Physical activity and sedentary time: association with metabolic health and liver fat

Kelly A. Bowden Davies[1,2,3], Victoria S. Sprung[2,3,4], Juliette A. Norman[2,3], Andrew Thompson[5], Katie L. Mitchell[6], Jo A. Harrold[6], Graham Finlayson[7], Catherine Gibbons[7], John P.H. Wilding[2,3], Graham J. Kemp[2,8], Mark Hamer[9], Daniel J. Cuthbertson[2,3]

[1]School of Biomedical Sciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK;

[2]Institute of Ageing & Chronic Disease, University of Liverpool, Liverpool, UK;

[3]Obesity and Endocrinology Research Group, Clinical Sciences Centre, University Hospital Aintree, Liverpool, UK;

[4]Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, UK;

[5]Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, UK;

[6]Department of Psychological Sciences, Institute of Psychology Health and Society, University of Liverpool, Liverpool, UK;

[7]Appetite Control and Energy Balance Research, School of Psychology, Faculty of Medicine and Health, University of Leeds, Leeds, UK;

[8]Liverpool Magnetic Resonance Imaging Centre (LiMRIC), University of Liverpool, Liverpool, UK;

[9]School Sport, Exercise Health Sciences, National Centre for Sport and Exercise Medicine - East Midlands, Loughborough University, Loughborough, UK

Corresponding author: Kelly A. Bowden Davies, School of Biomedical Sciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK; kelly.bowden-davies@newcastle.ac.uk; +44 (0)191 208 8311; fax unavailable.

ABSTRACT

Introduction/Purpose To investigate whether a) lower levels of daily physical activity (PA) and greater sedentary time accounted for contrasting metabolic phenotypes (higher liver fat/presence of metabolic syndrome [MetS+] vs lower liver fat/absence of metabolic syndrome [MetS-]) in individuals of similar BMI and b) the association of sedentary time on metabolic health and liver fat.

Methods Ninety-eight habitually active participants (53 female, 45 male; age 39±13 years; BMI 26.9±5.1 kg/m^2), underwent assessments of PA (SenseWear armband; wear time ~98%), cardio-respiratory fitness ($\dot{V}O_2$ peak), body composition (MRI and MRS) and multi-organ insulin sensitivity (OGTT). We undertook a) cross-sectional analysis comparing four groups: non-obese or obese, with and without metabolic syndrome (MetS+ vs MetS-) and b) univariate and multivariate regression for sedentary time and other levels of PA in relation to liver fat.

Results Light, moderate and vigorous PA did not account for differences in metabolic health between individuals, whether non-obese or obese, although MetS+ individuals were more sedentary, with a higher number, and prolonged bouts (~1-2 hours). Overall, sedentary time, average daily METS and $\dot{V}O_2$ peak were each independently associated with liver fat percentage. Each additional hour of daily sedentary time was associated with a 1.15% (95% CI, 1.14–1.50%) higher liver fat content.

Conclusions Greater sedentary time, independent of other levels of PA, is associated with being metabolically unhealthy; even in habitually active people, lesser sedentary time, and higher cardio-respiratory fitness and average daily METS is associated with lower liver fat.

KEY WORDS

INTRODUCTION

Strong epidemiologic evidence suggests an inverse relationship between physical activity (PA) levels and obesity, metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (1-5). Increased PA is recommended both for individuals and at a population level to improve metabolic health and help prevent these interrelated conditions. The independent protective effect of high cardio-respiratory fitness (CRF), an objective marker of PA, against all-cause mortality is well established (6, 7). There is a growing recognition that sedentary behaviour, which has an independent association with adverse health outcomes, should be minimised (2, 8, 9). Increasing moderate PA is protective against the aforementioned diseases and attenuates, but does not eliminate, the detrimental effects of sedentary behaviour (10). Breaking up prolonged periods of sedentary time (11) or replacing it with low-intensity PA (12) are beneficial for glycaemic control.

Obesity is strongly associated with poor cardio-metabolic health and overall mortality (13). However, not all obese individuals are *metabolically unhealthy* (MetS+) (14); conversely not all non-obese individuals are *metabolically healthy* (MetS-) (15). Some studies suggest that MetS+ may be a consequence of low PA (16, 17), but others have not supported this conclusion (18-20). With differences in methodology, cohort characteristics and definitions of metabolic phenotypes, these studies typically have not precisely defined the differences in PA characteristics between phenotypes. Only one study, of older adults, has *objectively* measured sedentary behaviour (19), which offers better reliability than self-report (21); no such study has been undertaken in young-middle aged adults. There are similarly conflicting results in studies of the association of metabolic health with objectively measured sedentary behaviour and quantitative measures of liver fat using magnetic resonance spectroscopy (MRS) or computed tomography (CT) (22-26). The accumulation of liver fat has been described as a major

25   contributor to the development of type 2 diabetes (27), is considered the hepatic manifestation

26   of MetS and closely linked with obesity and insulin resistance (28). Observing levels of PA,

27   including sedentary behaviour, in metabolic phenotypes of a given BMI category with further

28   quantification of liver fat may reveal associations which link habitual activity to health

29   outcomes and the predisposition for metabolic diseases.

30   This cross-sectional study will objectively monitor the habitual PA of young-middle aged

31   adults and extensively phenotype these individuals by assessment of metabolic health and

32   MRI-derived body composition. We hypothesise that, greater sedentary time and lower levels

33   of PA will be evident in metabolically unhealthy phenotypes (MetS+ vs MetS-) in BMI-

34   matched individuals; and secondly, higher MRS-quantified liver fat will be associated with

35   greater sedentary time and lower PA levels.

36   **METHODS**

37   **Participants**

38   Habitually active individuals who engaged in no more than 2 hours of exercise per week, were

39   recruited via local advertisements across University of Liverpool campuses and hospital

40   departments. Exclusions included cardiovascular, respiratory, kidney, liver and/or endocrine

41   complications, smoking and >14 units/week of alcohol consumption. The study conformed to

42   the *Declaration of Helsinki* and was approved by the North West Liverpool Central research

43   ethics committee (14/NW/1145; 14/NW/1147; 15/NW/0550). All participants were informed

44   of the methods verbally and in writing before providing written informed consent prior to any

45   assessments. Ninety-eight individuals (52 male, 46 female) with a mean age of 39±13 years

46   and BMI 27±5 kg/m^2 were recruited. Prior to each study visit, participants were required to

47   fast overnight for 12 hours (water was permitted *ad libitum*), abstain from alcohol and caffeine

48   for 24 hours and from exercise for 48 hours.

Study design

50   All participants completed measurement of baseline PA and dietary consumption over a period

51   of 4 days (including one weekend day) between January 2016 and Febuary 2017 followed by

52   assessment in the the order of a) anthropometry (including bio-impedance), fasting

53   biochemistry, an oral glucose tolerance test (OGTT) and assessment of CRF ($\dot{V}O_2$ peak) at

54   University Hospital Aintree and b) magnetic resonance imaging (MRI) and proton magnetic

55   resonance spectroscopy (^{1}H-MRS) at the University of Liverpool MRI Centre (LiMRiC). Due

56   to MRI scanner replacement during part of this study, MRI quantification of body fat was

57   conducted in only 72 individuals. Bio-impedance data was collected in all individuals, and $\dot{V}O_2$

58   peak calculations were based on both total body mass and fat free mass (FFM).

Individual phenotyping

60   Individuals were characterised into one of four groups based on BMI (non-obese <30 vs obese

61   ≥30 kg/m^2) and the presence or absence of MetS according to International Diabetes Federation

62   (IDF) criteria; we refer to these groups as i) 'non-obese MetS-', ii) 'non-obese MetS+', iii)

63   'obese MetS-' and iv) 'obese MetS+'.

Habitual assessment

65   *Physical activity monitoring* PA was monitored throughout using a validated (29) SenseWear

66   mini armband (BodyMedia Inc., Pittsburgh, PA, USA). Wear time (recorded as ~98%) was

67   monitored using SenseWear Professional software (version 8.0). Data included: daily average

68   step count, total energy expenditure, active energy expenditure and time spent in levels of PA

69   including: sleep, lying down, sedentary (<1.5 METS), light (1.5-3 METS), moderate (3-6

70   METS), vigorous (6-9 METS) and very vigorous (>9 METS). A Microsoft Excel template, as

71   previously described (30), was used to determine how sedentary time (not including sleep) was

72   accumulated and provided information on the frequency of bouts and the time accumulated in

73   a given bout category (<1 h: 1–5, 6–10, 11–20, 21–40, 41-60 min; 1-2 h: 61-80, 81-100, 101-

74   120 min; >2 h: 121-140, 141-160, 161-180 min). To examine 'frequently broken' periods of

75   sedentary time, the given bout categories at the lower end (<1 h) were shorter in duration. At

76   the higher end (> 1h), where fewer bouts are recorded, the given bout categories are greater in

77   duration. Based on previous observations (31), this approach was adopted to investigate

78   'patterns' of sedentary time, i.e. the frequency with which sedentary time is interrupted

79   (sedentary breaks) or the duration of uninterrupted periods of sedentary time (sedentary bouts).

80   Furthermore, moderate-vigorous PA (MVPA) of bouts greater or less than 10 minutes were

81   determined.

82   *Dietary analysis* Total energy consumption, carbohydrate, protein and fat content were

83   determined from 4-day dietary records by a registered nutritionist (KM) using Nutritics

84   (Nutrition Analysis Software for Professionals; https://www.nutritics.com/p/home).

85   **Other assessment measures**

86   *Anthropometric measurements* Stature (Model 220, Seca, Germany) and whole-body bio-

87   impedance analysis (Tanita, BC 420, Dolby Medical Stirling, UK) was conducted; this

88   provided total body mass, fat percentage, fat mass, fat free mass, muscle mass, total body water,

89   basal metabolic rate, bone mass and visceral fat indicator. Waist and hip circumference

90   measurements were taken in duplicate and blood pressure was determined from an average of

91   three measures (Dinamap, G & E Medical, USA).

92   *Biochemical measurements* Blood samples were collected and analysed using the Olympus

93   AU2700 analyser (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents

94   as follows: glucose with hexokinase, total cholesterol and HDL with cholesterol

95   esterase/oxidase and triacylglycerol with glycerol kinase. LDL was calculated according to the

96   Friedwald formula. Insulin was measured using radio-immunoassay (Invitrogen, UK). HOMA-

97   IR was calculated using fasting glucose and insulin concentrations.

98   *OGTT* Following a 12hr fast, blood samples were collected, a 75 g glucose drink was consumed

99   within 5 min and post-ingestion blood samples were drawn at 30, 60, 90 and 120 min. Matsuda

100   index was calculated to estimate whole body IS, and indices of hepatic-IR and skeletal muscle

101   IS were determined as previously described (32).

102   *CRF* A $\dot{V}O_2$ peak cardio-pulmonary exercise test (CPET) was performed on a treadmill (Model

103   77OCE, RAM Medisoft Group, Manchester, UK) in a temperature-controlled room. The CPET

104   provided breath-by-breath monitoring and analysis of expiratory gases and ventilation (Love

105   Medical Cardiopulmonary Diagnostics, Manchester, UK). The modified Bruce protocol was

106   employed, after an initial 2 min warm up at 2.2 km/h on a flat gradient, step-wise increments

107   in speed and gradient were employed each minute. $\dot{V}O_2$ peak was determined by exhaustion

108   plus one or more of: respiratory exchange ratio >1.15, heart rate >90% predicted maximum,

109   plateau in $\dot{V}O_2$.

110   *^{1}H-MRS* Liver and skeletal muscle fat were determined using a 1.5 T Siemens Symphony MRI

111   scanner as previously described (33).

Statistical analysis

113   All data were explored for normality using visual inspection of frequency distribution, and

114   logarithmically transformed where appropriate. Given the small sample size, power achieved

115   on each test was assessed and ranged from 46 to >99%; 20 of 26 achieved at least 80% power.

116   Age was analysed using a one factor between-groups ANOVA whereby a significant group

117   effect was observed ($P<0.05$). Between-group univariate general linear models (GLM) were

118   conducted for all other variables, with age as a covariate and Bonferroni correction for multiple

119   comparisons. Statistically significant interactions were explored and nominal *P*-values

120   reported. Univariate and multivariate linear regression were used to analyse components of PA

121   and fitness associated with liver fat. Decisions were made *a priori* to include all variables

122   reaching $P<0.1$ in univariate regression analysis alongside age and BMI in the multivariate

123   regression model. The statistical cut-off for inclusion in the final model is more stringent than

124   often used to guard against false discovery. The alpha level of statistical significance was set

125   at $P<0.05$. Data are presented as mean (95% CI), unless stated otherwise. Transformed data

126   were back-transformed to original units. *P* value >1 rounded to 1.000.

127   **RESULTS**

128   **Participant characteristics**

129   The numbers of individuals with each risk factor of MetS are summarised in Table 1, with the

130   PA and CRF data of the whole cohort combined. Calculated from their average of 4 d MVPA

131   (accumulated in bouts of >10 min), 61% of individuals met the World Health Organisation

132   (WHO) recommendations.

133   **Metabolic phenotyping**

134   The significant differences between the groups' components of MetS were in line with IDF

135   classification (Table 2). There was no significant difference between obese MetS- and obese

136   MetS+ BMI ($P=0.712$) but non-obese MetS+ BMI was 3 ± 2 kg/m^2 greater than non-obese

137   MetS- ($P=0.003$). In the general population, MRS defined liver fat >5.5% corresponds with

138 the prevalence of hepatic steatosis (34); 84 and 14 participants had liver fat <5.5% and ≥5.5%

139 respectively.

Dietary intake

141 Total energy consumption, carbohydrate, protein and fat did not differ significantly between

142 groups ($P>0.05$). Mean ±SD macronutrient percentages were 56±16% carbohydrate, 24±9%

143 protein, and 20±7% fat.

CRF

145 Obese MetS+ individuals had lower CRF than both obese and non-obese MetS- ($P\leq0.029$;

146 mean difference ≥7.5 mL·min^{-1}·kg^{-1}) but not non-obese MetS+ ($P=0.675$; mean difference 5.9

147 mL·min^{-1}·kg^{-1}) There was no difference between both non-obese groups and obese MetS-

148 ($P\geq0.080$) (Fig. 1a).

Multi-organ IS

150 Non-obese MetS- individuals had greater Matsuda index than non-obese MetS+ ($P=0.012$;

151 mean difference 2.0) (Fig. 1b); there was no difference between obese MetS- and both MetS+

152 groups ($P\geq0.141$). There was no group effect for skeletal muscle IS index ($P=0.220$). Hepatic-

153 IR index was greater in obese MetS+ than non-obese MetS- (Fig. 1c). There was a significant

154 group effect ($P=0.022$) for HOMA-IR.

MRS quantification of liver fat

156 Liver fat was higher in MetS+ in both non-obese and obese. Non-obese MetS- individuals had

157 4.6% lower liver fat than obese MetS+ ($P\leq0.005$) (Fig. 1d). Liver fat percentage in non-obese

158 MetS+ was not different to either obese group ($P\geq0.794$; mean difference ≥0.6 %); and liver

159   fat percentage in obese groups was not statistically different (P=0.336; mean difference 2.6

160   %).

Levels of physical activity: differences between the 4 metabolic phenotypes

162   *Average daily steps:* There was no group effect for average daily steps (Fig. 2a).

163   *Non-sleep sedentary time, lying time and sleep duration:* Non-sleep sedentary time (Fig. 2b)

164   was not different between non-obese groups (P=1.000; 49 min/day) and obese groups

165   (P=1.000; 23 min/day). Non-obese MetS- individuals had lower sedentary time than obese

166   MetS+ (P=0.04); there was no difference between obese MetS- and both MetS+ groups

167   (P≥0.199). There was no group effect for amount of time spent lying down (P=0.080) or

168   sleeping (P=0.117).

169   *Daily light PA time:* There was no difference in daily light activity between both non-obese

170   groups (P=0.711; mean difference 10 min/day) and both obese groups (P=1.000; 9 min/day).

171   However, both obese groups had less light activity than both non-obese MetS- (P≤0.015; mean

172   difference ≥69 min/day) (Fig. 2c).

173   *Daily moderate-vigorous PA time:* There was no difference between the groups' moderate-

174   vigorous activity (P=0.322) (Fig. 2d) and no significant differences were found for the way in

175   which MVPA was accumulated for bouts of 10 minutes or more, in either total minutes

176   accumulated or percentage of the time in relation to total MVPA.

177   *Average daily METS and PA duration:* Daily average METS (Fig. 2e) and PA duration (Fig.

178   2f) had significant group effects (P<0.0005 and P=0.020, respectively); for both measures,

179   non-obese MetS- had greater values than both obese groups, but were not different to non-

180   obese MetS+. Daily average METS in non-obese MetS- were 0.3 METS greater than both

181   obese groups (P<0.0005). The same was observed for PA duration, with non-obese MetS-

11

182   having greater duration that both obese groups ($P \leq 0.018$; mean difference ≥ 107 min/day).

183   There was no significant difference between obese MetS- and both MetS+ groups for average

184   daily METS and PA duration ($P \geq 0.079$ and $P \geq 0.450$ respectively).

185   *Patterns of waking sedentary time:* Analysis of sedentary behaviour was performed on waking

186   sedentary time examining the *duration of sedentary time* (Fig. 3a) and *the number of sedentary*

187   *bouts* (Fig. 3b) in a pre-determined bout category. There were no differences between the

188   groups in sedentary bout durations of < 1 h or > 2 h. However, significant differences were

189   apparent during bout durations lasting *between* 1 and 2 h. *Duration:* during bouts of 61-80 min,

190   non-obese MetS+ accumulated 33 min more sedentary time per day than non-obese MetS- (3,

191   60; $P=0.013$). During bouts of 81-100 min, MetS+ obese accumulated 34 min per day more

192   than obese MetS- (6, 62; $P=0.018$). During bouts of 101-120 min, obese MetS+ accumulated

193   28 min per day more than obese MetS- (5, 51; $P=0.018$). *Number of bouts:* as an average of 4

194   days, both MetS+ groups accumulated 1-2 more long bouts (between 1-2 h) of sedentary

195   behaviour, compared to their MetS- counterparts. Considering bouts of 61-80 min, non-obese

196   MetS+ had 0.5 more bouts per day (0.1, 0.9; $P=0.012$) than MetS-. Obese MetS+ had 0.4 more

197   bouts per day (0.1, 0.7; $P=0.019$) than MetS- of 81-100 min and 0.3 more bouts per day (0.1,

198   0.5; $P=0.017$) of 101-120 min.

199   *Levels of physical activity (regression analysis):* Univariable linear regression analysis

200   revealed that daily average steps, sedentary time, vigorous activity, METS and $\dot{V}O_2$ peak were

201   all significantly associated with liver fat. Carried forward in the multivariable analysis, three

202   of these factors remained statistically significant predictors of liver fat (Table 3). Greater daily

203   sedentary time is associated with higher liver fat, while higher overall daily METS and $\dot{V}O_2$

204   peak are associated with lower liver fat (Fig. 3). For a one hour increase in sedentary time, liver

205   fat increased by 1.15% (1.14–1.50%; $P=0.036$) while for a 1 mL·min^{-1}·kg^{-1} increase in CRF

206   ($\dot{V}O_2$ peak), liver fat reduced by 0.87% (0.25, 1.50; $P=0.007$).

DISCUSSION

The results of this extensive phenotypic analysis of objective measurements of PA and sedentary behaviour, metabolic and body composition measurements (including MRS-derived liver fat) in young-middle aged adults demonstrate two key messages. Firstly, in this cohort, overall habitual PA was not associated with different metabolic health status in individuals of similar BMI, and the accumulation of sedentary time was weakly associated with the presence of the MetS. Secondly, even in habitually active individuals, there is an association between greater sedentary time and increased liver fat, while the amount of moderate-vigorous PA (MVPA) appeared to have little independent association. These data highlight the potential importance of sedentary behaviour in determining optimal metabolic health and liver fat.

It is recognised that greater sedentary time increases the risk of becoming overweight/obese (35) and the risk of type 2 diabetes and cardiovascular disease, even after controlling for MVPA (8, 36). Whilst total volumes of habitual PA do not explain metabolic health in this cohort, those with MetS shown some evidence of being more sedentary, with a higher number of prolonged bouts of sedentary behaviour (between 1-2 hours). Frequent breaks in sedentary time have been shown to be beneficial to metabolic risk (31), health (37) and liver fat (24). To our knowledge, there are no studies which have investigated sedentary bouts greater than 1 hour. Interestingly, an extra hour of sedentary time has been associated with a 39% increased odds for MetS (38) and decreasing sedentary time accumulated in prolonged bouts may have beneficial effects om BMI and waist circumference (39). Futher research at durations of >1 hour may reveal insight into the the pattern in which sedentary time is accumulated and MetS. Even individuals who are physically active can still spent a significant amount of their waking day sedentary, (termed previously *sedentary exercisers'* (40)) which is associated with increased cardio-metabolic risk. Taken together, these findings suggest that public health and

231 chronic disease prevention strategies that largely focus on MVPA recommendations, might

232 benefit from new recommendations regarding interruption of prolonged sedentary time,

233 complimentary to those of PA.

234 Numerous prospective studies have confirmed the relationship between PA and liver fat (5, 41-

235 44) and compliance with national MVPA guidelines has been associated with a lower odds of

236 NAFLD (26). Furthermore, recent research in a population-based sample of adults has shown

237 that $\dot{V}O_2$ peak is strongly, inversely and independently related to the risk of liver fat (45). The

238 results presented are in agreement with previous research, greater levels of PA (here daily

239 METs) and higher CRF is independently associated with lower levels of liver fat. Importantly,

240 the associations between CRF and liver fat remained after adjustment for BMI; not all studies

241 have reported similar findings (46). The association between sedentary time and liver fat is

242 equivocal. Some authors have found no associations between PA or sedentary behaviour and

243 liver fat in 82 individuals (25, 26). Whereas others have concluded that PA and sedentary time

244 are indeed independently associated with the prevalence of NAFLD (22, 24). In *inactive*

245 individuals every hour of sedentary time was associated with increases of 1.74 L of total

246 abdominal fat, 0.62 L of visceral fat, 1.14 L of subcutaneous fat, and 1.86% liver fat (22).

247 Direct comparisons or broad conclusions are difficult due to differences in cohorts and

248 methodology. The findings of the current study suggest that sedentary time has an independent

249 effect on liver fat in active adults, however more data is required to confirm this. Our results

250 demonstrating that every hour of additional sedentary time translates to a 1.15% increase in

251 liver fat, can be put into context by comparing the effects of a 4 week aerobic cycling

252 intervention in sedentary obese men and women, where liver fat reduced by 1.7% (47). The

253 effects surgical, nutritional, lifestyle or pharmaceutical interventions aiming to reduce liver fat

254 has been recently reviewed (48).

255   This study utilises objective monitoring of PA, gold standard measurement of CRF and MRS-

256   derived liver fat in young-middle aged adults, all of which are key strengths. The results did

257   not support any strong evidence for a beneficial association of sedentary bouts <1 hour or

258   detrimental association of >2 hours perhaps due to study limitations which include the

259   relatively small sample size. Further limitations include: duration of PA assessment, the

260   monitor used to assess sedentary behaviour (SenseWear does not determine postural

261   differences), the comparatively healthy habitual PA habits of the participants which somewhat

262   limits the external validity of the findings, and the cross-sectional design which cannot

263   determine causality. Noteworthy is the higher BMI in unhealthy non-obese versus healthy non-

264   obese which confirms to the association of a greater BMI with greater metabolic risk. This

265   difference could not be controlled for as it was a component of our grouping analysis but

266   differences in age were statistically controlled for. While the present results demonstrate that

267   overall sedentary time needs to be considered independently of PA, objective PA monitoring

268   in a larger cohort with a prospective design will be required and future research should further

269   explore sedentary behaviour patterns (i.e. amount of sedentary breaks and duration of sedentary

270   bouts). The American Diabetes Association has recommended that adults should '*decrease the*

271   *amount of time spent daily in sedentary behaviour*' and that '*prolonged sitting should be*

272   *interrupted with bouts of light activity every 30 min*'. Importantly, these recommendations are

273   in addition to, not a substitute for, a physically active lifestyle. A 'cut-off' for harmful sedentary

274   behaviour patterns (i.e. frequency/duration) has not been defined in public health guidelines.

275   In summary, in habitually active adults, the amount of sedentary time is associated in this

276   single-measure observation with metabolic health and the quantity of liver fat. The findings of

277   this study highlight that public health policy designed to optimise the benefits of physical

278   activity may need to synergistically consider strategies to reduce sedentary behaviour.

285 **Conflict of Interest**

286 The authors declare that there is no conflict of interest associated with this manuscript. MH has

287 support from NIHR Leicester BRC. The results of the study to not constitute endorsement by

288 ACSM and are presented clearly, honestly, and without fabrication, falsification, or

289 inappropriate data manipulation.

290 **Author contribution statements**

291 DJC, GJK and JPHW conceived the study or parts of the study. KBD, VSS, JAN, DJC,

292 generated the data. GJK analysed the MRS data. KLM analysed the nutritional data. KBD and

293 AT statistically analysed and interpreted the data. All authors participated in preparation of the

294 manuscript and approved the final version for publication.

295 **REFERENCES**

296 1.   Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of
297 physical inactivity on major non-communicable diseases worldwide: an analysis of
298 burden of disease and life expectancy. The Lancet. 2012;380(9838):219-29. doi:
299 10.1016/s0140-6736(12)61031-9.
300 2.   Henson J, Yates T, Biddle SJ, Edwardson CL, Khunti K, Wilmot EG, et al.
301 Associations of objectively measured sedentary behaviour and physical activity with
302 markers of cardiometabolic health. Diabetologia. 2013;56(5):1012-20. Epub
303 2013/03/05. doi: 10.1007/s00125-013-2845-9. PubMed PMID: 23456209.

304 3.   Smith AD, Crippa A, Woodcock J, Brage S. Physical activity and incident type
305 2 diabetes mellitus: a systematic review and dose-response meta-analysis of prospective
306 cohort studies. Diabetologia. 2016;59(12):2527-45. Epub 2016/10/18. doi:
307 10.1007/s00125-016-4079-0. PubMed PMID: 27747395.
308 4.   Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung
309 AE, et al. Sedentary Behavior Research Network (SBRN) – Terminology Consensus
310 Project process and outcome. International Journal of Behavioral Nutrition and Physical
311 Activity. 2017;14(1):75. doi: 10.1186/s12966-017-0525-8.
312 5.   Romero-Gómez M, Zelber-Sagi S, Trenell M. Review: Treatment of NAFLD
313 with diet, physical activity and exercise. Journal of Hepatology. 2017;67:829-46. doi:
314 10.1016/j.jhep.2017.05.016. PubMed PMID: S0168827817320524.
315 6.   Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, Gibbons
316 LW. Physical fitness and all-cause mortality. A prospective study of healthy men and
317 women. Jama. 1989;262(17):2395-401. Epub 1989/11/03. PubMed PMID: 2795824.
318 7.   Ekelund LG, Haskell WL, Johnson JL, Whaley FS, Criqui MH, Sheps DS.
319 Physical fitness as a predictor of cardiovascular mortality in asymptomatic North
320 American men. The Lipid Research Clinics Mortality Follow-up Study. The New
321 England journal of medicine. 1988;319(21):1379-84. Epub 1988/11/24. doi:
322 10.1056/nejm198811243192104. PubMed PMID: 3185648.
323 8.   Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al.
324 Sedentary time and its association with risk for disease incidence, mortality, and
325 hospitalization in adults: a systematic review and meta-analysis. Annals of internal
326 medicine. 2015;162(2):123-32. Epub 2015/01/20. doi: 10.7326/m14-1651. PubMed
327 PMID: 25599350.
328 9.   Knaeps S, Lefevre J, Wijtzes A, Charlier R, Mertens E, Bourgois JG.
329 Independent Associations between Sedentary Time, Moderate-To-Vigorous Physical
330 Activity, Cardiorespiratory Fitness and Cardio-Metabolic Health: A Cross-Sectional
331 Study. PLOS ONE. 2016;11(7):e0160166. doi: 10.1371/journal.pone.0160166.
332 10.   Ekelund U, Steene-Johannessen J, Brown WJ, Fagerland MW, Owen N, Powell
333 KE, et al. Does physical activity attenuate, or even eliminate, the detrimental
334 association of sitting time with mortality? A harmonised meta-analysis of data from
335 more than 1 million men and women. Lancet (London, England).
336 2016;388(10051):1302-10. Epub 2016/08/01. doi: 10.1016/s0140-6736(16)30370-1.
337 PubMed PMID: 27475271.
338 11.   Henson J, Davies MJ, Bodicoat DH, Edwardson CL, Gill JM, Stensel DJ, et al.
339 Breaking up prolonged sitting with standing or walking attenuates the postprandial
340 metabolic response in postmenopausal women: a randomized acute study. Diabetes
341 care. 2016;39(1):130-8. Epub 2015/12/03. doi: 10.2337/dc15-1240. PubMed PMID:
342 26628415.
343 12.   Hamilton MT, Hamilton DG, Zderic TW. Sedentary behavior as a mediator of
344 type 2 diabetes. Medicine and sport science. 2014;60:11-26. Epub 2014/09/18. doi:
345 10.1159/000357332. PubMed PMID: 25226797; PubMed Central PMCID:
346 PMCPMC4364419.
347 13.   Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis
348 RJ, et al. Body-mass index and mortality among 1.46 million white adults. New

349 England Journal of Medicine. 2010;363(23):2211-9. doi: 10.1056/NEJMoa1000367.
350 PubMed PMID: 104970443.

351 14.   Phillips CM. Metabolically healthy obesity across the life course: epidemiology,
352 determinants, and implications. Annals Of The New York Academy Of Sciences.
353 2017;1391(1):85-100. doi: 10.1111/nyas.13230. PubMed PMID: 27723940.

354 15.   Stefan N, Schick F, Häring H-U. Causes, Characteristics, and Consequences of
355 Metabolically Unhealthy Normal Weight in Humans. Cell Metabolism.
356 2017;26(2):292-300. doi: 10.1016/j.cmet.2017.07.008. PubMed PMID: 28768170.

357 16.   Bell JA, Hamer M, van Hees VT, Singh-Manoux A, Kivimäki M, Sabia S.
358 Healthy obesity and objective physical activity. American Journal of Clinical Nutrition.
359 2015;102(2):268-75. doi: 10.3945/ajcn.115.110924. PubMed PMID: 110317970.

360 17.   Camhi SM, Crouter SE, Hayman LL, Must A, Lichtenstein AH. Lifestyle
361 behaviors in metabolically healthy and unhealthy overweight and obese Women: a
362 preliminary study. PLoS ONE. 2015;10(9):1-12. doi: 10.1371/journal.pone.0138548.
363 PubMed PMID: 109533967.

364 18.   Hankinson AL, Daviglus ML, Horn LV, Chan Q, Brown I, Holmes E, et al. Diet
365 composition and activity level of at risk and metabolically healthy obese American
366 adults. Obesity (19307381). 2013;21(3):637-43. doi: 10.1002/oby.20257. PubMed
367 PMID: 104070888.

368 19.   Bell JA, Kivimaki M, Batty GD, Hamer M. Metabolically healthy obesity: What
369 is the role of sedentary behaviour? Preventive Medicine: An International Journal
370 Devoted to Practice and Theory. 2014;62:35-7. doi: 10.1016/j.ypmed.2014.01.028.
371 PubMed PMID: 2014-14800-008.

372 20.   Phillips CM, Dillon C, Harrington JM, McCarthy VJC, Kearney PM, Fitzgerald
373 AP, et al. Defining Metabolically Healthy Obesity: Role of Dietary and Lifestyle
374 Factors. PLOS ONE. 2013;8(10). PubMed PMID: 000326022200011.

375 21.   Dyrstad SM, Hansen BH, Holme IM, Anderssen SA. Comparison of Self-
376 reported versus Accelerometer-Measured Physical Activity. Medicine & Science in
377 Sports & Exercise. 2014;46(1):99-106. doi: 10.1249/MSS.0b013e3l82a0595f. PubMed
378 PMID: 107924259. Language: English. Entry Date: 20140214. Revision Date:
379 20150712. Publication Type: Journal Article.

380 22.   Henson J, Edwardson CL, Morgan B, Horsfield MA, Khunti K, Davies MJ, et
381 al. Sedentary time and MRI-derived measures of adiposity in active versus inactive
382 individuals. Obesity (Silver Spring, Md). 2018;26(1):29-36. Epub 2017/12/22. doi:
383 10.1002/oby.22034. PubMed PMID: 29265769.

384 23.   Smith L, Thomas EL, Bell JD, Hamer M. The association between objectively
385 measured sitting and standing with body composition: a pilot study using MRI. BMJ
386 open. 2014;4(6):e005476. Epub 2014/06/12. doi: 10.1136/bmjopen-2014-005476.
387 PubMed PMID: 24916091; PubMed Central PMCID: PMCPMC4067859.

388 24.   Hallsworth K, Thoma C, Moore S, Ploetz T, Anstee QM, Taylor R, et al. Non-
389 alcoholic fatty liver disease is associated with higher levels of objectively measured
390 sedentary behaviour and lower levels of physical activity than matched healthy controls.
391 Frontline Gastroenterology. 2015;6(1):44-51. PubMed PMID: 25580206.

392 25.   Keating SE, Parker HM, Pavey TG, Baker MK, Caterson ID, George J, et al.
393 Objectively quantified physical activity and sedentary behavior in predicting visceral
394 adiposity and liver fat. Journal of Obesity. 2016:1-10. doi: 10.1155/2016/2719014.

395 PubMed PMID: 118432546. Language: English. Entry Date: 20161023. Revision Date:
396 20161023. Publication Type: Article. Journal Subset: Biomedical.
397 26.   Long MT, Pedley A, Massaro JM, Hoffmann U, Esliger DW, Vasan RS, et al.
398 Hepatic steatosis is associated with lower levels of physical activity measured via
399 accelerometry. Obesity. 2015;23(6):1259-66. doi: 10.1002/oby.21058. PubMed PMID:
400 2015-24029-019.
401 27.   Taylor R. Type 2 diabetes: etiology and reversibility. Diabetes care.
402 2013;36(4):1047-55. Epub 2013/03/23. doi: 10.2337/dc12-1805. PubMed PMID:
403 23520370; PubMed Central PMCID: PMCPMC3609491.
404 28.   Rector RS, Thyfault JP, Wei Y, Ibdah JA. Non-alcoholic fatty liver disease and
405 the metabolic syndrome: an update. World journal of gastroenterology.
406 2008;14(2):185-92. Epub 2008/01/12. PubMed PMID: 18186553; PubMed Central
407 PMCID: PMCPMC2675112.
408 29.   Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy
409 of armband monitors for measuring daily energy expenditure in healthy adults. Med Sci
410 Sports   Exerc.   2010;42(11):2134-40.   Epub   2010/04/14.   doi:
411 10.1249/MSS.0b013e3181e0b3ff. PubMed PMID: 20386334.
412 30.   Myers A, Gibbons C, Butler E, Dalton M, Buckland N, Blundell J, et al. A novel
413 integrative procedure for identifying and integrating three-dimensions of objectively
414 measured free-living sedentary behaviour. BMC Public Health. 2017;17(1):979. Epub
415 2017/12/29. doi: 10.1186/s12889-017-4994-0. PubMed PMID: 29282037; PubMed
416 Central PMCID: PMCPMC5745922.
417 31.   Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, et al. Breaks
418 in sedentary time: beneficial associations with metabolic risk. Diabetes care.
419 2008;31(4):661-6. PubMed PMID: 105761034. Language: English. Entry Date:
420 20080711. Revision Date: 20150711. Publication Type: Journal Article.
421 32.   Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin
422 resistance indexes derived from the oral glucose tolerance test. Diabetes care.
423 2007;30(1):89-94. Epub 2006/12/29. doi: 10.2337/dc06-1519. PubMed PMID:
424 17192339.
425 33.   Cuthbertson DJ, Shojaee-moradie F, Sprung VS, Jones H, Pugh CJA,
426 Richardson P, et al. Dissociation between exercise-induced reduction in liver fat and
427 changes in hepatic and peripheral glucose homeostasis in obese patients with Non-
428 Alcoholic Fatty Liver Disease. Clinical Science. 2016;130(2):93-104. doi:
429 10.1042/cs20150447.
430 34.   Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy
431 S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content:
432 prevalence of hepatic steatosis in the general population. American journal of
433 physiology Endocrinology and metabolism. 2005;288(2):E462-8. Epub 2004/09/02.
434 doi: 10.1152/ajpendo.00064.2004. PubMed PMID: 15339742.
435 35.   Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane
436 PH, et al. Interindividual variation in posture allocation: Possible role in human obesity.
437 Science. 2005;307(5709):584-6. doi: 10.1126/science.1106561. PubMed PMID:
438 edselc.2-52.0-12844251803.
439 36.   Wilmot EG, Edwardson CL, Achana FA, Davies MJ, Gorely T, Gray LJ, et al.
440 Sedentary time in adults and the association with diabetes, cardiovascular disease and

441 death: systematic review and meta-analysis. Diabetologia. 2012;55(11):2895-905.
442 Epub 2012/08/15. doi: 10.1007/s00125-012-2677-z. PubMed PMID: 22890825.

443 37. Honda T, Chen S, Yonemoto K, Kishimoto H, Chen T, Narazaki K, et al.
444 Sedentary bout durations and metabolic syndrome among working adults: a prospective
445 cohort study. BMC Public Health. 2016;16:888. Epub 2016/08/27. doi:
446 10.1186/s12889-016-3570-3. PubMed PMID: 27562190; PubMed Central PMCID:
447 PMCPMC5000401.

448 38. van der Berg JD, Stehouwer CD, Bosma H, van der Velde JH, Willems PJ,
449 Savelberg HH, et al. Associations of total amount and patterns of sedentary behaviour
450 with type 2 diabetes and the metabolic syndrome: The Maastricht Study. Diabetologia.
451 2016;59(4):709-18. Epub 2016/02/03. doi: 10.1007/s00125-015-3861-8. PubMed
452 PMID: 26831300; PubMed Central PMCID: PMCPMC4779127.

453 39. Bonn SE, Rimm EB, Matthews CE, Troiano RP, Bowles HR, Rood J, et al.
454 Associations of Sedentary Time with Energy Expenditure and Anthropometric
455 Measures. Med Sci Sports Exerc. 2018;50(12):2575-83. Epub 2018/07/27. doi:
456 10.1249/mss.0000000000001729. PubMed PMID: 30048408; PubMed Central
457 PMCID: PMCPMC6237631.

458 40. Bakrania K, Edwardson CL, Bodicoat DH, Esliger DW, Gill JM, Kazi A, et al.
459 Associations of mutually exclusive categories of physical activity and sedentary time
460 with markers of cardiometabolic health in English adults: a cross-sectional analysis of
461 the Health Survey for England. BMC Public Health. 2016;16:25. Epub 2016/01/13. doi:
462 10.1186/s12889-016-2694-9. PubMed PMID: 26753523; PubMed Central PMCID:
463 PMCPMC4709945.

464 41. Gerage AM, Ritti-Dias RM, Balagopal B, de Oliveira Conceição RD, Umpierre
465 D, Dos Santos Filho RD, et al. Physical activity levels and hepatic steatosis: a
466 longitudinal follow up study in adults. Journal Of Gastroenterology And Hepatology.
467 2017. doi: 10.1111/jgh.13965. PubMed PMID: 28857324.

468 42. Kwak MS, Kim D, Chung GE, Kim W, Kim JS. The preventive effect of
469 sustained physical activity on incident nonalcoholic fatty liver disease. Liver
470 international : official journal of the International Association for the Study of the Liver.
471 2017;37(6):919-26. Epub 2016/12/06. doi: 10.1111/liv.13332. PubMed PMID:
472 27917585.

473 43. Qiu S, Cai X, Sun Z, Li L, Zügel M, Steinacker JM, et al. Association between
474 physical activity and risk of nonalcoholic fatty liver disease: a meta-analysis.
475 Therapeutic Advances in Gastroenterology. 2017;10(9):701-13. doi:
476 10.1177/1756283X17725977.

477 44. Zelber-Sagi S, Godos J, Salomone F. Lifestyle changes for the treatment of
478 nonalcoholic fatty liver disease: a review of observational studies and intervention
479 trials. Therapeutic Advances in Gastroenterology. 2016;9(3):392-407. doi:
480 10.1177/1756283X16638830. PubMed PMID: PMC4830109.

481 45. PÄLve KS, Pahkala K, Suomela E, Aatola H, Hulkkonen J, Juonala M, et al.
482 Cardiorespiratory Fitness and Risk of Fatty Liver: The Young Finns Study. Medicine
483 & Science in Sports & Exercise. 2017;49(9):1834-41. doi:
484 10.1249/MSS.0000000000001288. PubMed PMID: 124667231. Language: English.
485 Entry Date: 20170823. Revision Date: 20170824. Publication Type: Article. Journal
486 Subset: Allied Health.

487   46.   Minder CM, Shaya GE, Michos ED, Keenan TE, Blumenthal RS, Nasir K, et al.
488 Relation between self-reported physical activity level, fitness, and cardiometabolic risk.
489 American Journal of Cardiology. 2014;113(4):637-43. doi:
490 10.1016/j.amjcard.2013.11.010. PubMed PMID: edselc.2-52.0-84895071550.
491   47.   Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson
492 MW, et al. Aerobic exercise training reduces hepatic and visceral lipids in obese
493 individuals without weight loss. Hepatology. 2009;50(4):1105-12. Epub 2009/07/29.
494 doi: 10.1002/hep.23129. PubMed PMID: 19637289.
495   48.   Tengowski MW, Fuerst T, Sirlin CB. Magnetic resonance imaging fatty liver
496 changes following surgical, lifestyle, or drug treatments in obese, non-alcoholic fatty
497 liver disease, or non-alcoholic steatohepatitis subjects. Imaging Med. 2017;9(6):195-
498 214.

499

Figure legends

Figure 1 Cardio-metabolic phenotyping, individual participant plots for: $\dot{V}O_2$ peak relative to fat free mass (FFM) (a), whole body insulin sensitivity (b), hepatic insulin resistance index (c) and liver intrahepatocellular lipid (IHCL) (d). Data are presented as mean ± SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. *P<0.05 group difference between BMI category, further group differences being given in the text.

Figure 2 Habitual physical activity and sedentary time, individual participant plots for: average daily steps (a), non-sleep sedentary time (<1.5 METS) (b), light activity (1.5−3 METS) (c), moderate-vigorous activity (>3 METS) (d), daily metabolic equivalents (METS) (e) and physical activity (PA) duration (f). Data are presented as mean ± SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. *P<0.05 group difference between BMI category, further group differences being given in the text.

Figure 3 Non-sleep sedentary behaviour, individual participant plots for: duration of sedentary bouts (a) and number of sedentary bouts in given bout category (b) between 1-2 h. Data are presented as mean ± SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. *P<0.05 group difference between BMI category, further group differences being given in the text.