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

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51 **<u>Abstract</u>**

52

53 *Purpose*

54 The effect of sedentary behaviour on regional fat deposition, independent of physical activity

55 remains equivocal. We examined the cross-sectional associations between objectively

56 measured sedentary time and markers of regional fat distribution (heart, liver, visceral,

57 subcutaneous and total body fat) in a population at a high risk of type 2 diabetes mellitus

58 (T2DM).

59

60 *Methods*

61 Participants were recruited from primary care to two diabetes prevention programmes.

62 Sedentary time (<25 counts per 15 seconds) was measured using Actigraph GT3X

63 accelerometers. Heart, liver, visceral, subcutaneous and total body fat were quantified using

64 magnetic resonance images (MRI). Fat volumes were calculated by multiplying the cross-

65 sectional areas of the fat-containing pixels by the slice thickness. The liver fat percentage was

66 measured using a representative region of interest created in the right lobe of the liver

67 avoiding the main portal veins. Linear regression models examined the association of

68 sedentary time with markers of regional fat deposition.

69

70 *Results*

71 Sixty-six participants (age=47.9±16.2years;male=50.0%) were included. Following

72 adjustment for several covariates, including glycaemia, whole body fat and moderate-to-

73 vigorous physical activity (MVPA), each 30 minutes of sedentary time was associated with

74 15.7cm^3 higher heart fat (p=0.008), 1.2% higher liver fat (p=0.026) and 183.7cm^3 higher

75 visceral fat (p=0.039).

76 _Conclusion_

77 This study provides new evidence suggesting that objectively measured sedentary behaviour

78 may have an independent association upon heart, liver and visceral fat in individuals at a high

79 risk of T2DM.

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

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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

126   whether objectively measured sedentary behaviour is associated with regional fat deposition,

127   independent of MVPA or total physical activity. Moreover, to our knowledge, there are

128   currently no reports examining the association between sedentary behaviour and liver fat.

129

130   It is also necessary to establish the association between sedentary behaviour and fat

131   distribution in those at high risk of chronic disease. Both national and international

132   recommendations and policies specify that chronic-disease prevention strategies should

133   include targeted interventions aimed at the identification and management of high risk

134   individuals (2). Moreover, sedentary time has been shown to be more strongly and adversely

135   associated with cardio-metabolic variables (including markers of adiposity) in high risk

136   individuals, (14) and those with established T2DM (3,4) after adjustment for MVPA and

137   other important confounders. Given that associations between sedentary time and markers of

138   adiposity (body mass index (BMI) and waist circumference) were weaker compared to other

139   cardio-metabolic variables (14), the association of sedentary behaviour may extend beyond

140   traditional measures of adiposity and may lie in the location of fat deposition. In particular,

141   within cells of non-adipose tissue that normally contain only small amounts of fat (ectopic

142   fat). Such ectopic depositions result in excess lipids being driven into alternative, non-

143   oxidative pathways, which in turn promotes metabolically relevant cellular dysfunction

144   (lipotoxicity).

145

146   The aim of this study, therefore, was to examine the association between objectively

147   measured sedentary time and heart, liver, visceral, subcutaneous and total body fat,

148   independent of MVPA and whole body fat in a population at high risk of T2DM.

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150

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 (**S**edentary **T**ime **An**d **D**iabetes). 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

176   invited to take part in the study. This approach has been shown to have good sensitivity and

177   specificity for identifying participants at a high risk of IGR (10).

178

179   <u>Project STAND</u>

180

181   Young adults who were at risk of developing T2DM were recruited from primary care

182   practices located across Leicestershire and the South East Midlands region. Practice

183   databases were searched for participants meeting the following inclusion criteria: a) aged 18-

184   40 years with a BMI in the obese range ($\geq$30kg/m^2;$\geq$27.5kg/m^2 for south Asians) or b) aged

185   18-40 years with a BMI in the overweight range $\geq$25kg/m^2 ($\geq$23kg/m^2 for south Asians) plus

186   one additional risk factor: a family history of T2DM or CVD, previous gestational diabetes,

187   polycystic ovarian syndrome, HbA1c $\geq$5.8% or IGR (38).

188

189   Individuals were excluded from both studies if they were taking steroids or had previously

190   diagnosed T2DM. Written Informed consent was obtained from all eligible participants and

191   both studies gained full ethical and governance approval.

192

193   <u>Covariates</u>

194

195   Information on current smoking status, family history of T2DM, medication status and

196   ethnicity (coded according to census criteria) was obtained following an interview-

197   administered questionnaire with a health care professional. Waist circumference was

198   measured over light clothing between the lower rib margin and the iliac crest. Height and

199   weight (Tanita TBE 611, Tanita, West Drayton, UK) were obtained by trained staff according

200   to standard operating procedures. The subsequent values were used to compute BMI (kg/m^2).

201   Systolic and diastolic blood pressure (mmHg) were taken three times in succession and the

202   mean of the last two used for analysis.

203

204   Social deprivation was determined by assigning an Index of Multiple Deprivation (IMD)

205   score to the participant's resident area (based on postcode) (32). IMD scores are publically

206   available continuous measures of compound social and material deprivation. Areas are

207   ranked from least deprived to most deprived based upon several dimensions linked to health

208   outcomes (including; income, employment, education, living environment and health).

209

210   Venous blood samples were obtained following an overnight fast, and all assays were

211   measured in the same laboratory. Analysis was conducted by individuals blinded to the

212   patients' identity, using stable methods, standardised to external quality assurance values.

213   HbA1c was analysed using the Bio-Rad Variant II HPLC system (Bio-Rad Clinical

214   Diagnostics, Hemel Hempstead, UK) and total cholesterol was measured using standard

215   enzymatic techniques.

216

217   <u>Quantification of sedentary time</u>

218

219   All eligible participants were asked to wear a tri-axial accelerometer at the baseline visit

220   (Actigraph GT3X, Pensacola, FL, USA), for a minimum of seven consecutive days during

221   waking hours. These accelerometers translate raw accelerations into activity counts. Cut-

222   points modified by Troiano et al. were used to categorise an epoch as sedentary (<25 counts

223   per 15 seconds) or MVPA (≥505 counts per 15 seconds). These intensity thresholds were

224   calculated as a weighted average determined from previous treadmill or track walking studies

225   (34). Total physical activity volume represented the summation of counts within each epoch.

226 Non-wear time was defined as a minimum of 60 minutes of continuous zero counts and days

227 with at least 600 minutes of wear time were considered valid (13, 14). In order to be included

228 in the analysis, participants were required to have a minimum of four valid days (35).

229

230 A data analysis tool (KineSoft version 3.3.75, Kinesoft, New Brunswick, Canada;

231 www.kinesoft.org) was used to process the accelerometer data.

232

233 <u>Measure of adiposity</u>

234

235 Magnetic Resonance Imaging (MRI) was performed at Glenfield Hospital, Leicester, UK,

236 where heart, liver, visceral, subcutaneous and total body fat (includes liver, intra-abdominal,

237 subcutaneous and visceral fat) was quantified. MRI is a reliable modality for the assessment

238 of adipose tissue and is capable of measuring fat distribution with a high spatial resolution

239 (22).

240

241 Scanning was performed using either a 1.5 Tesla Avanto (WA) or a 3.0 Tesla Skyra system

242 (STAND) (Siemens Medical, Erlangen, Germany). Flexible body array coils were applied to

243 the thorax and abdomen for signal reception. For lipid volume quantification, a 2-point Dixon

244 gradient-echo pulse sequence was used to separate tissue water signal from lipid signal and to

245 create two separate image sets with signal intensity showing 'fat' and 'water' content (21). 3-

246 D images were acquired axially with 5 mm slice thickness and in-plane resolution of

247 1.56 mm, interpolated to 0.78 mm. The field of view was 500 mm (left-right) by 375 mm

248 (anterior-posterior). Images were acquired in three contiguous blocks, covering the thoracic,

249 abdominal and pelvic regions, with each block acquired in a breath-hold at full inspiration to

250 minimise motion–related artefacts and to negate changes in slice position. The acquisition

251 time for each block was 18s. All scans were performed by the same team of trained staff

252 according to standardised procedures.

253

254 Analysis of the MR images was performed using image analysis software produced in-house

255 (Java Image Manipulation, Version 7). All analysis was undertaken by the same researcher

256 who was blinded to the clinical, anthropometric and physical activity data.

257

258 For analysis, the 'fat' and 'water' images were mathematically combined to create a 'fat

259 percentage' image. Fat-containing pixels were then defined as those with a pixel intensity

260 between 51 and 99% (100% being due to image artefact). The images were reconstructed into

261 15 mm thick contiguous slices, from the top of the pulmonary trunk extending to the bottom

262 of the symphysis pubis. Volumes of interest for the whole body and heart were created by

263 outlining the perimeter of the body and heart respectively on each relevant slice using a

264 mouse-controlled pointer and excluding those pixels outside the structures. The region of

265 interest surrounding the heart included myocardial, epicardial (pericardial) and immediate

266 extra-pericardial (thoracic) fat.

267

268 The visceral (and retroperitoneal) fat was further separated, by outlining the abdominal and

269 chest wall muscles and excluding the pixels for the subcutaneous fat. The fat volume was

270 calculated automatically by multiplying the cross-sectional areas of the fat-containing pixels,

271 summed over all slices on which the tissue was outlined, by the slice thickness. This created

272 three fat volumes: total body fat, visceral fat from the top of the pulmonary trunk to the

273 bottom of the symphysis pubis, and the heart fat volume. The liver fat percentage was also

274 measured using a representative region of interest created in the right lobe of liver avoiding

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

276 body fat.

277

278 <u>Statistical Analysis</u>

279

280 IBM SPSS Statistics v20.0 (Chicago, IL, USA) was used to conduct all statistical analyses.

281 Linear regression analysis was used on the combined study cohorts to examine the

282 independent association of sedentary time (independent variable), with various markers of

283 regional fat deposition (dependent variable). We display results per 30 minutes of sedentary

284 time for ease of interpretation.

285

286 Model 1 was adjusted for age (continuous), gender, ethnicity (white European/south

287 Asian/other), social deprivation (continuous), family history of T2DM (yes/no), smoking

288 status (current/ex/never smoked), total cholesterol, HbA1c, systolic blood pressure, blood

289 pressure medication (ACE inhibitors (yes/no)), beta-blockers (yes/no), lipid lowering

290 medication (yes/no), time accelerometer worn (average number of minutes per day) and

291 MVPA. We also undertook the same model, but adjusted for total physical activity volume

292 (counts per day) rather than MVPA given that others have suggested this mediates significant

293 associations between sedentary behaviour and metabolic health (24). In order to examine the

294 extent to which total adiposity attenuated these relationships, model 2 was further adjusted

295 for whole body fat. Models were assessed for normality and multi co-linearity was assessed

296 through the variance inflation factor (VIF). To further represent the strength of sedentary time

297 with markers of adiposity, variables were also examined as tertiles using analysis of

298 covariance procedures.

299

300   Significant observations were followed up with interaction terms to assess associations

301   between sedentary time and study, sex, level of MVPA, whole body fat and HbA1c. All

302   interactions were adjusted for the covariates listed in model 1.

303

304   Two-tailed p values of 0.05 or less were considered statistically significant for main effects.

305    $p<0.1$ was considered significant for interactions. To allow for direct comparisons across fat

306   deposition markers, results of the generalised linear regression analysis are also presented as

307   the standardised beta co-efficient (β)±standard error(SE).

308

309   **Results**

310

311   Table 1 displays the demographic, anthropometric, MRI-derived and accelerometer

312   characteristics of included participants. In total, 32 participants from Project STAND

313   (age=33.1±6.0 years; male=34.4%) and 34 participants from WA (age=61.9±8.0 years;

314   male=64.7%) had valid measures of objective activity and MRI data.

315

316   There were no statistical differences (p>0.05) in anthropometric, metabolic, and social

317   deprivation measures between participants who were included in this analysis vs. those not

318   included (did not undergo an MRI scan).

319

320   Model 1 illustrates the linear relationship between each 30 minute block of sedentary time

321   and markers of regional fat deposition. Following adjustment for various confounders,

322   including HbA1c, and MVPA, 30 minutes of sedentary time was associated with 20.5cm^3

323   higher heart fat ((95% CI) 5.4, 35.6), 1.4% higher liver fat (0.3, 2.5) and a 409.2cm^3 higher

324   visceral fat (127.6, 690.8). All significant associations seen in Model 1 persisted after further

325   adjustment for whole body fat in Model 2 (15.7cm^3 higher heart fat ((95% CI) 0.5, 30.8),

326   1.2% higher liver fat (0.3, 2.3) and a 191.3cm^3 higher visceral fat (2.7, 368.8).

327

328   No significant associations were observed for whole body and subcutaneous fat (Table 2).

329   Supplementary Table 1 also displays the overall associations (presented as standardised $\beta \pm$

330   SE) in the combined cohort for total sedentary time with MRI-derived markers of regional fat

331   deposition.

332

333   In order to provide visual representation of reported associations, figure 1 illustrates the

334   associations between total sedentary time and heart fat, liver fat and visceral fat when

335   examined as tertiles, after adjustment for the covariates listed above. Compared to those in

336   the lowest tertile of sedentary time, those in the highest tertile had, on average, 13.2cm^3

337   higher heart fat (p<0.001), 1.6% higher liver fat (p<0.001) and a 556.3cm^3 higher visceral fat

338   (p<0.001).

339

340   Interaction analyses indicated a significant effect for study group with the older cohort (WA)

341   demonstrating stronger associations of sedentary time with visceral fat (presented as

342   unstandardised β (95% CI)) (WA = 800.0 (345.3, 1255.9) vs. STAND = 69.4 (-297.8, 436.6)

343   (p for interaction=0.010). Sex interactions also indicated that sedentary time had a larger

344   impact on visceral fat in males (male = 779.1 (171.4, 1386.9) vs. female = 133.4 (-269.0,

345   544.8) (p for interaction=0.049) (Table 3). No other significant interactions for associations

346   with measures of ectopic fat were observed for study group, sex, whole body fat, MVPA or

347   HbA1c level (p>0.1).

348

349   The findings above were unaffected if waist circumference or BMI rather than whole body fat

350   was used in Model 2 (data not shown).

351

352   **<u>Discussion</u>**

353

354   This study conducted in individuals at high risk of T2DM, demonstrated that sedentary time

355   was associated with heart, liver and visceral fat, independent of measured confounders,

356   including glycaemia, whole body fat and MVPA. The findings from this study extend

357   previous cross-sectional results observed in the general population, by demonstrating the

358   association of objectively measured sedentary behaviour with markers of regional fat

359   deposition. To our knowledge, this is the first study to show associations between sedentary

360   time and liver, heart and visceral fat in a population with a high risk of chronic disease.

361

362   The observation that sedentary time is associated with liver fat, independent of adiposity, is a

363   novel finding and may suggest an independent association between sedentary time and liver

364   fat accumulation. Nevertheless, the associations observed between sedentary time and heart

365   and visceral fat are in contrast to the majority of (11,25,29), but not all (20) previous

366   literature, which has tended to show either weak or no associations. The discrepancy in

367   findings between studies may be partially explained by the fact that sedentary time has

368   previously been quantified using self-report (29), which has high measurement error (27), or

369   undertaken in generally healthy, low risk populations compared to the present analysis, which

370   specifically targeted individuals with a high risk of chronic disease and underlying metabolic

371   dysfunction.

372

373   Visceral, hepatic, and cardiac adiposity, rather than obesity *per se*, have all been causally

374   associated with glucose, insulin metabolism and subsequent metabolic dysfunction (6).

375   These mechanisms may induce multiple autocrine, paracrine and endocrine influences, which

376   include the pro-inflammatory cytokine response (28). Therefore, the associations observed for

377   regional and ectopic fat in the present study may help to partially explain the relatively strong

378   association between sedentary time and glucose metabolism consistently reported in those

379   with a high risk of, or diagnosed, T2DM (3,4,14). Although a causal link between sedentary

380   behaviour and differential regional and ectopic fat distribution has not been directly

381   elucidated, there is some supporting evidence. As this analysis and others have found only

382   relatively weak associations between sedentary behaviour and markers of overall adiposity

383   (4,13,14), it is likely that potential mechanisms are beyond total energy balance. One possible

384   candidate could be through the actions of lipoprotein lipase (LPL). Research using animal

385   models of sedentary behaviour have shown that muscle inactivity causes rapid and dramatic

386   reductions in LPL activity (12). In turn, it has been suggested that reductions in LPL mass

387   and activity may directly promote intra-abdominal visceral fat accumulation (17). Therefore,

388   if generalisable to humans, it may be plausible that muscle inactivity induced by

389   prolonged/chronic sitting related sedentary behaviour causes reductions in postural muscle

390   LPL activity. This in turn may help to promote the deposition of triglycerides into cells of

391   non-adipose tissue, fuelling the detrimental phenomenon of ectopic over-accumulation (31).

392   However, this potential mechanism lacks confirmation in human research and thus remains

393   suggestive rather than definitive. Our study supports the need for further experimental

394   research in humans focusing on lipid metabolism and distribution.

395

396   Sedentary time in the current study was shown to have a stronger association with visceral fat

397   in older, compared to younger adults and in males compared to females. Although visceral fat

398   is known to increase with age, clear sex dimorphisms also exist, largely due to anatomical

399   differences in adipose tissue deposition (6). For example, even after correcting for total body

400   fat mass, women have been shown to have a lower ratio of visceral adipose tissue to total

401   body fat mass compared to men (19). The underlying mechanisms driving these observations

402   are largely unknown; it is likely to be a complex phenotype that includes sex hormones and

403   adipose tissue storage dysfunction in several sites, including the heart and liver (6).

404   Therefore, the preliminary findings from this study further highlight the importance of

405   carefully considering the population under investigation in future experimental and

406   epidemiologic investigations.

407

408   The present study has several strengths: most notably the use of objective methodologies to

409   estimate exposures and outcomes in a high risk of T2DM population recruited through

410   primary care. This is particularly important as our population is representative of those who

411   are likely to be identified as being at high risk of type 2 diabetes mellitus within routine care

412   and referred on to available prevention programmes. Furthermore, all participants were from

413   the same geographical location, with similar risk, metabolic and physical activity profiles. All

414   measurements (including MRI scans) were also performed by the same team of trained staff,

415   following identical standard operating procedures.

416

417   However, the following limitations should be considered. Firstly, given the high risk nature

418   of the cohort, the results may have limited generalisability and the small sample size may

419   restrict the external validity of our findings. Secondly, the cross-sectional design limits

420   inference about the direction of causality between the sedentary variables and MRI markers;

421   reverse causality remains a possibility, particularly as the relationship between adiposity and

422   sedentary time may be bi-directional (9). It is also plausible that unmeasured lifestyle

423 variables (e.g. snacking, alcohol consumption) and pre-existing co-morbidities may have

424 confounded the observed relationships. Thirdly, cardiac images were un-gated and we were

425 unable to distinguish between pericardial, epicardial and pericoronary fat. However, it could

426 be argued that measuring whole heart fat reduces any potential bias, particularly related to

427 measurement in leaner individuals. Fourthly, accelerometers rely on categorising movement

428 (acceleration), as opposed to distinguishing between specific postures (sitting, lying and

429 standing behaviours), which may lead to an under-estimation of the true association between

430 sedentary time and markers of adiposity.

431

432 In conclusion, the present study provides new evidence suggesting that objectively measured

433 sedentary behaviour is associated with heart, liver and visceral fat in individuals at a high risk

434 of T2DM. Interestingly, since the associations remained after adjustment for whole body fat

435 and MVPA, it may suggest that sedentary behaviour is linked to selective depositions of fat

436 which cannot be fully explained by an increase in overall adiposity and may act via an

437 independent mechanism. However, given the limitations, more research is needed to

438 determine the distinct pathological effects of each type of fat and how these endpoints might

439 be associated with different behaviours, in particular sitting-related sedentary time.

440

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454

455 **Conflict of Interest**

456

457 The authors declare no conflict of interest. The results of the present study do not constitute

458 endorsement by ACSM.

459

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613 **Legends to figures**

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

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

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

617 Tertiles of sedentary time with heart fat (Figure 1A), visceral fat (Figure 1B) and liver fat

618 (Figure 1C). Estimated marginal means are adjusted for age, gender, smoking status, family

619 history of T2DM, ethnicity, social deprivation, ACE inhibitors, beta blockers, lipid lowering

620 medication, systolic blood pressure, cholesterol, HbA1c, MVPA, time accelerometer worn

621 and whole body fat. Tertile cut-points for sedentary time were 9.6h and 10.9h per day.

622 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

623 (10.9–14.0). $p<0.001$ for trend **(Figure 1A, Figure 1B, Figure 1C)**. Bars represent mean and

624 error bars are 95% confidence intervals.

625 **Supplementary Tables**

626 Supplementary Table 1. Associations of total sedentary time with markers of MRI-derived

627 regional fat distribution when adjusted for either MVPA or total physical activity volume.