EM-0161; 18-EM-0185) and were conducted in accordance with the Declaration of Helsinki (2013). Written informed consent was obtained for each participant before their participation.

Participants

Overall, data for 179 volunteers (111 men, 68 women) were included in the present analysis. Figure 1 describes the sample reduction process. Primary care services, community events, poster advertisement, existing research databases and word-of-mouth were used to recruit study volunteers. Participants were aged between 28-80 years and were classified as either having T2DM or being at high-risk of T2DM due to obesity, elevated hepatic steatosis and/or non-diabetic hyperglycaemia. Female participants were either postmenopausal (for at least 12 months) or were not pregnant or lactating (self-reported). The exclusion criteria of the primary studies were as follows: those undertaking a weight loss dietary intervention (with the purpose of reducing body mass), those engaging in regular purposeful exercise (see Supplementary Digital Content Table 1 for the definition used in each study), and those exhibiting weight instability within the last three months (≥ 3 kg weight change). In the present analysis,

132 participants who were not from a white European or South Asian ethnic background were
133 excluded *(n* = 10).

134 **INSERT FIGURE 1**

135 *Study procedures*

136 All data were collected between April 2015 and July 2022 from study screening and/or baseline
137 assessment visits. Identical research techniques and standard operating procedures were used
138 between studies for each outcome in this analysis. The exception was body fat percentage
139 which was measured via dual-energy X-ray absorptiometry (DEXA) in one study(27)
140 (NCT03315988; *n* = 16) and via bioelectrical impedance analysis (BIA) in all other studies (*n*
141 = 163). Participants were instructed to avoid alcohol, caffeine, and structured exercise in the
142 24 h before study visits. Information on participants' demographics, medical history and
143 medication use was obtained by a healthcare professional.

144 *Anthropometric measurements*

145 Height was measured to the nearest 0.5 cm using a stadiometer, while body weight was
146 measured to the nearest 0.1 kg using integrated weighing scales (Tanita TBE 611, Tanita, West
147 Drayton, UK). These values were then used to calculate participants' BMI ($kg.m^{-2}$). Body fat
148 percentage was measured by BIA (Tanita TBE 611, Tanita, West Drayton, UK) or DEXA
149 (Lunar Prodigy, GE Corporation, Illinois, US).

150 *Device-measured physical activity and sedentary time*

151 To assess physical activity and sedentary time, participants were requested to wear a wrist-
152 worn tri-axial accelerometer (GENEActiv, ActivInsights Ltd, Kimbolton, UK) after their
153 baseline assessment visit for at least six days. Participants were instructed to continue their
154 daily activities as usual and wear the device on their non-dominant wrist at all times, if possible.

A log was provided to record any periods where the device was removed, along with participants' wake and sleep times for each date of accelerometer wear. Data were recorded at 100Hz, downloaded using GENEActiv PC software (version 3.2., GENEActiv, ActivInsights Ltd, Kimbolton UK) and processed using an R-package GGIR(28) (http://cran.r-project.org). Data were extracted, the average magnitude of dynamic acceleration corrected for gravity (Euclidean Norm minus 1 g (ENMO)) averaged over 5-s epochs and expressed in milligravitational units (mg). Files were excluded if they showed post-calibration error greater than (10 mg) or did not contain at least four valid days of measurement, each consisting of ≥ 16 hours of wear time. The duration of the sleep window was calculated using automated sleep detection(28) (HDCZA sleep detection algorithm(29)). Physical activity variables, calculated as average minutes per day, were classified as time spent sedentary (< 40 mg excluding the sleep period)(30), in light physical activity (LPA; 40-100 mg), moderate physical activity (MPA; 100-400 mg), vigorous physical activity (VPA; > 400 mg)(31) and moderate-to-vigorous physical activity (MVPA; > 100 mg). MVPA data were expressed in bouts of ≥ 1 minute (where 80% of the activity was above the threshold) to avoid capturing very short incidental activity(31).

Blood sampling and biochemical analysis

Venous blood samples were taken from an antecubital vein in the fasted state on the morning of study visits. Blood samples were drawn into chilled EDTA monovettes (Sarstedt, Leicester, UK) and centrifuged immediately at 3500 rpm for 10 minutes at 4°C. Plasma supernatant was then removed, aliquoted and stored at -80°C for later analysis. A semi-automated clinical chemistry benchtop analyser (Pentra 400, Horiba Medical, Montpellier, France) was used to determine plasma concentrations of glucose (Horiba Medical, Montpellier, France) and NEFA (Randox Laboratories Ltd, County Antrim, UK) using colorimetric methods. Plasma insulin concentrations were measured by an enzyme-linked immunosorbent assay (Mercodia, Uppsala,

Sweden). The coefficient of variation for the glucose, NEFA, and insulin analyses were 0.57%, 0.64%, and 4.61%, respectively. The adipose tissue insulin resistance index (ADIPO-IR) was calculated as fasting plasma NEFA ($mmol.L^{-1}$) *x* fasting plasma insulin ($pmol.L^{-1}$)(7).

Statistical analysis

Statistical analyses were performed using SPSS version 27.0 (SPSS Inc., Chicago, Illinois). Kolmogorov-Smirnov tests were performed to check the distribution of the data. Participant characteristics are shown as mean ± SD for normally distributed data, median (interquartile range) for non-normally distributed data, and number (percentage) for categorical data. Generalised linear models with a normal distribution and identity link function were used to assess the independent associations of physical activity and sedentary time (exposure variables) with ADIPO-IR (outcome variable). Three models were ran as follows: (1) basic model adjusted for study and waking hours (continuous); (2) adjusted for the previous variables plus age (continuous), sex (men/women) and ethnicity (white European/South Asian); (3) adjusted for the previous variables plus BMI (continuous). LPA and VPA were not considered separately in the analysis due to multicollinearity between LPA and sedentary time, while median VPA was less than one minute per day. Where significant associations were observed, interaction terms were subsequently added to model 3 to assess whether these associations were moderated by ethnicity, sex, age, and BMI. To facilitate interpretation, significant interactions between continuous variables were stratified into tertiles to describe the direction of the interaction. Statistical significance was considered $p < 0.05$ for main effects and $p < 0.10$ for interactions (given that interaction analyses have lower statistical power).

Sensitivity analysis

As some participants were taking statins only ($n = 36$), metformin only ($n = 5$) or both ($n = 10$) (which lower circulating NEFA(32) and glucose), we performed a sensitivity analysis by

removing individuals taking these medications (n = 126) to determine whether their use impacted the associations of physical activity and sedentary time with ADIPO-IR. Furthermore, given that our sample included both participants with type 2 diabetes (n = 32) and those at high-risk (n = 147), we also examined associations of physical activity and sedentary time with ADIPO-IR within these individual groups. Independent sample t-tests for normally distributed and Mann-Whitney U tests for non-normally distributed data were used to assess differences in participant characteristics between the whole cohort and the sensitivity cohort (i.e. with those taking statins and/or metformin removed). For the sensitivity analysis, identical generalised linear models and interaction terms were subsequently run in the sensitivity cohort. Additional models were also run to confirm whether the main results were consistent when body fat percentage was included as a covariate in place of BMI.

Results

Participant characteristics

The demographic, metabolic and physical activity characteristics of all participants are presented in Table 1. A total of 179 participants (median age: 66.2 (66.0-70.8) years; 62.0% male) with valid physical activity data were pooled in this analysis. All participants were classified as either having or being at high-risk of T2DM (with either overweight or obesity [median BMI: 31.1 $kg.m^{-2}$; IQR: 28.0-34.4 $kg.m^{-2}$], elevated hepatic steatosis, and/or non-diabetic hyperglycaemia). Furthermore n = 5 (2.8%) were taking metformin only (no statins), n = 36 (20.2%) were taking statins only (no metformin) and n = 10 (5.6%) were taking both metformin and statins. On average, participants were sedentary for 670 mins (11 hours 10 mins) per day and were performing 24 mins of MVPA per day.

The participant characteristics of the cohort stratified by either white European (n = 122 [68.2%]; median age: 66.6 years; 62.3% male) or South Asian ethnicity (n = 57 [31.8%]; median age: 66.0 years; 61.4% male) are shown in Supplementary Digital Content (Table 2). BMI and body fat percentage were significantly higher in white Europeans compared to South Asians (all $p < 0.05$), while vigorous physical activity levels were significantly lower (p = 0.003). All other demographic, metabolic and physical activity variables were similar between ethnicities.

INSERT TABLE 1

Sedentary time

Table 2 shows the associations of device-assessed sedentary time with ADIPO-IR. In Model 1, sedentary time (per 30 minutes) was positively associated with ADIPO-IR (2.34 Arbitrary Unit [AU] [1.02, 3.66]). Following further adjustments for demographics (Model 2) and BMI (Model 3), the positive association remained such that each 30 minutes of sedentary time was

associated with a 1.80 AU (0.51, 3.06) higher ADIPO-IR. To explore whether this association was independent of participants' MVPA levels, we conducted an additional model which was further adjusted for MVPA. This model revealed that sedentary time was positively associated with ADIPO-IR independent of MVPA (2.52 AU [1.05, 3.96]; $p < 0.001$).

INSERT TABLE 2

Moderate-to-vigorous physical activity

Associations between device-assessed MVPA and ADIPO-IR are presented in Table 2. In Models 1-3, MVPA was not significantly associated with ADIPO-IR.

Interaction analyses

Interaction analyses found associations were not modified by either ethnicity ($p = 0.894$), sex ($p = 0.415$) or age ($p = 0.171$). However, results were modified by BMI ($p = 0.005$; Figure 2). Across BMI tertiles, the association between sedentary time and ADIPO-IR strengthened at higher BMIs, with the most pronounced relationship seen with BMI values $\geq$ 33.2 $kg.m^{-2}$ (tertile 3).

INSERT FIGURE 2

Sensitivity analyses

Following the removal of participants taking statins and/or metformin, a total of 126 participants were included in a sensitivity analyses. No significant differences in participant characteristics were evident between this cohort and the whole study cohort ($p \geq 0.072$; see Table 3, Supplementary Digital Content). For this sensitivity cohort, Supplementary Digital Content Table 4 shows the generalised linear model analyses examining associations of sedentary time and MVPA with ADIPO-IR; while Supplementary Digital Content Table 5 details the related interaction analyses. Overall, the pattern of results in this sensitivity cohort

was similar to those reported for the full cohort. In the other sensitivity analysis, we examined associations between our exposure and outcome variables separately in those with (n = 32) and at high-risk of T2DM (n = 147) (see Supplementary Digital Content Table 6). Again, the pattern of results was similar to the combined (full) cohort, however, the p-values approached statistical significance in the smaller T2DM cohort (see Supplementary Digital Content Table 7 and 8). Furthermore, the results of the main study analysis remained consistent when models were adjusted for body fat percentage in place of BMI (see Supplementary Digital Content Table 9 and 10).

Discussion

Our primary finding is that sedentary time is positively associated with ADIPO-IR, independent of MVPA and other confounding variables. This association remained evident in sensitivity analyses excluding participants taking glucose- and lipid-lowering medications and was particularly strong in those with higher BMI values. Conversely, no associations were seen between MVPA and ADIPO-IR.

Adipose tissue insulin resistance is defined as an impaired cellular (adipocyte) response to insulin, resulting in exaggerated lipolysis and/or impaired lipogenesis(3). It manifests in response to adipocyte stress, commonly associated with obesity, adipocyte hypertrophy and low-grade inflammation(3). Our analyses demonstrate that sedentary time is positively related to ADIPO-IR, with every 30 minutes of device-measured sedentary time associated with a 2.5-unit higher ADIPO-IR. Importantly, this relationship is independent of key confounding variables. To contextualise the magnitude of this association, our previous meta-regression(21) identified an opposing yet similar strength association whereby each kg of exercise-induced weight loss was associated with a 2.7-unit lower ADIPO-IR. Adipose tissue inflammation may mechanistically link sedentary time and adipose tissue insulin resistance, with numerous studies showing that greater volumes of sedentary time are positively related to biomarkers of chronic inflammation(22). Given the pathophysiological link between exaggerated adipose lipolysis, ectopic lipid deposition (skeletal muscle, liver, pancreas)(16), and insulin resistance; impaired adipose tissue insulin sensitivity may mechanistically link excess sedentary time with hyperglycaemia and cardiovascular disease risk.

Accumulating evidence suggests that sedentary time and MVPA are independent behaviours with distinct metabolic health impacts(33). Our data support this notion as the association between sedentary time and ADIPO-IR remained after statistically controlling for MVPA.

Although our observational data cannot elucidate mechanisms, our findings are supported by data from bedrest studies which provide an extreme physiological model of sedentary behaviour(34). Specifically, formal exercise training is unable to overcome many adverse metabolic effects, including alterations to lipid metabolism and ectopic fat deposition (34). Our data imply that chronic low-level muscle contraction is necessary for the maintenance of adipocyte sensitivity to insulin, although experimental trials are needed to confirm this notion and identify responsible mechanisms.

Within our analyses we explored whether BMI, age, sex and ethnicity moderated associations between activity behaviours and ADIPO-IR(24–26). These variables were chosen *a priori* given that each influence body composition, fat localisation, and metabolic characteristics of adipocytes. BMI was found to moderate the relationship between sedentary time and ADIPO-IR, with stronger associations seen in those with higher levels of BMI. It is possible that the more deleterious cardiometabolic profile typically seen in those with higher BMI values, including higher circulating insulin and NEFA concentrations, provide greater scope for sedentary time to influence ADIPO-IR. The lack of mediating influence of ethnicity (white European vs. South Asian) was unexpected in our study as South Asians have been found to be more insulin resistant than white Europeans when adiposity is normalised(35). This notion was evident within our dataset whereby South Asians had a similar ADIPO-IR despite a lower BMI and body fat percentage. Furthermore, ethnic-based differences in adipocyte structure and function have been reported(36), prompting our hypothesis that stronger associations between activity behaviours and ADIPO-IR would be seen in South Asians than in white Europeans.

Within our study, we conducted sensitivity analyses which: 1) excluded participants who were taking statins or metformin (~30% of the study cohort) and 2) assessed associations between our exposure and outcome variables separately in participants with T2DM (18% of the cohort) and those at-high risk (82% of the cohort). The first analysis was necessary as statins lower

343 circulating NEFA concentrations(32), while metformin improves glucose regulation in people
344 with impaired glycaemic control. The second analysis was also warranted given that people
345 with T2DM exhibit a more severe metabolic profile, and many were also taking metformin.
346 Overall, these sensitivity analyses demonstrated that the independent association between
347 sedentary time and ADIPO-IR was consistent across our primary and sensitivity analyses;
348 albeit with marginally weaker β-coefficients in the non-medicated sample as well as the group
349 composed solely of individuals with T2DM. However, it should be noted that the small sample
350 sizes in these groups may have contributed to the weaker effect in the latter analyses. One
351 difference between the whole cohort and sensitivity cohort (non-medicated sample) was that
352 BMI moderated the association between sedentary time and ADIPO-IR in the former, but not
353 the latter. Given that the β-coefficients within interaction analyses were similar between
354 cohorts across tertiles of BMI, reduced statistical power with the smaller sample size may
355 similarly explain the absence of the BMI interaction in the sensitivity cohort.

356 In our analyses, MVPA was unrelated to ADIPO-IR, in the both the whole study and sensitivity
357 cohorts. Based on our recent systematic review and meta-analysis(21), this outcome was
358 somewhat unexpected. Specifically, although limited to pre-to-post intervention analyses
359 (without non-exercise control groups), we previously found that structured exercise training
360 reduced ADIPO-IR. Furthermore, many observational studies have documented inverse
361 associations between MVPA and indices of whole-body insulin sensitivity (including fasting
362 insulin)(37, 38), while insulin sensitivity is typically improved in response to physical activity
363 interventions in healthy individuals and those with insulin resistance/hyperglycaemia(39, 40).
364 Given that our sample had notably raised circulating insulin concentrations, with circulating
365 NEFA modestly elevated, it is not clear why an inverse association between MVPA and
366 ADIPO-IR was not apparent. It may be relevant that vigorous-intensity physical activity was
367 negligible in our sample, while participants' moderate-intensity physical activity would

primarily derive from incidental movement behaviours given our study exclusion criteria prohibiting regular, purposeful exercise. Consequently, the intensity of these behaviours may have been insufficient to influence the components of ADIPO-IR.

Key strengths of this study include the 24-hour assessment of physical activity/movement behaviours with highly sensitive accelerometery and the diversity of the sample which permitted interaction analyses. Limitations include the indirect measurement of adipose tissue insulin resistance via ADIPO-IR, rather than direct measurement through an insulin-clamp with stable-isotope tracer (glycerol and/or palmitate tracer). Participants' VPA totalled less than one minute per day meaning specific relationships with this movement behaviour could not be assessed; therefore, further analyses are required in cohorts exhibiting a greater range of times spent in VPA. Furthermore, the causal nature of these findings cannot be determined from our cross-sectional analyses, with intervention studies needed to confirm our findings. Alternative compositional approaches such as isotemporal substitution and compositional data analysis could be employed to provide some insight. However, given the cross-sectional (observational) nature of our data, the current approach was adopted to avoid overstating the implications of our findings and should be interpreted simply as hypothesis-generating with which to inform future prospective studies.

In a population with or at high risk of T2DM, this study has shown that sedentary time is positively associated with adipose tissue insulin resistance, independent of MVPA, with stronger associations seen in people with higher BMIs. Conversely, MVPA was unrelated to adipose tissue insulin resistance. Our observational findings suggest that greater sedentary time may impair the ability of insulin to regulate adipose tissue lipolysis and/or lipogenesis, which may potentially contribute to ectopic lipid deposition, insulin resistance and heightened cardiometabolic risk.

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Conflict of Interest

The authors declare no conflicts of interest. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results do not constitute endorsement by the ACSM.

Supplementary Digital Content

Supplementary digital content (Tables 1 to 10). DOC

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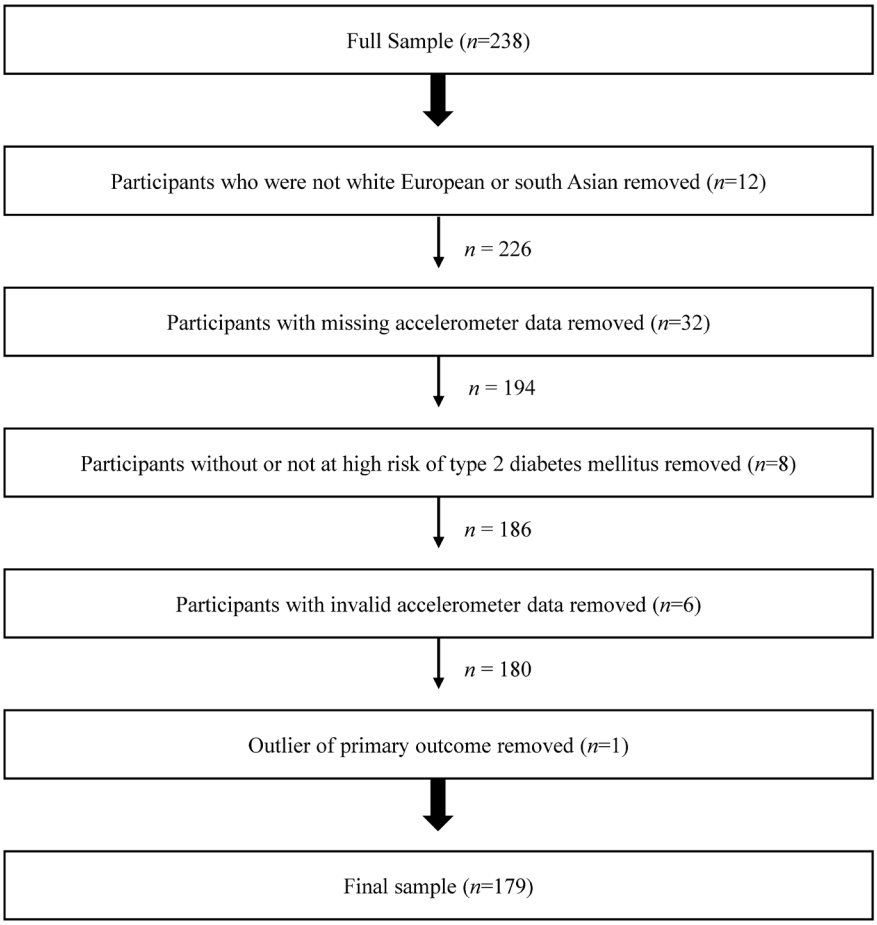
Figure legends:

Figure 1. Study sample reduction process.

Figure 2. Forest plot showing the interaction of BMI within the association between device-measured sedentary time (per 30 min) and ADIPO-IR (n = 179).

542 Fig 1



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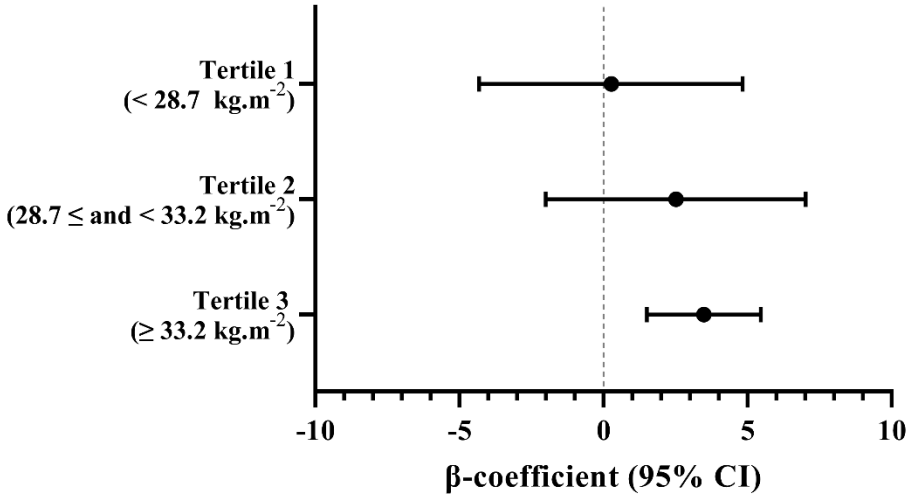

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576 Table 1. Participant characteristics (all cohort and stratified by sex)

Demographic variables	**All (*n*=179)**	**Male (*n*=111)**	**Female (*n*=68)**
Age (years)	66.2 (66.0 – 70.8)	66.0 (58.1 – 71.0)	66.4 (62.0 – 70.6)
Ethnicity (White European) [%]	122 [68.2]	76 [68.5]	46 [67.6]
BMI ($kg.m^{-2}$)	31.1 (28.0 – 34.4)	31.7 (28.0 – 34.3)	30.9 (28.0 – 34.7)
Body fat (%) (*n*=176)	36.4 ± 8.0	32.8 ± 7.3	42.2 ± 5.1
Number of participants diagnosed with T2DM	32 [17.9]	32 [28.8]	0 [0]
Metformin use (no statins) [%] (*n*=177)	5 [2.8]	5 [4.5]	0 [0]
Statin use (no metformin) [%] (*n*=177)	36 [20.2]	24 [21.6]	12 [17.6]
Metformin and statin use [%] (*n*=177)	10 [5.6]	10 [9]	0 [0]
Fasted metabolic variables			
Glucose ($mmol.L^{-1}$)	5.2 (4.4 – 5.9)	5.6 (5.0 – 6.4)	5.0 (4.7 – 5.4)
Insulin ($pmol.L^{-1}$)	75.4 (52.8 – 103.5)	77.4 (56.9 – 107.5)	68.1 (45.9 – 93.2)
NEFA ($mmol.L^{-1}$)	0.52 (0.38 – 0.68)	0.50 (0.36 – 0.67)	0.54 (0.40 – 0.78)
HOMA-IR (AU)	3.43 ± 1.93	3.83 ± 2.05	2.80 ± 1.55
ADIPO-IR (AU)	43.0 ± 26.6	43.6 ± 26.4	42.1 ± 27.3
Physical activity variables			
Waking hours ($mins.d^{-1}$)	968 ± 76	971 ± 75	964 ± 77
Sedentary time ($mins.d^{-1}$)	670 ± 99	677 ± 97	659 ± 103
Light PA ($mins.d^{-1}$)	225 ± 72	219 ± 75	233 ± 65
Vigorous PA ($mins.d^{-1}$)	0.9 (0.2 – 2.0)	0.9 (0.2 – 2.2)	0.9 (0.4 – 1.8)
1-minute bouted MVPA ($mins.d^{-1}$)	24 (11 – 43)	27 (13 – 49)	18 (10 – 37)

577 Data are presented as mean ± SD, median (interquartile range) or number [column percentage].

578 ADIPO-IR, adipose tissue insulin resistance index; AU, arbitrary unit, BMI, body mass index; HOMA-
579 IR, homeostatic assessment for insulin resistance; MVPA, moderate-to-vigorous physical activity;
580 NEFA, non-esterified fatty acid; PA, physical activity

Table 2. Associations of device-measured sedentary time and physical activity with ADIPO-IR

***n*=179**	**Sedentary Time (per min)**		**Sedentary Time (per 30 mins)**		**1-minute bouted MVPA (per min)**		**1-min bouted MVPA (per 10 mins)**	
	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**
Model 1								
ADIPO-IR	0.078 (0.034, 0.122)	**<0.001**	2.34 (1.02, 3.66)	**<0.001**	-0.065 (-0.192, 0.062)	0.315	-0.65 (-1.92, 0.63)	0.315
Model 2								
ADIPO-IR	0.079 (0.034, 0.123)	**<0.001**	2.37 (1.02, 3.69)	**<0.001**	-0.060 (-0.191, 0.071)	0.372	-0.60 (-1.91, 0.71)	0.372
Model 3								
ADIPO-IR	0.060 (0.017, 0.102)	**0.006**	1.80 (0.51, 3.06)	**0.006**	0.023 (-0.104, 0.150)	0.718	0.23 (-1.04, 1.50)	0.718

Model 1: adjusted for study and waking hours.

Model 2: adjusted for Model 1 + age, sex, and ethnicity.

Model 3: adjusted for Model 2 + BMI

Supplementary Digital Content

Supplementary Table 1. Summary of included studies

	Running period	Study design	Inclusion criteria	Exclusion criteria	Primary outcome
Stand Up (NCT02453204)	April 2015-March 2017	Randomised interventional (crossover model)	-Aged 65 to 79 years -Able to walk without any assistive device and not requiring assistance from another person	-Regular purposeful exercise (≥75 minutes of self-reported vigorous exercise per week) -Inability to stand -Use of glucose lowering medication and/or steroid	Insulin area under the curve
Go For It (ISRCTN12337078)	May 2015-July 2018	Randomised interventional (crossover model)	-Aged 50 to 74 years -2 h glucose 7.8, 11.1 mmol.L^{-1} after an OGTT OR has HbA1c: 5.70% to 6.49% at baseline or within the last 5 years OR BMI ≥27.5 kg/m^2 (WE) or ≥25 kg/m^2 (SA) -Weight stable (less than ± 5 kg in the last 6 months)	-Regular purposeful exercise (≥3 x 20-minute bouts of vigorous exercise) -Individuals who stand for extended periods of time -Use of glucose lowering medication -Dieters/restrained eaters	6-hour postprandial area under the incremental glucose curve
Plant Your Health (NCT03315988)	October 2017-January 2019	Interventional single group assignment	-Aged 18 to 75 years -2h glucose ≥7.8 mmol/L after a standard OGTT OR HbA1c ≥5.7% and ≤ 8% within last 36 months -Overweight or obese (BMI ≥ 25 kg/m^2 if WE or BMI ≥ 23 kg/m^2 if SA) -Regular meat and/or fish eater (at least 3 times per week)	-HbA1c > 8.0% (64 mmol/mol) -Use of glucose lowering medication (within the last 60 d) -Current smokers -Current use of vegan/vegetarian diet -Significant weight change (≥10%) over the last 3 months -Clinical eating disorder -Current or recent (within 6 months) oral antibiotics or steroid use -Ongoing cardiovascular disease	Effect of an 8-week vegan diet on TMAO plasma levels and 3-hour post-challenge glucose levels compared to baseline

	Running period	Study design	Inclusion criteria	Exclusion criteria	Primary outcome
Up For 5 (NCT03482596)	February 2018-July 2019	Interventional single group assignment	-Aged 40 to 75 years -Overweight (WE: BMI > 25 - <30 kg/m^2, SA: > 23 <27.5 kg/m^2) with HbA1c 5.7, 7.5% (42), within the previous 36 months) OR Obese (WE: BMI ≥ 30 kg/m^2, SA: ≥ 27.5 kg/m^2) -Large proportions of their day spent sitting (self-reported)	-Regular purposeful exercise (at least once a week) -HbA1c >7.5% -Overweight with HbA1c <5.7% -Use of glucose lowering medication -Type 1 diabetes -Recent cardiovascular event (within the last 12 months) -Current smoker -Steroid use	Postprandial glucose incremental area under the curve
DELIVER (NCT04004273)	October 2018-July 2022	Randomised interventional (controlled) trial	-Aged 30 to 75 years -BMI: 27 to 45 kg.m^{-2} -Waist circumference ≥ 94 cm (or ≥ 90 cm if SA) -Clinically elevated liver fat (≥ 5.56% assessed via ^{1}H-MRS) *Additional criteria for those diagnosed type 2 diabetes:* -Treatment via lifestyle or metformin only within the last 6 months -HbA1c 6.5 – 10%	-Contraindications to magnetic resonance procedures -Regular purposeful exercise training of vigorous intensity –≥ 3 sessions per week) -Weight instability or planned/ on-going dietary intervention -Current smoker -Uncontrolled hypertension - systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg -Use of additional glucose lowering medication to metformin within the last 6 months -Taking insulin	Liver saturated lipid index (%) determined by proton magnetic resonance spectroscopy

	Running period	Study design	Inclusion criteria	Exclusion criteria	Primary outcome
The Yoga Study (NCT03549390)	October 2018-March 2020	Randomised interventional (crossover model)	-Aged 18 to 75 years -HbA1c >5.7% and BMI ≥23kg/m2* / ≥25kg/m2 (WE) OR BMI ≥27.5kg/m2* / ≥30kg/m2 (WE) -Do not engage in regular sports or strenuous physical activity -No medical conditions that affect balance and ability to undertake yoga postures -No other current medical conditions	-Regular purposeful exercise or strenuous leisure time exercise (>120 minutes self-reported exercise per week) -HbA1c >8.0% -Use of glucose lowering medication -Inability to stand or undertake light-moderate physical activity -Ongoing cardiovascular disease -Steroid abuse -Current smoker -Pregnant/lactation	Insulin area under the curve

BMI; body mass index, OGTT; oral glucose tolerance test, SA; South Asian, TMAO; Trimethylamine N-oxide, WE; white Europe

Supplementary Table 2. Participant characteristics stratified by ethnicity

Demographic variables (*n*=179)	**White European (*n*=122)***	**South Asian (*n*=57)****	***p*-value**
Age (years)	66.6 (61.8 – 71.0)	66.0 (56.0 – 69.5)	0.300
Sex (male) [%]	76 [62.3]	35 [61.4]	
BMI ($kg.m^{-2}$)	32.6 (28.8 – 36.1)	28.3 (25.6 – 31.4)	**<0.001**
Body fat (%)	37.2 ± 8.2	34.6 ± 7.3	**0.049**
Number of participants diagnosed with T2DM	28 [23]	4 [7]	
Metformin use (no statins) [%]	4 [3.3]	1 [1.8]	
Statins use (no metformin) [%]	25 [14]	11 [19.3]	
Both use (metformin and statins)	9 [5]	1 [1.8]	
Fasted metabolic variables			
Glucose ($mmol.L^{-1}$)	5.2 (4.8 – 6.0)	5.2 (4.9 – 5.7)	0.512
Insulin ($pmol.L^{-1}$)	75.0 (54.4 – 105.5)	76.2 (51.5 – 102.4)	0.974
NEFA ($mmol.L^{-1}$)	0.53 (0.38 – 0.69)	0.50 (0.40 – 0.64)	0.530
HOMA-IR (AU)	3.52 ± 2.03	3.25 ± 1.71	0.379
ADIPO-IR (AU)	43.6 ± 26.9	41.9 ± 26.3	0.687
Physical activity variables			
Waking hours ($mins.d^{-1}$)	966 ± 74	973 ± 80	0.535
Sedentary time ($mins.d^{-1}$)	674 ± 101	662 ± 97	0.485
Light PA ($mins.d^{-1}$)	222 ± 76	230 ± 61	0.494
Vigorous PA ($mins.d^{-1}$)	0.7 (0.2 – 1.7)	1.4 (0.4 – 3.4)	**0.003**
1-minute bouted MVPA ($mins.d^{-1}$)	25 (11 – 45)	23 (12 – 42)	0.867

Data are presented as mean ± SD, median (interquartile range) or number [column percentage].

ADIPO-IR, adipose tissue insulin resistance index; AU, arbitrary unit; BMI, body mass index; HOMA-IR, homeostatic assessment for insulin resistance; MVPA, moderate-to-vigorous physical activity; NEFA, non-esterified fatty acid; PA, physical activity.

*White European ethnicity was defined as those identifying themselves as “white/Caucasian” and descending from any European country.

**South Asian ethnicity was defined as those identifying as “Asian” or “Asian British (Indian, Pakistani, Bangladeshi)”.

Supplementary Table 3. Participant characteristics for whole study cohort versus those not taking statins and/or metformin (sensitivity cohort).

Demographic variables	**All (*n*=179)**	**Sensitivity cohort (*n*=126)**	***p*-value**
Age (years)	66.2 (66.0 – 70.8)	66.1 (57.4 – 71.2)	0.993
Sex (male) [%]	111 [62.0]	70 [55.6]	
Ethnicity (White European) [%]	122 [68.2]	84 [66.7]	
BMI ($kg.m^{-2}$)	31.1 (28.0 – 34.4)	30.6 (25.1 – 33.7)	0.480
Body fat (%)	36.4 ± 8.0	36.4 ± 8.0	0.972
Fasted metabolic variables			
Glucose ($mmol.L^{-1}$)	5.2 (4.4 – 5.9)	5.2 (4.8 – 5.6)	0.090
Insulin ($pmol.L^{-1}$)	75.4 (52.8 – 103.5)	75.2 (51.0 – 103.6)	0.779
NEFA ($mmol.L^{-1}$)	0.52 (0.38 – 0.68)	0.50 (0.37 – 0.65)	0.445
HOMA-IR (AU)	3.43 ± 1.93	3.20 ± 1.76	0.268
ADIPO-IR (AU)	43.0 ± 26.6	41.4 ± 26.4	0.600
Physical Activity			
Waking hours ($mins.d^{-1}$)	968 ± 76	963 ± 75	0.547
Sedentary time ($mins.d^{-1}$)	670 ± 99	663 ±93	0.559
Light PA ($mins.d^{-1}$)	225 ± 72	224 ± 72	0.914
Vigorous PA ($mins.d^{-1}$)	0.9 (0.2 – 2.0)	1.1 (0.4 – 2.2)	0.244
1-minute bouted MVPA ($mins.d^{-1}$)	24 (11 – 43)	24 (12 – 46)	0.690

Data are presented as mean ± SD, median (interquartile range) or number [column percentage].

ADIPO-IR, adipose tissue insulin resistance index; AU, arbitrary unit; BMI, body mass index; HOMA-IR, homeostatic assessment for insulin resistance; MVPA, moderate-to-vigorous physical activity; NEFA, non-esterified fatty acid; PA, physical activity.

Supplementary Table 4. Associations of device-measured sedentary time and physical activity with ADIPO-IR after excluding participants on statins and/or metformin.

***n*=126**	**Sedentary Time (per min)**		**Sedentary Time (per 30 mins)**		**1-minute bouted MVPA (per min)**		**1-minute bouted MVPA (per 30 mins)**	
	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**
Model 1								
ADIPO-IR	0.083 (0.033, 0.134)	**0.001**	2.49 (0.99, 4.02)	**0.001**	-0.102 (-0.237, 0.033)	0.140	-1.02 (-2.37, 0.33)	0.140
Model 2								
ADIPO-IR	0.083 (0.032, 0.134)	**0.001**	2.49 (0.96, 4.02)	**0.001**	-0.108 (-0.249, 0.033)	0.133	-1.08 (-2.49, 0.33)	0.133
Model 3								
ADIPO-IR	0.062 (0.012, 0.112)	**0.015**	1.86 (0.36, 3.36)	**0.015**	-0.038 (-0.177, 0.100)	0.587	-0.38 (-1.77, 1.00)	0.587

Model 1 adjusted for study and waking hours.

Model 2 adjusted for Model 1 + age, sex, and ethnicity.

Model 3 adjusted for Model 2 + BMI.

Supplementary Table 5. Interaction analyses with ethnicity, sex, age, and BMI for device-measured sedentary time after excluding participants on statins and/or metformin.

	Explanatory Variable	***n***	***p*-value for interaction**	Category 1 **β (95% CI)**	Category 2 **β (95% CI)**	Category 3 **β (95% CI)**
Ethnicity				**White European**	**South Asian**	
	Sedentary Time (per 30 mins)	126	0.627	2.07 (-3.00, 7.14)	1.41 (-0.96, 3.78)	
Sex				**Male**	**Female**	
	Sedentary Time (per 30 mins)	126	0.598	2.19 (0.27, 4.08)	1.50 (-2.97, 5.94)	
Age				**< 63 years**	**63 ≤ and < 69 years**	**≥ 69 years**
	Sedentary Time (per 30 mins)	126	0.494	3.87 (-1.68, 9.42)	0.57 (-5.16, 6.24)	1.35 (-1.08, 3.75)
BMI				**< 28.7 kg.m^{-2}**	**28.7 ≤ and < 33.2 kg.m^{-2}**	**≥ 33.2 kg.m^{-2}**
	Sedentary Time (per 30 mins)	126	0.249	0.78 (-5.76, 7.32)	2.88 (-3.36, 9.09)	3.36 (0.45, 6.24)

Supplementary Table 6. Participant characteristics for participants not diagnosed with T2DM versus those diagnosed with T2DM.

Demographic variables	**Participants with non-T2DM (*n*=147)**	**Participants with T2DM (*n*=32)**	***p*-value**
Age (years)	67.0 (61.0 – 71.0)	64.4 (57.2 – 68.9)	0.098
Sex (male) [%]	79 [53.7]	32 [100]	
Ethnicity (White European) [%]	94 [63.9]	28 [87.5]	
BMI ($kg.m^{-2}$)	30.1 (27.3 – 34.3)	33.0 (30.9 – 40.6)	**0.002**
Body fat (%)	37.3 ± 8.2	32.4 ± 5.3	**<0.001**
Fasted metabolic variables			
Glucose ($mmol.L^{-1}$)	5.1 (5.0 – 5.6)	7.23 (6.2 – 8.0)	**<0.001**
Insulin ($pmol.L^{-1}$)	72.9 (51.0 – 101.4)	89.0 (65.8 – 106.5)	0.082
NEFA ($mmol.L^{-1}$)	0.50 (0.37 – 0.65)	0.67 (0.45 – 0.71)	0.067
HOMA-IR (AU)	3.10 ± 1.71	4.97 ± 2.16	**<0.001**
ADIPO-IR (AU)	41.5 ± 26.7	50.3 ± 25.4	0.091
Physical Activity			
Waking hours ($mins.d^{-1}$)	969 ± 78	964 ± 67	0.739
Sedentary time ($mins.d^{-1}$)	674 ± 97	621 ± 111	0.242
Light PA ($mins.d^{-1}$)	218 ± 65	254 ± 93	**0.047**
Vigorous PA ($mins.d^{-1}$)	1.1 (0.4 – 2.2)	0.2 (0.0 – 1.2)	**<0.001**
1-minute bouted MVPA ($mins.d^{-1}$)	23 (11 – 42)	30 (11 – 53)	0.256

Data are presented as mean ± SD, median (interquartile range) or number [column percentage].

ADIPO-IR, adipose tissue insulin resistance index; AU, arbitrary unit; BMI, body mass index; HOMA-IR, homeostatic assessment for insulin resistance; MVPA, moderate-to-vigorous physical activity; NEFA, non-esterified fatty acid; PA, physical activity.

Supplementary Table 7. Associations of device-measured sedentary time and physical activity with ADIPO-IR on those not diagnosed with T2DM

***n*=147**	**Sedentary Time (per min)**		**Sedentary Time (per 30 mins)**		**1-minute bouted MVPA (per min)**		**1-minute bouted MVPA (per 30 mins)**	
	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**
Model 1								
ADIPO-IR	0.077 (0.023 to 0.131)	**0.005**	2.31 (0.69 to 3.93)	**0.005**	-0.022 (-0.162 to 0.117)	0.754	-0.22 (-1.62 to 1.17)	0.754
Model 2								
ADIPO-IR	0.080 (0.025 to 0.134)	**0.004**	2.4 (0.75 to 4.02)	**0.004**	-0.015 (-0.161 to 0.131)	0.841	-0.15 (-1.61 to 1.31)	0.841
Model 3								
ADIPO-IR	0.051 (-0.001 to 0.103)	0.055	1.53 (-0.03 to 3.09)	0.055	0.076 (-0.061 to 0.213)	0.276	0.76 (-0.61 to 2.13)	0.276

Model 1 adjusted for study and waking hours.

Model 2 adjusted for Model 1 + age, sex, and ethnicity.

Model 3 adjusted for Model 2 + BMI.

Supplementary Table 8. Associations of device-measured sedentary time and physical activity with ADIPO-IR on those diagnosed with T2DM

***n*=32**	**Sedentary Time (per min)**		**Sedentary Time (per 30 mins)**		**1-minute bouted MVPA (per min)**		**1-minute bouted MVPA (per 30 mins)**	
	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**
Model 1								
ADIPO-IR	0.060 (-0.006 to 0.127)	0.076	1.8 (-0.18 to 3.81)	0.076	-0.107 (-0.363 to 0.148)	0.411	-1.07 (-3.63 to 1.48)	0.411
Model 2								
ADIPO-IR	0.058 (-0.006 to 0.122)	0.076	1.74 (-0.18 to 3.66)	0.076	-0.135 (-0.383 to 0.113)	0.287	-1.35 (-3.83 to 1.13)	0.287
Model 3								
ADIPO-IR	0.056 (-0.008 to 0.120)	0.086	1.68 (-0.24 to 3.60)	0.086	-0.111 (-0.388 to 0.166)	0.432	-1.11 (-3.88 to 1.66)	0.432

Model 1 adjusted for study and waking hours.

Model 2 adjusted for Model 1 + age, sex, and ethnicity.

Model 3 adjusted for Model 2 + BMI.

Supplementary Table 9. Associations of device-measured sedentary time and physical activity with ADIPO-IR

***n*=176**	**Sedentary Time[a] (per min)**		**Sedentary Time[a] (per 30 mins)**		**1-minute bouted MVPA[b] (per min)**		**1-minute bouted MVPA[b] (per 10 mins)**	
	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**	***β* (95% CI)**	***p*-value**
Model 1								
ADIPO-IR	0.084 (0.039, 0.129)	**<0.001**	2.52 (1.17, 3.87)	**<0.001**	-0.079 (-0.209, 0.050)	0.230	-0.79 (-2.09, 0.50)	0.230
Model 2								
ADIPO-IR	0.086 (0.041, 0.131)	**<0.001**	2.58 (1.23, 3.93)	**<0.001**	-0.072 (-0.207, 0.062)	0.294	-0.72 (-2.07, 0.62)	0.294
Model 3								
ADIPO-IR	0.068 (0.024, 0.111)	**0.002**	2.04 (0.72, 3.33)	**0.002**	-0.009 (-0.138, 0.119)	0.886	-0.09 (-1.38, 1.19)	0.886
Model 4								
ADIPO-IR	0.086 (0.037, 0.135)	**<0.001**	2.58 (1.11, 4.05)	**<0.001**	0.110 (-0.032, 0.252)	0.129	1.10 (-0.32, 2.52)	0.129

Model 1 adjusted for study and waking hours.

Model 2 adjusted for Model 1 + age, sex, and ethnicity.

Model 3 adjusted for Model 2 + body fat percentage

Model 4 adjusted for Model 3 + MVPA[a]/ Sedentary time[b]

Supplementary Table 10. Interaction analyses with ethnicity, sex, age, and body fat percentage for device-measured sedentary time.

				Category 1	Category 2	Category 3
	Explanatory Variable	***n***	***p*-value for interaction**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Ethnicity				**White European**	**South Asian**	
	Sedentary Time (per 30 mins)	176	0.767	1.92 (-2.55, 6.42)	2.28 (0.15, 4.44)	
Sex				**Male**	**Female**	
	Sedentary Time (per 30 mins)	176	0.030	1.08 (-0.48, 2.61)	3.48 (-0.24, 7.17)	
Age				**< 63 years**	**63 ≤ and < 69 years**	**≥ 69 years**
	Sedentary Time (per 30 mins)	176	0.209	4.8 (0.15, 9.48)	-0.06 (-4.8, 4.65)	1.44 (-0.66, 3.54)
Body fat %				**< 31.54 %**	**31.54 ≤ and < 39.48 %**	**≥ 39.48 %**
	Sedentary Time (per 30 mins)	176	0.005	2.19 (-2.82, 7.14)	1.74 (-3.09, 6.51)	3.75 (1.56, 5.91)