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±16.2 years; 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 15.7 cm$^3$ higher heart fat ($p=0.008$), 1.2% higher liver fat ($p=0.026$) and 183.7 cm$^3$ 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 (≥30kg/m²; ≥27.5kg/m² for south Asians) or b) aged
18-40 years with a BMI in the overweight range ≥25kg/m² (≥23kg/m² for south Asians) plus
one additional risk factor: a family history of T2DM or CVD, previous gestational diabetes,
polycystic ovarian syndrome, HbA1c ≥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/m²).
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 ($\beta$)±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±6.0 years; male=34.4%) and 34 participants from WA (age=61.9±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 20.5cm$^3$ higher heart fat ((95% CI) 5.4, 35.6), 1.4% higher liver fat (0.3, 2.5) and a 409.2cm$^3$ 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 (15.7 cm$^3$ higher heart fat (95% CI) 0.5, 30.8), 1.2% higher liver fat (0.3, 2.3) and a 191.3 cm$^3$ 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, 13.2 cm$^3$ higher heart fat (p<0.001), 1.6% higher liver fat (p<0.001) and a 556.3 cm$^3$ 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 $\beta$ (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


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 Tables

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.