

Full title: Associations of sedentary time with fat distribution in a high risk population

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Main text word count = 3433; Number of Tables = 4; Number of Figures = 1; Number of
references = 39

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Abstract

Purpose

The effect of sedentary behaviour on regional fat deposition, independent of physical activity remains equivocal. We examined the cross-sectional associations between objectively measured sedentary time and markers of regional fat distribution (heart, liver, visceral, subcutaneous and total body fat) in a population at a high risk of type 2 diabetes mellitus (T2DM).

Methods

Participants were recruited from primary care to two diabetes prevention programmes. Sedentary time (<25 counts per 15 seconds) was measured using Actigraph GT3X accelerometers. Heart, liver, visceral, subcutaneous and total body fat were quantified using magnetic resonance images (MRI). Fat volumes were calculated by multiplying the cross-sectional areas of the fat-containing pixels by the slice thickness. The liver fat percentage was measured using a representative region of interest created in the right lobe of the liver avoiding the main portal veins. Linear regression models examined the association of sedentary time with markers of regional fat deposition.

Results

Sixty-six participants (age=47.9±16.2years;male=50.0%) were included. Following adjustment for several covariates, including glycaemia, whole body fat and moderate-to-vigorous physical activity (MVPA), each 30 minutes of sedentary time was associated with 15.7cm³ higher heart fat ($p=0.008$), 1.2% higher liver fat ($p=0.026$) and 183.7cm³ higher visceral fat ($p=0.039$).

Conclusion

This study provides new evidence suggesting that objectively measured sedentary behaviour may have an independent association upon heart, liver and visceral fat in individuals at a high risk of T2DM.

Keywords; Type 2 diabetes, Sedentary behaviour, High risk, Fat distribution, MRI, Primary care

Introduction

Abdominal obesity is known to predispose individuals to cardiovascular disease (CVD) and type 2 diabetes (T2DM), with regional fat deposits being postulated to be of greater importance than overall adiposity in causing metabolic and cardiovascular disturbance (5, 31). Several studies have implicated pericardial and liver fat as particular pathogenic risk factors (23,26), with excess visceral adiposity also being associated with dyslipidemia, systemic inflammation, insulin resistance, T2DM and all-cause mortality (1,7,15,18).

Despite the well-documented positive effects of moderate-to-vigorous physical activity (MVPA) on regional fat deposition (16), the associative role of sedentary behaviour, independent of physical activity, is less well understood and the available literature equivocal.

Over the past decade there has been an accumulation of epidemiological evidence from both cross-sectional and prospective observational studies indicating that sedentary behaviour (best conceptualised as any non-exercise sitting time (30)) may be independently associated with several deleterious health outcomes, including T2DM, obesity, the metabolic syndrome, cardiovascular disease and cardiovascular mortality (8,33,36,37). However, previous cross-sectional and longitudinal studies conducted in the general population have shown no association between sedentary behaviour and visceral fat accumulation in adults (20,25,29). Although associations have previously been observed between objectively measured sedentary time and pericardial fat (11,20), the relationships were either attenuated after adjustment for MVPA (11) or MVPA was quantified using self report (20), thus raising issues regarding response bias and poor levels of validity (27). It therefore remains unclear

whether objectively measured sedentary behaviour is associated with regional fat deposition, independent of MVPA or total physical activity. Moreover, to our knowledge, there are currently no reports examining the association between sedentary behaviour and liver fat.

It is also necessary to establish the association between sedentary behaviour and fat distribution in those at high risk of chronic disease. Both national and international recommendations and policies specify that chronic-disease prevention strategies should include targeted interventions aimed at the identification and management of high risk individuals (2). Moreover, sedentary time has been shown to be more strongly and adversely associated with cardio-metabolic variables (including markers of adiposity) in high risk individuals, (14) and those with established T2DM (3,4) after adjustment for MVPA and other important confounders. Given that associations between sedentary time and markers of adiposity (body mass index (BMI) and waist circumference) were weaker compared to other cardio-metabolic variables (14), the association of sedentary behaviour may extend beyond traditional measures of adiposity and may lie in the location of fat deposition. In particular, within cells of non-adipose tissue that normally contain only small amounts of fat (ectopic fat). Such ectopic depositions result in excess lipids being driven into alternative, non-oxidative pathways, which in turn promotes metabolically relevant cellular dysfunction (lipotoxicity).

The aim of this study, therefore, was to examine the association between objectively measured sedentary time and heart, liver, visceral, subcutaneous and total body fat, independent of MVPA and whole body fat in a population at high risk of T2DM.

Methods

Subjects

The present study reports a baseline convenience subsample (n=66) from the Walking Away from Type 2 Diabetes Study (WA) and Project STAND (Sedentary Time And Diabetes). When combined, the full cohort for both studies included 1,026 participants (WA=833, Project STAND=193). Both of these diabetes prevention studies were conducted by the same research group within the same geographical area (Leicestershire and South East Midlands, United Kingdom (UK)) and baseline data collection was undertaken during 2010. All measurements were performed by the same team of trained staff who followed identical standard operating procedures. A detailed description of both trial methods have been published elsewhere (38,39).

Walking Away

Participants (aged 30-74 years) were recruited from 10 primary care practices within the Leicestershire region (city and county), UK. Individuals at high risk of impaired glucose regulation (IGR) (composite of impaired glucose tolerance (IGT) and/or impaired fasting glycaemia (IFG)) or T2DM were identified using a modified version of the automated Leicester Risk Score, specifically designed to be administered in primary care (10). The Morbidity, Information Query and Export Syntax (MIQUEST) programme was used to assess medical records and rank individuals for diabetes risk using predefined weighted variables commonly held on practice databases (age, gender, BMI, family history of T2DM and use of antihypertensive medication). Those scoring within the 90th percentile in each practice were

invited to take part in the study. This approach has been shown to have good sensitivity and specificity for identifying participants at a high risk of IGR (10).

Project STAND

Young adults who were at risk of developing T2DM were recruited from primary care practices located across Leicestershire and the South East Midlands region. Practice databases were searched for participants meeting the following inclusion criteria: a) aged 18-40 years with a BMI in the obese range ($\geq 30\text{kg/m}^2$; $\geq 27.5\text{kg/m}^2$ for south Asians) or b) aged 18-40 years with a BMI in the overweight range $\geq 25\text{kg/m}^2$ ($\geq 23\text{kg/m}^2$ for south Asians) plus one additional risk factor: a family history of T2DM or CVD, previous gestational diabetes, polycystic ovarian syndrome, HbA1c $\geq 5.8\%$ or IGR (38).

Individuals were excluded from both studies if they were taking steroids or had previously diagnosed T2DM. Written Informed consent was obtained from all eligible participants and both studies gained full ethical and governance approval.

Covariates

Information on current smoking status, family history of T2DM, medication status and ethnicity (coded according to census criteria) was obtained following an interview-administered questionnaire with a health care professional. Waist circumference was measured over light clothing between the lower rib margin and the iliac crest. Height and weight (Tanita TBE 611, Tanita, West Drayton, UK) were obtained by trained staff according to standard operating procedures. The subsequent values were used to compute BMI (kg/m^2).

Systolic and diastolic blood pressure (mmHg) were taken three times in succession and the mean of the last two used for analysis.

Social deprivation was determined by assigning an Index of Multiple Deprivation (IMD) score to the participant's resident area (based on postcode) (32). IMD scores are publically available continuous measures of compound social and material deprivation. Areas are ranked from least deprived to most deprived based upon several dimensions linked to health outcomes (including; income, employment, education, living environment and health).

Venous blood samples were obtained following an overnight fast, and all assays were measured in the same laboratory. Analysis was conducted by individuals blinded to the patients' identity, using stable methods, standardised to external quality assurance values. HbA1c was analysed using the Bio-Rad Variant II HPLC system (Bio-Rad Clinical Diagnostics, Hemel Hempstead, UK) and total cholesterol was measured using standard enzymatic techniques.

Quantification of sedentary time

All eligible participants were asked to wear a tri-axial accelerometer at the baseline visit (Actigraph GT3X, Pensacola, FL, USA), for a minimum of seven consecutive days during waking hours. These accelerometers translate raw accelerations into activity counts. Cut-points modified by Troiano et al. were used to categorise an epoch as sedentary (<25 counts per 15 seconds) or MVPA (≥ 505 counts per 15 seconds). These intensity thresholds were calculated as a weighted average determined from previous treadmill or track walking studies (34). Total physical activity volume represented the summation of counts within each epoch.

Non-wear time was defined as a minimum of 60 minutes of continuous zero counts and days with at least 600 minutes of wear time were considered valid (13, 14). In order to be included in the analysis, participants were required to have a minimum of four valid days (35).

A data analysis tool (KineSoft version 3.3.75, Kinesoft, New Brunswick, Canada; www.kinesoft.org) was used to process the accelerometer data.

Measure of adiposity

Magnetic Resonance Imaging (MRI) was performed at Glenfield Hospital, Leicester, UK, where heart, liver, visceral, subcutaneous and total body fat (includes liver, intra-abdominal, subcutaneous and visceral fat) was quantified. MRI is a reliable modality for the assessment of adipose tissue and is capable of measuring fat distribution with a high spatial resolution (22).

Scanning was performed using either a 1.5 Tesla Avanto (WA) or a 3.0 Tesla Skyra system (STAND) (Siemens Medical, Erlangen, Germany). Flexible body array coils were applied to the thorax and abdomen for signal reception. For lipid volume quantification, a 2-point Dixon gradient-echo pulse sequence was used to separate tissue water signal from lipid signal and to create two separate image sets with signal intensity showing ‘fat’ and ‘water’ content (21). 3-D images were acquired axially with 5 mm slice thickness and in-plane resolution of 1.56 mm, interpolated to 0.78 mm. The field of view was 500 mm (left-right) by 375 mm (anterior-posterior). Images were acquired in three contiguous blocks, covering the thoracic, abdominal and pelvic regions, with each block acquired in a breath-hold at full inspiration to minimise motion-related artefacts and to negate changes in slice position. The acquisition

time for each block was 18s. All scans were performed by the same team of trained staff according to standardised procedures.

Analysis of the MR images was performed using image analysis software produced in-house (Java Image Manipulation, Version 7). All analysis was undertaken by the same researcher who was blinded to the clinical, anthropometric and physical activity data.

For analysis, the ‘fat’ and ‘water’ images were mathematically combined to create a ‘fat percentage’ image. Fat-containing pixels were then defined as those with a pixel intensity between 51 and 99% (100% being due to image artefact). The images were reconstructed into 15 mm thick contiguous slices, from the top of the pulmonary trunk extending to the bottom of the symphysis pubis. Volumes of interest for the whole body and heart were created by outlining the perimeter of the body and heart respectively on each relevant slice using a mouse-controlled pointer and excluding those pixels outside the structures. The region of interest surrounding the heart included myocardial, epicardial (pericardial) and immediate extra-pericardial (thoracic) fat.

The visceral (and retroperitoneal) fat was further separated, by outlining the abdominal and chest wall muscles and excluding the pixels for the subcutaneous fat. The fat volume was calculated automatically by multiplying the cross-sectional areas of the fat-containing pixels, summed over all slices on which the tissue was outlined, by the slice thickness. This created three fat volumes: total body fat, visceral fat from the top of the pulmonary trunk to the bottom of the symphysis pubis, and the heart fat volume. The liver fat percentage was also measured using a representative region of interest created in the right lobe of liver avoiding

the main portal veins. Subcutaneous fat was calculated by subtracting visceral fat from total body fat.

Statistical Analysis

IBM SPSS Statistics v20.0 (Chicago, IL, USA) was used to conduct all statistical analyses. Linear regression analysis was used on the combined study cohorts to examine the independent association of sedentary time (independent variable), with various markers of regional fat deposition (dependent variable). We display results per 30 minutes of sedentary time for ease of interpretation.

Model 1 was adjusted for age (continuous), gender, ethnicity (white European/south Asian/other), social deprivation (continuous), family history of T2DM (yes/no), smoking status (current/ex/never smoked), total cholesterol, HbA1c, systolic blood pressure, blood pressure medication (ACE inhibitors (yes/no)), beta-blockers (yes/no), lipid lowering medication (yes/no), time accelerometer worn (average number of minutes per day) and MVPA. We also undertook the same model, but adjusted for total physical activity volume (counts per day) rather than MVPA given that others have suggested this mediates significant associations between sedentary behaviour and metabolic health (24). In order to examine the extent to which total adiposity attenuated these relationships, model 2 was further adjusted for whole body fat. Models were assessed for normality and multi co-linearity was assessed through the variance inflation factor (VIF). To further represent the strength of sedentary time with markers of adiposity, variables were also examined as tertiles using analysis of covariance procedures.

Significant observations were followed up with interaction terms to assess associations between sedentary time and study, sex, level of MVPA, whole body fat and HbA1c. All interactions were adjusted for the covariates listed in model 1.

Two-tailed p values of 0.05 or less were considered statistically significant for main effects. $p < 0.1$ was considered significant for interactions. To allow for direct comparisons across fat deposition markers, results of the generalised linear regression analysis are also presented as the standardised beta co-efficient (β) \pm standard error (SE).

Results

Table 1 displays the demographic, anthropometric, MRI-derived and accelerometer characteristics of included participants. In total, 32 participants from Project STAND (age=33.1 \pm 6.0 years; male=34.4%) and 34 participants from WA (age=61.9 \pm 8.0 years; male=64.7%) had valid measures of objective activity and MRI data.

There were no statistical differences ($p > 0.05$) in anthropometric, metabolic, and social deprivation measures between participants who were included in this analysis vs. those not included (did not undergo an MRI scan).

Model 1 illustrates the linear relationship between each 30 minute block of sedentary time and markers of regional fat deposition. Following adjustment for various confounders, including HbA1c, and MVPA, 30 minutes of sedentary time was associated with 20.5cm³ higher heart fat ((95% CI) 5.4, 35.6), 1.4% higher liver fat (0.3, 2.5) and a 409.2cm³ higher visceral fat (127.6, 690.8). All significant associations seen in Model 1 persisted after further

adjustment for whole body fat in Model 2 (15.7cm³ higher heart fat ((95% CI) 0.5, 30.8), 1.2% higher liver fat (0.3, 2.3) and a 191.3cm³ higher visceral fat (2.7, 368.8).

No significant associations were observed for whole body and subcutaneous fat (Table 2). Supplementary Table 1 also displays the overall associations (presented as standardised $\beta \pm$ SE) in the combined cohort for total sedentary time with MRI-derived markers of regional fat deposition.

In order to provide visual representation of reported associations, figure 1 illustrates the associations between total sedentary time and heart fat, liver fat and visceral fat when examined as tertiles, after adjustment for the covariates listed above. Compared to those in the lowest tertile of sedentary time, those in the highest tertile had, on average, 13.2cm³ higher heart fat ($p < 0.001$), 1.6% higher liver fat ($p < 0.001$) and a 556.3cm³ higher visceral fat ($p < 0.001$).

Interaction analyses indicated a significant effect for study group with the older cohort (WA) demonstrating stronger associations of sedentary time with visceral fat (presented as unstandardised β (95% CI)) (WA = 800.0 (345.3, 1255.9) vs. STAND = 69.4 (-297.8, 436.6) (p for interaction=0.010). Sex interactions also indicated that sedentary time had a larger impact on visceral fat in males (male = 779.1 (171.4, 1386.9) vs. female = 133.4 (-269.0, 544.8) (p for interaction=0.049) (Table 3). No other significant interactions for associations with measures of ectopic fat were observed for study group, sex, whole body fat, MVPA or HbA1c level ($p > 0.1$).

The findings above were unaffected if waist circumference or BMI rather than whole body fat was used in Model 2 (data not shown).

Discussion

This study conducted in individuals at high risk of T2DM, demonstrated that sedentary time was associated with heart, liver and visceral fat, independent of measured confounders, including glycaemia, whole body fat and MVPA. The findings from this study extend previous cross-sectional results observed in the general population, by demonstrating the association of objectively measured sedentary behaviour with markers of regional fat deposition. To our knowledge, this is the first study to show associations between sedentary time and liver, heart and visceral fat in a population with a high risk of chronic disease.

The observation that sedentary time is associated with liver fat, independent of adiposity, is a novel finding and may suggest an independent association between sedentary time and liver fat accumulation. Nevertheless, the associations observed between sedentary time and heart and visceral fat are in contrast to the majority of (11,25,29), but not all (20) previous literature, which has tended to show either weak or no associations. The discrepancy in findings between studies may be partially explained by the fact that sedentary time has previously been quantified using self-report (29), which has high measurement error (27), or undertaken in generally healthy, low risk populations compared to the present analysis, which specifically targeted individuals with a high risk of chronic disease and underlying metabolic dysfunction.

Visceral, hepatic, and cardiac adiposity, rather than obesity *per se*, have all been causally associated with glucose, insulin metabolism and subsequent metabolic dysfunction (6). These mechanisms may induce multiple autocrine, paracrine and endocrine influences, which include the pro-inflammatory cytokine response (28). Therefore, the associations observed for regional and ectopic fat in the present study may help to partially explain the relatively strong association between sedentary time and glucose metabolism consistently reported in those with a high risk of, or diagnosed, T2DM (3,4,14). Although a causal link between sedentary behaviour and differential regional and ectopic fat distribution has not been directly elucidated, there is some supporting evidence. As this analysis and others have found only relatively weak associations between sedentary behaviour and markers of overall adiposity (4,13,14), it is likely that potential mechanisms are beyond total energy balance. One possible candidate could be through the actions of lipoprotein lipase (LPL). Research using animal models of sedentary behaviour have shown that muscle inactivity causes rapid and dramatic reductions in LPL activity (12). In turn, it has been suggested that reductions in LPL mass and activity may directly promote intra-abdominal visceral fat accumulation (17). Therefore, if generalisable to humans, it may be plausible that muscle inactivity induced by prolonged/chronic sitting related sedentary behaviour causes reductions in postural muscle LPL activity. This in turn may help to promote the deposition of triglycerides into cells of non-adipose tissue, fuelling the detrimental phenomenon of ectopic over-accumulation (31). However, this potential mechanism lacks confirmation in human research and thus remains suggestive rather than definitive. Our study supports the need for further experimental research in humans focusing on lipid metabolism and distribution.

Sedentary time in the current study was shown to have a stronger association with visceral fat in older, compared to younger adults and in males compared to females. Although visceral fat

is known to increase with age, clear sex dimorphisms also exist, largely due to anatomical differences in adipose tissue deposition (6). For example, even after correcting for total body fat mass, women have been shown to have a lower ratio of visceral adipose tissue to total body fat mass compared to men (19). The underlying mechanisms driving these observations are largely unknown; it is likely to be a complex phenotype that includes sex hormones and adipose tissue storage dysfunction in several sites, including the heart and liver (6). Therefore, the preliminary findings from this study further highlight the importance of carefully considering the population under investigation in future experimental and epidemiologic investigations.

The present study has several strengths: most notably the use of objective methodologies to estimate exposures and outcomes in a high risk of T2DM population recruited through primary care. This is particularly important as our population is representative of those who are likely to be identified as being at high risk of type 2 diabetes mellitus within routine care and referred on to available prevention programmes. Furthermore, all participants were from the same geographical location, with similar risk, metabolic and physical activity profiles. All measurements (including MRI scans) were also performed by the same team of trained staff, following identical standard operating procedures.

However, the following limitations should be considered. Firstly, given the high risk nature of the cohort, the results may have limited generalisability and the small sample size may restrict the external validity of our findings. Secondly, the cross-sectional design limits inference about the direction of causality between the sedentary variables and MRI markers; reverse causality remains a possibility, particularly as the relationship between adiposity and sedentary time may be bi-directional (9). It is also plausible that unmeasured lifestyle

variables (e.g. snacking, alcohol consumption) and pre-existing co-morbidities may have confounded the observed relationships. Thirdly, cardiac images were un-gated and we were unable to distinguish between pericardial, epicardial and pericoronary fat. However, it could be argued that measuring whole heart fat reduces any potential bias, particularly related to measurement in leaner individuals. Fourthly, accelerometers rely on categorising movement (acceleration), as opposed to distinguishing between specific postures (sitting, lying and standing behaviours), which may lead to an under-estimation of the true association between sedentary time and markers of adiposity.

In conclusion, the present study provides new evidence suggesting that objectively measured sedentary behaviour is associated with heart, liver and visceral fat in individuals at a high risk of T2DM. Interestingly, since the associations remained after adjustment for whole body fat and MVPA, it may suggest that sedentary behaviour is linked to selective depositions of fat which cannot be fully explained by an increase in overall adiposity and may act via an independent mechanism. However, given the limitations, more research is needed to determine the distinct pathological effects of each type of fat and how these endpoints might be associated with different behaviours, in particular sitting-related sedentary time.

Acknowledgements

The research was supported by The National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care - Leicestershire, Northamptonshire and Rutland (NIHR CLAHRC – LNR), the University of Leicester Clinical Trials Unit and the NIHR Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit which is a partnership between University Hospitals of Leicester NHS Trust,

Loughborough University and the University of Leicester. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. MRI scans (for the WA cohort only) were funded by Unilever Discover, UK. Project STAND was funded by the Medical Research Council and National Prevention Research Initiative funding partners (MRC Project no.91409). Dr G McCann is funded by a post-doctoral NIHR fellowship.

Conflict of Interest

The authors declare no conflict of interest. The results of the present study do not constitute endorsement by ACSM.

References

1. Calabro P, Yeh ET. Intra-abdominal adiposity, inflammation, and cardiovascular risk: new insight into global cardiometabolic risk. *Curr Hypertens Rep.* 2008; 10(1):32-8.
2. Chatterton H, Younger T, Fischer A, Khunti K, Programme Development Group. Risk identification and interventions to prevent type 2 diabetes in adults at high risk: summary of NICE guidance. *BMJ.* 2012; 345:e4624.
3. Cooper AJ, Brage S, Ekelund U, Wareham NJ, Griffin SJ, Simmons RK. Association between objectively assessed sedentary time and physical activity with metabolic risk factors among people with recently diagnosed type 2 diabetes. *Diabetologia.* 2014; 57(1):73-82.

- 473 4. Cooper AR, Sebire S, Montgomery AA et al. Sedentary time, breaks in sedentary time
474 and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia*. 2012;
475 55(3):589-99.
- 476
- 477 5. Despres JP. Excess visceral adipose tissue/ectopic fat the missing link in the obesity
478 paradox? *J Am Coll Cardiol*. 2011; 57(19):1887-9.
- 479
- 480 6. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature*. 2006;
481 444(7121):881-7.
- 482
- 483 7. Ebbert JO, Jensen MD. Fat depots, free fatty acids, and dyslipidemia. *Nutrients*. 2013;
484 5(2):498-508.
- 485
- 486 8. Edwardson CL, Gorely T, Davies MJ et al. Association of sedentary behaviour with
487 metabolic syndrome: a meta-analysis. *PLoS One*. 2012; 7(4):e34916.
- 488
- 489 9. Golubic R, Wijndaele K, Sharp SJ et al. Physical activity, sedentary time and gain in
490 overall and central body fat: 7-year follow-up of the ProActive trial cohort. *Int J Obes*
491 *(Lond)*. 2014.
- 492
- 493 10. Gray LJ, Davies MJ, Hiles S et al. Detection of impaired glucose regulation and/or
494 type 2 diabetes mellitus, using primary care electronic data, in a multiethnic UK community
495 setting. *Diabetologia*. 2012; 55(4):959-66.
- 496

- 497 11. Hamer M, Venuraju SM, Urbanova L, Lahiri A, Steptoe A. Physical activity,
498 sedentary time, and pericardial fat in healthy older adults. *Obesity (Silver Spring)*. 2012;
499 20(10):2113-7.
500
- 501 12. Hamilton MT, Hamilton DG, Zderic TW. Exercise physiology versus inactivity
502 physiology: an essential concept for understanding lipoprotein lipase regulation. *Exerc Sport*
503 *Sci Rev*. 2004; 32(4):161-6.
504
- 505 13. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and
506 cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J*. 2011; 32(5):590-
507 7.
508
- 509 14. Henson J, Yates T, Biddle SJ et al. Associations of objectively measured sedentary
510 behaviour and physical activity with markers of cardiometabolic health. *Diabetologia*. 2013;
511 56(5):1012-20.
512
- 513 15. Hocking S, Samocha-Bonet D, Milner KL, Greenfield JR, Chisholm DJ. Adiposity
514 and insulin resistance in humans: the role of the different tissue and cellular lipid depots.
515 *Endocr Rev*. 2013; 34(4):463-500.
516
- 517 16. Kay SJ, Fiatarone Singh MA. The influence of physical activity on abdominal fat: a
518 systematic review of the literature. *Obes Rev*. 2006; 7(2):183-200.
519

17. Kobayashi J, Tashiro J, Murano S, Morisaki N, Saito Y. Lipoprotein lipase mass and activity in post-heparin plasma from subjects with intra-abdominal visceral fat accumulation. *Clin Endocrinol (Oxf)*. 1998; 48(4):515-20.
18. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring)*. 2006; 14(2):336-41.
19. Kuk JL, Lee S, Heymsfield SB, Ross R. Waist circumference and abdominal adipose tissue distribution: influence of age and sex. *Am J Clin Nutr*. 2005; 81(6):1330-4.
20. Larsen BA, Allison MA, Kang E, Saad S, Laughlin GA, Araneta MR, Barrett-Connor E, Wassel CL. Associations of Physical Activity and Sedentary Behavior with Regional Fat Deposition. *Med Sci Sports Exerc*. 2013.
21. Le-Petross H, Kundra V, Szklaruk J, Wei W, Hortobagyi GN, Ma J. Fast three-dimensional dual echo dixon technique improves fat suppression in breast MRI. *J Magn Reson Imaging*. 2010; 31(4):889-94.
22. Machann J, Thamer C, Stefan N et al. Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. *Radiology*. 2010; 257(2):353-63.

- 543 23. Mahabadi AA, Massaro JM, Rosito GA et al. Association of pericardial fat,
544 intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the
545 Framingham Heart Study. *Eur Heart J*. 2009; 30(7):850-6.
546
- 547 24. Maher C, Olds T, Mire E, Katzmarzyk PT. Reconsidering the sedentary behaviour
548 paradigm. *PLoS One*. 2014; 9(1):e86403.
549
- 550 25. McGuire KA, Ross R. Incidental physical activity and sedentary behavior are not
551 associated with abdominal adipose tissue in inactive adults. *Obesity (Silver Spring)*. 2012;
552 20(3):576-82.
553
- 554 26. Nazare JA, Smith JD, Borel AL et al. Ethnic influences on the relations between
555 abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the
556 International Study of Prediction of Intra-Abdominal Adiposity and Its Relationship With
557 Cardiometabolic Risk/Intra-Abdominal Adiposity. *Am J Clin Nutr*. 2012; 96(4):714-26.
558
- 559 27. Neilson HK, Robson PJ, Friedenreich CM, Csizmadi I. Estimating activity energy
560 expenditure: how valid are physical activity questionnaires? *Am J Clin Nutr*. 2008;
561 87(2):279-91.
562
- 563 28. Richardson VR, Smith KA, Carter AM. Adipose tissue inflammation: feeding the
564 development of type 2 diabetes mellitus. *Immunobiology*. 2013; 218(12):1497-504.
565

- 566 29. Saunders TJ, Tremblay MS, Despres JP, Bouchard C, Tremblay A, Chaput JP.
567 Sedentary behaviour, visceral fat accumulation and cardiometabolic risk in adults: a 6-year
568 longitudinal study from the Quebec Family Study. *PLoS One*. 2013; 8(1):e54225.
569
- 570 30. Sedentary Behaviour Research N. Letter to the editor: standardized use of the terms
571 "sedentary" and "sedentary behaviours". *Appl Physiol Nutr Metab*. 2012; 37(3):540-2.
572
- 573 31. Snel M, Jonker JT, Schoones J et al. Ectopic fat and insulin resistance:
574 pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol*. 2012;
575 2012:983814.
576
- 577 32. The English Indices of Deprivation: Summary. *Available at:*
578 [http://webarchive.nationalarchives.gov.uk/20120919132719/http://www.communities.gov.uk/](http://webarchive.nationalarchives.gov.uk/20120919132719/http://www.communities.gov.uk/documents/communities/pdf/576659.pdf)
579 [documents/communities/pdf/576659.pdf](http://webarchive.nationalarchives.gov.uk/20120919132719/http://www.communities.gov.uk/documents/communities/pdf/576659.pdf). Accessed January 11, 2012. 2007
580
- 581 33. Thorp AA, Owen N, Neuhaus M, Dunstan DW. Sedentary behaviors and subsequent
582 health outcomes in adults a systematic review of longitudinal studies, 1996-2011. *Am J Prev*
583 *Med*. 2011; 41(2):207-15.
584
- 585 34. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical
586 activity in the United States measured by accelerometer. *Med Sci Sports Exerc*. 2008;
587 40(1):181-8.
588
- 589 35. Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments
590 in field-based research. *Med Sci Sports Exerc*. 2005; 37(11 Suppl):S531-43.

36. Wijndaele K, Orrow G, Ekelund U et al. Increasing objectively measured sedentary time increases clustered cardiometabolic risk: a 6 year analysis of the ProActive study. *Diabetologia*. 2014; 57(2):305-12.
37. Wilmot EG, Edwardson CL, Achana FA et al. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia*. 2012; 55(11):2895-905.
38. Wilmot EG, Edwardson CL, Biddle SJ et al. Prevalence of diabetes and impaired glucose metabolism in younger 'at risk' UK adults: insights from the STAND programme of research. *Diabet Med*. 2013; 30(6):671-5.
39. Yates T, Davies MJ, Henson J et al. Walking away from type 2 diabetes: trial protocol of a cluster randomised controlled trial evaluating a structured education programme in those at high risk of developing type 2 diabetes. *BMC Fam Pract*. 2012; 13:46,2296-13-46.

Legends to figures

Figure 1A. Tertiles of sedentary time with heart fat.

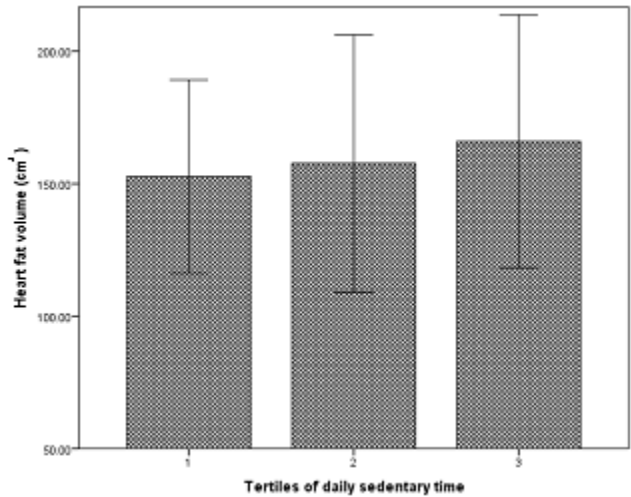


Figure 1B. Tertiles of sedentary time with visceral fat.

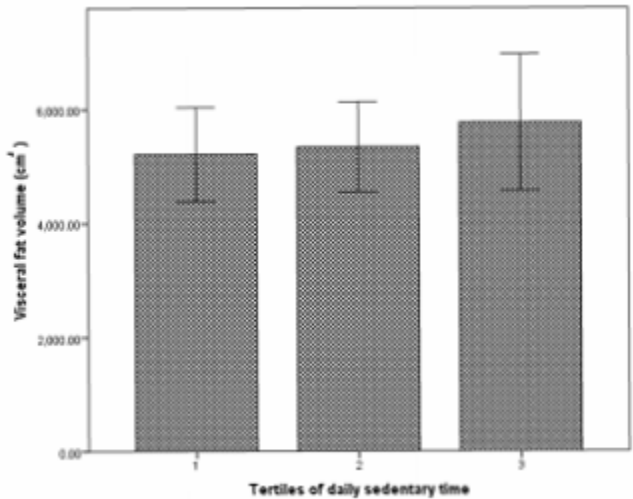
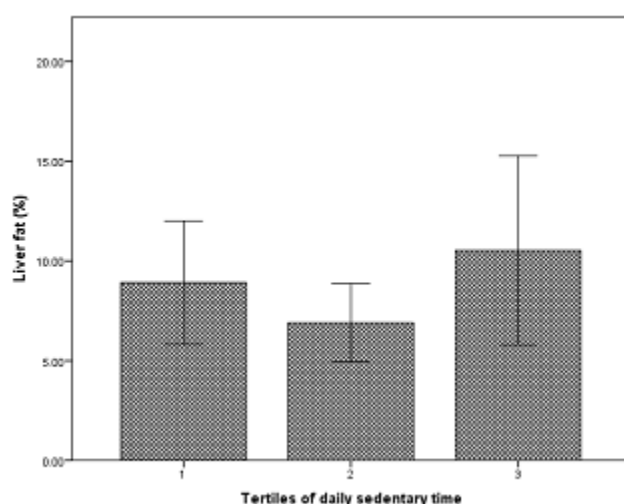


Figure 1C. Tertiles of sedentary time with liver fat.



Tertiles of sedentary time with heart fat (Figure 1A), visceral fat (Figure 1B) and liver fat (Figure 1C). Estimated marginal means are adjusted for age, gender, smoking status, family history of T2DM, ethnicity, social deprivation, ACE inhibitors, beta blockers, lipid lowering medication, systolic blood pressure, cholesterol, HbA1c, MVPA, time accelerometer worn and whole body fat. Tertile cut-points for sedentary time were 9.6h and 10.9h per day. Medians and ranges for tertile 1=8.8 h (7.7–9.6); tertile 2=10.3 h (9.6–10.8); tertile 3=11.8 h (10.9–14.0). $p < 0.001$ for trend (**Figure 1A, Figure 1B, Figure 1C**). Bars represent mean and error bars are 95% confidence intervals.

Supplementary Table

Supplementary Table 1. Associations of total sedentary time with markers of MRI-derived regional fat distribution when adjusted for either MVPA or total physical activity volume. Supplementary Table 1: Associations of total sedentary time with markers of MRI-derived regional fat distribution when adjusted for either MVPA or total physical activity volume

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Model 1				
	Sedentary time <i>Standardised β (SE)^a</i> <i>(adjustment for</i> <i>MVPA)</i>	<i>p</i>	Sedentary time <i>Standardised β (SE)^b</i> <i>(adjustment for total</i> <i>physical activity</i> <i>volume)</i>	<i>p</i>
Heart fat (cm ³)	0.59 (0.21)	0.001	0.60 (0.22)	0.012
Liver fat (%)	0.48 (0.20)	0.003	0.52 (0.21)	0.019
Visceral fat (cm ³)	0.53 (0.20)	<0.001	0.47 (0.19)	0.022
Subcutaneous fat (cm ³)	0.31 (0.21)	0.179	0.20 (0.21)	0.416
Whole body fat (cm ³)	0.43 (0.22)	0.052	0.31 (0.22)	0.175
Model 2				
	Sedentary time <i>Standardised β (SE)^a</i>	<i>p</i>	Sedentary time <i>Standardised B (SE)^b</i>	<i>p</i>
Heart fat (cm ³)	0.46 (0.20)	0.008	0.49 (0.22)	0.035
Liver fat (%)	0.39 (0.20)	0.026	0.40 (0.21)	0.044
Visceral fat (cm ³)	0.25 (0.29)	0.039	0.25 (0.12)	0.046
Model 1 was adjusted for age, gender, smoking status, family history of T2DM, ethnicity, social deprivation, ACE inhibitors, beta blockers, lipid lowering medication, systolic blood pressure, cholesterol, HbA1c, time accelerometer worn and ^a MVPA or ^b total physical activity				
Model 2 was adjusted for the above covariates and whole body fat				

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