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

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## 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  $39\pm 13$  years; BMI  $26.9\pm 5.1$  kg/m<sup>2</sup>), 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**

Body composition, magnetic resonance spectroscopy, metabolic syndrome, insulin regulation, cardio-respiratory fitness, metabolic equivalents

## 1 INTRODUCTION

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

13 Obesity is strongly associated with poor cardio-metabolic health and overall mortality (13).  
14 However, not all obese individuals are *metabolically unhealthy* (MetS+) (14); conversely not  
15 all non-obese individuals are *metabolically healthy* (MetS-) (15). Some studies suggest that  
16 MetS+ may be a consequence of low PA (16, 17), but others have not supported this conclusion  
17 (18-20). With differences in methodology, cohort characteristics and definitions of metabolic  
18 phenotypes, these studies typically have not precisely defined the differences in PA  
19 characteristics between phenotypes. Only one study, of older adults, has *objectively* measured  
20 sedentary behaviour (19), which offers better reliability than self-report (21); no such study has  
21 been undertaken in young-middle aged adults. There are similarly conflicting results in studies  
22 of the association of metabolic health with objectively measured sedentary behaviour and  
23 quantitative measures of liver fat using magnetic resonance spectroscopy (MRS) or computed  
24 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  $39\pm 13$  years  
46 and BMI  $27\pm 5$  kg/m<sup>2</sup> 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.

#### 49 **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 February 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 ( $^1H$ -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).

#### 59 **Individual phenotyping**

60 Individuals were characterised into one of four groups based on BMI (non-obese  $<30$  vs obese  
61  $\geq 30$  kg/m<sup>2</sup>) 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+’.

#### 64 **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 770CE, 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 *<sup>1</sup>H-MRS* Liver and skeletal muscle fat were determined using a 1.5 T Siemens Symphony MRI  
111 scanner as previously described (33).

## 112 **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  $3\pm 2$  kg/m<sup>2</sup> 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.

#### 140 **Dietary intake**

141 Total energy consumption, carbohydrate, protein and fat did not differ significantly between  
142 groups ( $P>0.05$ ). Mean  $\pm$ SD macronutrient percentages were  $56\pm 16\%$  carbohydrate,  $24\pm 9\%$   
143 protein, and  $20\pm 7\%$  fat.

#### 144 **CRF**

145 Obese MetS+ individuals had lower CRF than both obese and non-obese MetS- ( $P\leq 0.029$ ;  
146 mean difference  $\geq 7.5$  mL $\cdot$ min $^{-1}\cdot$ kg $^{-1}$ ) but not non-obese MetS+ ( $P=0.675$ ; mean difference  $5.9$   
147 mL $\cdot$ min $^{-1}\cdot$ kg $^{-1}$ ) There was no difference between both non-obese groups and obese MetS-  
148 ( $P\geq 0.080$ ) (Fig. 1a).

#### 149 **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 ( $P\geq 0.141$ ). 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.

#### 155 **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+ ( $P\leq 0.005$ ) (Fig. 1d). Liver fat percentage in non-obese  
158 MetS+ was not different to either obese group ( $P\geq 0.794$ ; mean difference  $\geq 0.6\%$ ); and liver

159 fat percentage in obese groups was not statistically different ( $P=0.336$ ; mean difference 2.6  
160 %).

#### 161 **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 ( $P\geq 0.199$ ). 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- ( $P\leq 0.015$ ; mean  
172 difference  $\geq 69$  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-

182 having greater duration than both obese groups ( $P \leq 0.018$ ; mean difference  $\geq 107$  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  $1 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  increase in CRF  
206 ( $\dot{V}O_2$  peak), liver fat reduced by 0.87% (0.25, 1.50;  $P=0.007$ ).

## 207 **DISCUSSION**

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

217 It is recognised that greater sedentary time increases the risk of becoming overweight/obese  
218 (35) and the risk of type 2 diabetes and cardiovascular disease, even after controlling for MVPA  
219 (8, 36). Whilst total volumes of habitual PA do not explain metabolic health in this cohort,  
220 those with MetS shown some evidence of being more sedentary, with a higher number of  
221 prolonged bouts of sedentary behaviour (between 1-2 hours). Frequent breaks in sedentary time  
222 have been shown to be beneficial to metabolic risk (31), health (37) and liver fat (24). To our  
223 knowledge, there are no studies which have investigated sedentary bouts greater than 1 hour.  
224 Interestingly, an extra hour of sedentary time has been associated with a 39% increased odds  
225 for MetS (38) and decreasing sedentary time accumulated in prolonged bouts may have  
226 beneficial effects on BMI and waist circumference (39). Further research at durations of >1  
227 hour may reveal insight into the the pattern in which sedentary time is accumulated and MetS.  
228 Even individuals who are physically active can still spent a significant amount of their waking  
229 day sedentary, (termed previously *sedentary exercisers*' (40)) which is associated with  
230 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.

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## 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 do 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.

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## 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  $\pm$  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  $\pm$  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  $\pm$  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.