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1 **Effect of frequent interruptions of sedentary time on nutrient metabolism in sedentary**  
2 **overweight male and female adults**

3

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35 **Running title:** Breaking-up sedentary time and nutrient metabolism

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48 **ABSTRACT (248/250 WORDS)**

49 **Objective:** This study compared 24-h nutrient oxidation responses between a sedentary  
50 condition (SED) and a condition where short 5-min bouts of moderate-intensity physical activity  
51 were performed hourly for 9 consecutive hours over 4-d (MICRO). To determine whether any  
52 shifts in fuel use were due solely to increases in energy expenditure we also studied a condition  
53 consisting of a single isoenergetic 45-min bout of moderate-intensity exercise (ONE). **Methods:**  
54 Twenty sedentary overweight or obese adults (10M/10F; 32.4±6.3 years; BMI, 30.6±2.9kg/m<sup>2</sup>)  
55 completed all three conditions (MICRO, SED, and ONE) in a randomized order. Each condition  
56 consisted of a 3 d free-living run-in followed by a 24-h stay in a whole-room calorimeter to  
57 measure total energy expenditure (TEE) and substrate utilization. Dietary fat oxidation was also  
58 assessed during the chamber stay by administering a [1-<sup>13</sup>C] oleic acid tracer at breakfast.  
59 Energy intake was matched across conditions. **Results:** Both MICRO and ONE increased TEE  
60 relative to SED resulting in a negative energy balance. HOMA-IR improved in both activity  
61 conditions. MICRO increased 24-h carbohydrate oxidation compared to both ONE and SED  
62 (p<0.01 for both). ONE was associated with higher 24-h total fat oxidation compared to SED,  
63 and higher 24-h dietary fat oxidation compared to both SED and MICRO. Differences in  
64 substrate oxidation remained significant after adjusting for energy balance. **Conclusion:** In  
65 overweight and obese men and women, breaking up sitting time increased reliance upon  
66 carbohydrate as fuel over 24-h while a single energy-matched continuous bout of exercise  
67 preferentially relies upon fat over 24-h.

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75 **News and Noteworthy**

76 • Insulin sensitivity as assessed by HOMA-IR was improved after 4-days of physical activity

77 independent of frequency and duration of activity bouts.

78 • Temporal patterns of activity across the day differentially affect substrate oxidation.

79 • Frequent interruptions of sedentary time with short bouts of walking primarily increases 24-h

80 carbohydrate oxidation whereas an energy-matched single continuous bout of moderate

81 intensity walking primarily increased 24-h fat oxidation.

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## 87 **Introduction**

88 Sedentary behavior is associated with several adverse health outcomes including obesity,  
89 cardiometabolic diseases, diabetes, certain types of cancer and premature mortality (23, 26).  
90 These associations have been observed across sex, age, ethnicity, and even among individuals  
91 who meet the current intensity-based physical activity guidelines (i.e., 150-min/week of  
92 moderate intensity or 75-min/week of vigorous exercise) (26). Isotemporal substitution modeling  
93 suggests that replacing 1h of sedentary time with either light or moderate-to-vigorous intensity  
94 physical activity (MVPA) in inactive adults was associated with lower mortality and risk factors of  
95 metabolic disease, with MVPA associated with the most potent health-enhancing time-  
96 dependent behavior (7, 24). Independent of potential confounders and time spent in other  
97 activities, reallocation of 30-min/day of sedentary time with an equal amount of MVPA is  
98 associated with lower blood triglycerides, glucose and insulin, and higher insulin sensitivity (7).  
99 Additionally, observational studies suggest that interrupting sedentary time with frequent bouts of  
100 physical activity is associated with lower plasma glucose and insulin, waist circumference,  
101 inflammatory marker C-reactive protein even in individuals who regularly exercise (15, 17).  
102 Adults whose sedentary time was mostly uninterrupted had less healthy cardiometabolic  
103 profiles, based on increased blood glucose and triglycerides, compared to those who had more  
104 frequent breaks in sedentary time (15-17), even when controlling for total sedentary time,  
105 MVPA, age, sex and ethnicity (15, 17).

106 A number of acute and short-term studies ( $\leq 3$  days) have shown that frequent interruptions of  
107 prolonged sedentary activities with short bouts of walking decrease postprandial plasma  
108 glucose and insulin concentrations compared to a prolonged sedentary condition (2, 5, 9-11, 18,  
109 21, 27, 34). For example, Dunstan et al. (10) compared plasma glucose and insulin responses  
110 to uninterrupted sitting and 2-min bouts of activity every 20-min for 5-h in overweight adults.  
111 Sitting was either interrupted by light (3.2 km/h) or moderate intensity (5.8-6.4 km/h) treadmill

112 walking. Relative to uninterrupted sitting, glucose and insulin iAUC in response to standardized  
113 meal were both significantly reduced after the activity-break conditions. A possible hypothesis  
114 for the reduction in plasma glucose concentrations is that carbohydrate oxidation is increased to  
115 support the increased energy expenditure. To test this hypothesis, we used room calorimetry  
116 and stable isotope tracers to compare the short-term effects (4-d) of activity microbouts (5-min  
117 of moderate-intensity physical activity performed hourly for 9 consecutive hours) to a sedentary  
118 condition on 24-h nutrient oxidation in physically inactive adults with overweight and obesity.  
119 Because a shift in carbohydrate oxidation with activity microbouts might be solely due to  
120 increased energy expenditure we also studied an energy matched single bout of moderate-  
121 intensity physical activity performed in the morning. Only few studies have controlled for energy  
122 expenditure by comparing the effect of frequent interruptions of sedentary behavior with bouts of  
123 physical activity to an isocaloric continuous bout of physical activity, which remains the most  
124 common form of recommended physical activity (5, 12, 18, 27, 34). A finding that short frequent  
125 bouts of activity promote carbohydrate oxidation in sedentary persons may inform on an  
126 approach to personalize exercise prescription for individuals who have difficulty complying with  
127 traditional physical activity recommendations and reducing the exposure to increased plasma  
128 glucose observed in highly sedentary populations.

129

## 130 **Methods and Study Design**

### 131 *Participants*

132 Eligible participants were aged 19-45 years, with a body mass index (BMI) between 27 and 33  
133 kg/m<sup>2</sup>, weight stable for >3 months, had fasting plasma insulin concentrations below 25  $\mu$ U/mL,  
134 and self-reported > 6h/day sitting. Women were pre-menopausal but could use oral  
135 contraceptives. Exclusion criteria included clinically diagnosed diabetes, taking glucose- and/or  
136 lipid-lowering medication, dyslipidemia, smoking, or being physically active (>150-min/week

137 moderate-intensity exercise). Participants were recruited between October 2014 and October  
138 2016 from newspapers advertisements, public announcement, and flyers in the Denver and  
139 Aurora areas, Colorado, USA (see CONSORT diagram Figure 1).

140

#### 141 *Study design*

142 Following a screening visit, each eligible subject completed three separate 4-day trial conditions  
143 that consisted of three days in free-living conditions followed by 24-h in a whole-room  
144 calorimeter. The three trial conditions were administered in random order:

145 - *Sedentary (SED)*: During the 3-day free-living period, subjects were asked to maintain  
146 usual levels of daily activity, and were asked to refrain from structured exercise. On day  
147 4, subjects remained sedentary in the whole-room calorimeter.

148 - *Sedentary + 1 continuous bout of activity (ONE)*: During the 3-day free-living period,  
149 subjects were instructed to perform 45-min of moderate-intensity walking once per day in  
150 the morning and maintained usual levels of daily activity the rest of the day. On day 4,  
151 participants remained sedentary in the whole-room calorimeter except to perform one  
152 bout of 45-min moderate-intensity treadmill walking at 10:00AM.

153 - *Sedentary + microbouts of activity (MICRO)*: During the 3-day run-in period, subjects  
154 performed 5-min of moderate intensity walking bouts each hour for 9 consecutive hours  
155 in daily life and maintained usual levels of daily activity the rest of the time. On day 4,  
156 participants performed 5-min moderate-intensity treadmill walking every hour for 9  
157 consecutive hours from 1000h to 1800h and remained sedentary the rest of the day.

158

159 The study conditions were separated by a 28-day wash out period and women were studied in  
160 the follicular phase of the menstrual cycle. All the visits were conducted at the Clinical and  
161 Translational Research Center of University of Colorado Hospital (CTRC). This study was



162 approved by the Colorado Multiple Institutional Review Board (COMIRB) and was in accordance  
163 with the Declaration of Helsinki.

164

#### 165 *Screening visit*

166 At screening, written consent was obtained and participants were screened for exclusion  
167 criteria. This included a medical history and physical examination. The short version of the  
168 International Physical Activity Questionnaire (IPAQ) (6) was completed to assess eligibility  
169 based on inclusion criteria for habitual physical activity and time spent sedentary. Subjects then  
170 performed a test on a motorized treadmill to determine the walking pace that was prescribed for  
171 the ONE and MICRO conditions. The walking test started at a pace of 2.4 mph and the pace  
172 increased by increments of 0.3 mph every 2-min. At each level, subjects rated their perceived  
173 effort on a Borg scale from 0 (very light) to 20 (maximal exertion). The aim was to identify the  
174 speed that participants associated with a level of effort reaching 13 (somewhat hard). The  
175 walking test was stopped when the participant rated the speed of the treadmill with an RPE of  
176 16 (hard to very hard). The treadmill speed associated with 13 RPE was the pace that was  
177 prescribed on day 4 for ONE and MICRO. For the three days of the run-in period, subjects were  
178 instructed to walk at a pace similar to what was established with the walking test, i.e. the  
179 participants perceived moderate-intensity walking pace, an RPE of 13.

180

#### 181 *Randomization*

182 Participants were randomized to one of three possible trial-condition orders using balanced  
183 blocks prepared for male and female participants. The study statistician (Z.P.) prepared the  
184 computer-generated randomization lists and sealed envelopes for randomization. Once  
185 informed consent was obtained, a study member opened the sealed randomization envelope  
186 revealing the trial-condition order.

187

188 *Run-in diet and physical activity*

189 A 3-day standard diet was provided by the CTRC Metabolic Kitchen during the run-in to each  
190 inpatient study visit. The macronutrient composition of the diet was 30% fat, 55% carbohydrate  
191 (CHO) and 15% protein of total energy intake. Daily energy needs were calculated based on an  
192 estimate of resting metabolic rate (RMR) derived from the average of 1) direct measurement by  
193 hood indirect calorimetry and 2) an estimate using the following equation:  $[(23.9 \times \text{FFM in kg}) +$   
194  $372]$ , where fat-free mass (FFM) was measured by dual energy X-ray absorptiometry (DXA,  
195 Hologic Delphi-W, Bedford, MA) (20, 35). The estimated RMR was then multiplied by an activity  
196 factor (1.4-1.7) based on the time spent physically active self-reported in the IPAQ. Participants  
197 were instructed to eat all food and bring back leftovers to the Metabolic Kitchen. They were also  
198 asked to abstain from consuming alcohol and to consume the same amount of caffeine (number  
199 of cups) for 24-h before each whole-room calorimeter stay. Daily energy needs in the chamber  
200 were estimated using the same equations as described above but applying an activity factor of  
201 1.3. Compliance with the activity prescriptions (SED, ONE or MICRO) during the 3-day run in  
202 period was objectively assessed with the use of an inclinometer (ActivPAL, Glasgow, UK) and  
203 accelerometer (Actigraph GT3X+, Fort Walton Beach, FL). Results on physical activity during  
204 the run-in diet were reported elsewhere (8).

205

206 *Inpatient study day*

207 Figure 2 depicts the inpatient study protocol. Participants reported to the CTRC at 0730h,  
208 voided and were weighed. An IV catheter was placed into the antecubital vein for blood  
209 sampling. Subjects then entered the whole-room calorimeter for a 23-h stay. Subjects remained  
210 seated for the first hour to achieve a steady state within the individual and the whole-room  
211 calorimeter. A fasting blood sample was obtained at 0855h. Breakfast, lunch, dinner and snack  
212 were given at 0900h, 1300h, 1830h and 2130h and contained 30%, 35%, 25% and 10% of daily  
213 energy needs, respectively. The macronutrient composition of each meal was 65% CHO, 20%

214 fat and 15% protein of total energy intake; a moderately-high carbohydrate diet was used to  
215 optimize our ability to observe differences in fuel utilization between the three conditions.  $1\text{-}^{13}\text{C}$   
216 oleic acid (20mg/kg of FFM, 99% enrichment, Cambridge Isotopic Laboratories, MA) was mixed  
217 and administered with the liquid breakfast meal. Breath and blood samples were then collected  
218 every hour for 14-h from 0900h-2200h, and at 0300h. Final blood and breath samples were  
219 collected at 0700h the following day. In MICRO, blood samples were collected prior each  
220 activity bout x 9 bouts. Blood samples were obtained by having subjects extend their arm  
221 through a port-hole imbedded in the wall of the whole-room calorimeter. Lights-out and a sleep  
222 opportunity was scheduled from 2230h to 0630h. Urine was collected in one jug for the “waking  
223 time” from the start of the study day to 2230h (bedtime), and in a second jug during “sleep time”  
224 from 2230h (bedtime) to 0700h the following morning. Study participants exited the whole-room  
225 calorimeter at 0700h.

226

### 227 *Energy expenditure and substrate oxidation*

228 Twenty-three hour respiratory gas exchange data were extrapolated to 24-h values. TEE and  
229 substrate oxidation were determined using  $\text{O}_2$  consumption and  $\text{CO}_2$  production determined  
230 from the flow rates and differences in gas concentrations between air entering and air exiting  
231 the calorimeter and nitrogen excretion in the urine, as previously described (25). EE and  
232 substrate oxidation (13) were determined over 24-h, waking time and sleeping time. Twenty-four  
233 hour energy balance was calculated as the difference between 24-h energy intake and 24-h  
234 TEE. Activity energy expenditure (AEE) was calculated as  $\text{TEE} - 10\% \text{ of TEE} - \text{SMR}$ , where  
235 10% TEE is the thermic effect of food and SMR (sleep metabolic rate) was the sleep metabolic  
236 rate measured from 0100h to 0300h (30).

237

### 238 *Dietary fatty acid oxidation*

239 Participants collected an hourly breath sample for  $^{13}\text{CO}_2$  by blowing through a tube into two 15  
240 ml vacutainer tubes. Breath  $\text{CO}_2$  was sampled directly from the vacutainer with a syringe, and  
241  $^{13}\text{CO}_2/^{12}\text{CO}_2$  was measured with isotopic ratio mass spectrometer (IRMS, Delta V, Thermo  
242 Electron, Bremen, Germany). The average baseline enrichment value was subtracted from the  
243 subsequent values for each subject, and each time point was expressed as the increase in  
244 enrichment relative to the subject's own baseline. By using time matched  $\text{CO}_2$  production rates  
245 from the whole-room calorimeter,  $1\text{-}^{13}\text{C}$  oleic acid oxidation was calculated as the instantaneous  
246 percentage recovery of  $^{13}\text{C}$  in expired  $\text{CO}_2$  per hour for 14-h and after 24-h cumulative oxidation  
247 rates were also calculated over 24-h as previously described (4) and after correction for  $\text{CO}_2$   
248 entrapment in the bicarbonate pool and TCA cycle (1).

249

#### 250 *Plasma analysis*

251 Whole blood was added to a preservative (3.6 mg EDTA plus 2.4 mg glutathione in distilled  
252 water). Plasma and serum were separated after spinning and stored at  $-80^\circ\text{C}$  until analyzed.  
253 EDTA plasma samples were assayed for triglycerides (TG), glucose and insulin. Insulin  
254 concentrations were measured using a standard double antibody radioimmunoassay (EMD  
255 Millipore, St. Charles, Missouri). Serum glucose concentrations were determined using the  
256 hexokinase method, TG were measured using the enzymatic assay and non-esterified fatty  
257 acids (NEFA) by using commercial enzymatic assay (Wako Diagnostics, Mountain View, CA);  
258 all samples were run on the Beckman Coulter AU480 Chemistry Analyzer (Brea, CA).

259

#### 260 *Data and statistical analysis*

261 The sample size was calculated using data from a previous study (11). In that study, the authors  
262 observed in 19 overweight men that interruptions of prolonged sitting with moderate-intensity  
263 activity (2-min every 20-min) reduced 5-h glucose and insulin incremental area under the curve  
264 (iAUC) by 29% and 23%, respectively, compared to uninterrupted sitting. Assuming the effect

265 sizes between conditions would be similar to this study, we estimated that 20 paired  
266 observations were needed to achieve an 80% power to detect a direct treatment effect, while  
267 adopting a two-tailed testing and  $\alpha < 0.05$ .

268 iAUCs were calculated with the trapezoidal rule for plasma metabolites and insulin. Linear  
269 mixed models (LMM) were used to test differences in total substrate use, TG, FFA, glucose and  
270 insulin iAUCs and dietary fat oxidation with intervention as repeated effect, sequence, period  
271 and intervention as fixed effects and subjects as random effect with a compound symmetry  
272 covariance. Energy balance was taken into account as a covariate when necessary. Least  
273 significant difference (LSD) post-hoc tests were used to examine between condition differences.  
274 Carryover effects were expected to be minimal because of the minimum 28-days washout  
275 period between consecutive interventions. Pearson correlation coefficients were calculated to  
276 examine the relationships among the outcomes, i.e. TEE, AEE, energy balance, substrate use,  
277 dietary fatty acid oxidation, and plasma metabolites and insulin iAUCs. Data are expressed as  
278 mean  $\pm$  SD, unless otherwise stated. All statistical analyses were performed with SPSS (v22.0,  
279 IBM, SPSS Statistics Inc., Chicago, IL).

280

## 281 **Results**

### 282 *Participant characteristics*

283 The Consolidated Standards of Reporting Trials (CONSORT) diagram is shown as Figure 1.  
284 Twenty-five sedentary overweight adults ( $n=12\text{M}/13\text{F}$ ;  $31.6\pm 6.5$  years;  $\text{BMI}=30.5\pm 2.7$   $\text{kg}/\text{m}^2$ )  
285 were recruited to participate in the study. Data are shown for twenty participants ( $10\text{M}/10\text{F}$ ;  
286  $32.4\pm 6.3$  years;  $\text{BMI}$ ,  $30.6\pm 2.9$   $\text{kg}/\text{m}^2$ ) who completed all the procedures. Participant's  
287 characteristics are displayed in Table 1.

288

### 289 *Twenty-four hour energy intake, expenditure, balance and nutrient oxidation*

290 Daily patterns of EE and respiratory quotient (RQ) are presented in Supplemental Figure 1.  
291 Twenty-four hour energy intake, expenditure and balance are shown in Figure 3A. By design,  
292 both TEE and AEE were matched between the two active conditions and significantly higher  
293 than SED ( $p < 0.0001$  for both). Because total energy intake was the same across all three study  
294 conditions, participants were in negative energy balance in both ONE ( $-1.50 \pm 0.17$  MJ/d,  $p < 0.01$ )  
295 and MICRO ( $-1.64 \pm 0.17$  MJ/d,  $p < 0.01$ ) compared to SED. Twenty-four hour non-protein  
296 respiratory quotient (NPRQ) was lower in ONE compared to both SED and MICRO conditions  
297 ( $0.881 \pm 0.006$  versus  $0.900 \pm 0.006$  and  $0.900 \pm 0.009$  respectively,  $p < 0.05$  for both; Figure 3D),  
298 even when accounting for differences in energy balance. MICRO was associated with higher  
299 24-h carbohydrate oxidation compared to both ONE ( $p < 0.01$ ) and SED ( $p < 0.001$ ; Figure 3C). In  
300 contrast ONE was associated with higher 24-h total fat oxidation compared to SED ( $p < 0.001$ )  
301 and higher 24-h dietary fat oxidation compared to both SED and MICRO ( $p < 0.05$  for both;  
302 Figure 3C and E). When taking energy balance into account, both 24-h total and dietary fat  
303 oxidation were higher and 24-h carbohydrate oxidation lower in ONE compared to MICRO  
304 ( $p < 0.05$  for all). When expressing nutrient oxidation as a percentage of TEE, we observed that  
305 ONE had greater reliance on fat as fuel and less on carbohydrate compared to SED. Nutrient  
306 oxidation as percentage of TEE was not altered in MICRO (Figure 3B).

307

308 In ONE, changes in both 24-h total and dietary fat oxidation were positively correlated with the  
309 increase in 24-h EE ( $R^2 = 0.66$ ,  $p < 0.001$ ;  $R^2 = 0.58$ ,  $p < 0.01$ , respectively) but these associations  
310 were not observed in MICRO even though the increase in 24-h EE was similar. In both active  
311 conditions, changes in 24-h fat oxidation were negatively correlated with changes in 24-h  
312 carbohydrate oxidation (ONE:  $R = -0.79$ ,  $p < 0.0001$ ; MICRO:  $R = -0.84$ ,  $p < 0.0001$ ).

313

314 *Substrate oxidation during waking and sleeping periods*

315 As expected, EE was significantly higher in ONE and MICRO compared to SED ( $p < 0.0001$  for  
316 both; Figure 4C) during the waking time when physical activity was performed. Waking NPRQ  
317 was lower in ONE compared to SED and MICRO ( $0.894 \pm 0.006$  versus  $0.911 \pm 0.005$  and  
318  $0.914 \pm 0.009$  respectively,  $p < 0.05$  for both). During sleep, NPRQ was lower in both ONE and  
319 MICRO compared to SED ( $0.847 \pm 0.009$  and  $0.852 \pm 0.008$  versus  $0.875 \pm 0.011$ , respectively,  
320  $p < 0.05$  for both). In both active conditions, carbohydrate oxidation decreased during the night.  
321 Compared to SED this was associated with greater fat oxidation in ONE, but increased protein  
322 oxidation in MICRO ( $p < 0.05$  for all; Figure 4E).

323

#### 324 *Plasma metabolites and index of insulin sensitivity*

325 Twenty-four-hour profiles of glucose, TG, insulin, and FFA are shown in Supplemental Figure 2.  
326 There were no differences between conditions in fasting concentrations or 24-h iAUC values for  
327 plasma glucose, insulin, FFA and TG (Figure 5). After 4-d of both active conditions HOMA-IR  
328 was decreased compared to SED (SED =  $1.67 \pm 0.87$ , ONE =  $1.20 \pm 0.52$ , MICRO =  $1.42 \pm 0.70$ ;  
329  $p < 0.05$  for both), indicating an improvement in fasting insulin sensitivity. In addition, insulin iAUC  
330 measured from the first to the last microbout of activity (1000h – 1800h) was reduced in ONE  
331 (ONE =  $-16599 \pm 10871 \mu\text{IU/mL}\cdot 9\text{h}^{-1}$ ;  $p < 0.0001$ ) and MICRO (MICRO =  $-11570 \pm 8866 \mu\text{IU/mL}\cdot 9\text{h}^{-1}$ ;  
332  $p < 0.01$ ) compared to SED (SED =  $-5283 \pm 10667 \mu\text{IU/mL}\cdot 9\text{h}^{-1}$ ) (Figure 5). No differences were  
333 noted over this same time period in plasma glucose, TG and FFA.

334

## 335 **Discussion**

336 In this crossover study, we showed in sedentary overweight or obese adults that four days of  
337 frequent interruptions in sedentary time with short moderate intensity walking breaks every hour  
338 for nine hours leads to greater reliance upon carbohydrate as fuel compared to a sedentary  
339 control condition. In contrast and to our surprise, a single isoenergetic continuous bout of

340 moderate-intensity walking led to greater total and dietary fat oxidation. These findings suggest  
341 that when energy expenditure is equal between the two active conditions, breaking up  
342 sedentary time impacts daily patterns in fuel utilization differently than when exercise is  
343 performed as a single bout in the morning.

344

345 Breaking up sedentary behavior with short bouts of activity resulted in improved postprandial  
346 glucose and insulin metabolism in several previous acute and short intervention studies (2, 5, 9-  
347 11, 18, 21, 27, 34). In the present study, we observed a significant reduction in plasma insulin  
348 iAUC when participants were performing microbouts of activity, but postprandial glucose  
349 concentration was not reduced over the 9h active period. There are several possible reasons for  
350 the discrepancy in postprandial glucose responses in response to short frequent breaks of  
351 prolonged sitting compared to sedentary control. Previous studies supplied liquid meal  
352 replacement shakes as the study day energy intake. While the use of liquid meal replacement  
353 shakes ensures accurate standardization of macronutrient intake it does not reflect the  
354 postprandial responses of foods that are regularly consumed by the target population. The  
355 current study administered food preference questionnaires and provided meals composed of  
356 whole-foods including dietary fiber. Dietary fiber has been shown to attenuate postprandial  
357 plasma glucose responses (22, 32). Additionally, the current study did not include participants  
358 with impaired glucose tolerance or type 2 diabetes as has been done in previous studies (10,  
359 11, 21). Along this line, Blankenship *et al.* (2014) studied overweight and obese participants  
360 similar to the present study but also did not observe differences in plasma glucose responses to  
361 a meal tolerance test administered at the end of a day after a protocol involving either frequent  
362 long or short activity breaks (conditions matched for EE) (5). However, glycemic variability  
363 measured by continuous glucose monitoring was reduced in the two frequent break conditions,  
364 indicating improved glucose control (5). Peddie *et al.* (2013) showed a reduction in postprandial  
365 glucose iAUC in young healthy male adults compared to sedentary condition when interrupting



366 sitting with walking breaks of 1-min and 40-s every 30-min (30-min total walking) over 9h (27).  
367 Similarly, Bailey et al showed in overweight young adults a beneficial effect on postprandial  
368 glycemia when sitting was interrupted by 2-min bouts of light walking every 20-min (28-min total)  
369 for 6-h (2). In the current study, independent of changes in substrate use, both modalities of  
370 physical activity improved insulin sensitivity after four days as indicated by the decrease in  
371 HOMA-IR in the fasted state the following morning and the decrease in postprandial insulin  
372 iAUC during the active period. Taken together, both exercise interventions improved indexes of  
373 insulin sensitivity but through a differential metabolic response to varying frequencies of activity  
374 bouts. These studies suggest that while less apparent in non-diabetic populations, frequent  
375 interruptions of sedentary time help control glucose metabolism via a better insulin sensitivity  
376 and greater use of carbohydrate as fuel in postprandial state and over 24-h thus lowering  
377 glycemia mean and variability. The magnitude of the effects may be related to the frequency of  
378 the interruptions. Interestingly, these effects seem to be independent of energy balance, and  
379 likely related to the frequent interruptions of sedentary time.

380

381 To distinguish the effect of energy expenditure from those resulting from the frequent  
382 interruptions of sedentary time, we included an isoenergetic single continuous bout of moderate  
383 intensity walking in the study design. At the same energy expenditure and energy deficit,  
384 frequent interruptions of sedentary activity with short bouts of moderate intensity physical  
385 activity primarily rely upon carbohydrate substrate to maintain energetic homeostasis over 24-h,  
386 while a single bout of moderate-intensity activity favors the oxidation of both 24-h total and  
387 dietary fatty acids, as previously reported (4). One could assume that the greater use of fat  
388 oxidation observed with physical activity performed as a continuous long bout may result over  
389 the long-run in a greater weight loss than what could be attained by performing multiple short  
390 bouts of activity. Jackicic et al. (19) showed that multiple short bouts and time-matched long  
391 continuous bouts of activity induced similar weight loss after 18 months of intervention in

392 sedentary overweight women. It is however important to note that the short bouts were of 10-  
393 min duration which could be long enough to trigger fat oxidation compared to 5-min bout of  
394 activity. Future studies looking at the long-term impact of microbouts of activity versus long  
395 bouts of activity on body weight regulation will be needed to further test this hypothesis.

396

397 The differential effects between these two active conditions was also observed over the waking  
398 and sleeping periods when examined separately. During the waking period, frequent  
399 interruptions in sedentary time was associated with greater carbohydrate oxidation while the  
400 performance of a single bout of walking led to an increase in both carbohydrate and lipid  
401 oxidation. As suggested by the tight correlation between carbohydrate and fat oxidation  
402 observed over 24-h for each active condition, nutrient oxidation is likely the result of competition  
403 between substrates entering the TCA cycle (28). One may assume the potential following  
404 scenario. Glucose was preferentially used with the short bouts of activity because it was readily  
405 available as skeletal muscle glycogen, especially during the first minutes of the bout of activity.  
406 The regular muscle contractions spread across the day with the frequent interruptions of  
407 sedentary time may have further stimulated the translocation from the cytoplasm to the  
408 membrane of the glucose transporter GLUT4, as previously shown (3). This triggered the  
409 uptake and oxidation of glucose by the cell to provide energy. Because the microbouts of  
410 activity were performed in the postprandial state only, glucose was constantly available and  
411 competing against fat. When performing 45-min of walking, glycogen storage was at least partly  
412 depleted thus allowing fat to be oxidized for energy expenditure and glycogen pools were re-  
413 filled. Because of the close relationship between energy and fat balances (14, 33), we observed  
414 that at the equal energy expenditure, fat oxidation was increased but only when physical activity  
415 was performed as one single bout.

416

417 During the sleeping periods, carbohydrate oxidation was reduced in the two active conditions  
418 compared to the sedentary condition. Interestingly, while this was in favor of increased fat  
419 oxidation after 45-min of moderate-intensity walking performed in the morning, it was associated  
420 with an increase in protein oxidation following a day performing microbouts of activity. However,  
421 measurement of protein oxidation via urinary nitrogen excretion is not a direct measure of  
422 protein oxidation but an assessment of protein deamination. The greater disappearance of  
423 protein may rather reflect a use of protein for gluconeogenesis to replenish muscle glycogen  
424 than a use of protein as fuel for the body. Over 24-h, the microbouts of activity likely trigger the  
425 use of glycogen stores and its replenishment, thus enhancing glycogen turnover; future studies  
426 will be needed to test this hypothesis. These differences and changes in nocturnal nutrient  
427 metabolism are important given the growing body of data pointing towards a key role of sleep in  
428 the regulation of energy homeostasis and metabolism (29). For example, we showed that  
429 higher rates of nocturnal fat oxidation are associated with lower weight gain over five years in  
430 adults (31). Future research is needed to better understand the changes induced by the two  
431 different types of physical activity interventions on the changes observed in waking and sleeping  
432 nutrient metabolism.

433

434 A major strength of this study is that it was a randomized controlled trial testing energy-matched  
435 active conditions to isolate the respective effects of the frequency of interruptions of sedentary  
436 time from energy expenditure. Also, diet, alcohol and caffeine consumption were controlled, and  
437 nutrient metabolism was measured over 24-h in a whole-room calorimeter. There are some  
438 methodological factors that limit generalisability of the present findings. Comparing the active  
439 conditions to the sedentary conditions in stable energy balance would have been more rigorous,  
440 and most likely representative of what happened in daily life in chronic situations. We however  
441 mathematically and statistically accounted for differences in energy balance, and the two active  
442 conditions were in similar energy imbalance. Another limitation was the artificial elevation of

443 dietary carbohydrate oxidation that may have potentialized the use of carbohydrate as fuel  
444 during the MICRO condition. Additionally, while dietary fat oxidation was measured, we cannot  
445 comment on the source of carbohydrate that was oxidized during the study day. Future studies  
446 using both fat and carbohydrate stable isotope tracers will be needed.

447

448

#### 449 **Conclusions and future directions**

450 In conclusion, we showed that while four days of frequent interruptions in sitting time primarily  
451 relies upon carbohydrate as fuel, a single long bout of activity primarily influences lipid  
452 metabolism. This suggests that the beneficial effects of interrupting sedentary time on glucose  
453 control that was previously reported is likely related to a greater reliance upon carbohydrate as  
454 fuel. This effect does not appear to be related to energy expenditure and balance, but rather to  
455 increasing the frequency of muscle contractions spread across the day. Underlying mechanisms  
456 at play as well as the role of moderating factors such as weight status, insulin resistance and  
457 sex need to be examined in the future.

458

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462

#### 463 **Conflict of interests**

464 The authors declare no conflict on interests.

465

466

#### 467 **Author contributions**

468 AB and DHB designed the study, collected, analyzed and interpreted data, and drafted the  
469 manuscript. NPDJ collected, analyzed and interpreted data, and drafted the manuscript. ELM,  
470 CAR analyzed and interpreted data and drafted the manuscript. DG, AL, CM collected and  
471 analyzed data. ZP performed statistical analysis and drafted the manuscript. All authors gave  
472 final approval of the manuscript to be published and agreed to be accountable for all aspects of  
473 the work in ensuring that questions related to the accuracy or integrity of any part of the work  
474 are appropriately investigated and resolved.

475

#### 476 **Disclosure**

477 The contents are solely the responsibility of the authors and do not represent the views of the  
478 U.S. Department of Veterans Affairs, the United States Government, the Center for Disease  
479 Control, National Institute of Occupational Health and Safety and the Mountain and Area Plains  
480 Education and Research Center.

481

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486 Education and Research Center.

487

#### 488 **References**

- 489 1. **Antoun E, Momken I, Bergouignan A, Villars C, Platat C, Schoeller DA, Blanc S,**  
490 **and Simon C.** The [1-13C] acetate recovery factor to correct tracer-derived dietary fat oxidation  
491 is lower in overweight insulin-resistant subjects. *European e-Journal of Clinical Nutrition and*  
492 *Metabolism* 5: e173-e179, 2010.
- 493 2. **Bailey DP, and Locke CD.** Breaking up prolonged sitting with light-intensity walking  
494 improves postprandial glycemia, but breaking up sitting with standing does not. *Journal of*  
495 *science and medicine in sport* 18: 294-298, 2015.
- 496 3. **Bergouignan A, Latouche C, Heywood S, Grace MS, Reddy-Luthmoodoo M, Natoli**  
497 **AK, Owen N, Dunstan DW, and Kingwell BA.** Frequent interruptions of sedentary time

- 498 modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: Ancillary  
 499 analysis from randomized clinical trials. *Scientific reports* 6: 32044, 2016.
- 500 4. **Bergouignan A, Momken I, Lefai E, Antoun E, Schoeller DA, Platat C, Chery I,**  
 501 **Zahariev A, Vidal H, Gabert L, Normand S, Freyssenet D, Laville M, Simon C, and Blanc S.**  
 502 Activity energy expenditure is a major determinant of dietary fat oxidation and trafficking, but the  
 503 deleterious effect of detraining is more marked than the beneficial effect of training at current  
 504 recommendations. *The American journal of clinical nutrition* 98: 648-658, 2013.
- 505 5. **Blankenship JM, Granados K, and Braun B.** Effects of subtracting sitting versus  
 506 adding exercise on glycemic control and variability in sedentary office workers. *Applied*  
 507 *physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 39:  
 508 1286-1293, 2014.
- 509 6. **Booth M.** Assessment of physical activity: an international perspective. *Res Q Exerc*  
 510 *Sport* 71: 2000.
- 511 7. **Buman MP, Winkler EA, Kurka JM, Hekler EB, Baldwin CM, Owen N, Ainsworth BE,**  
 512 **Healy GN, and Gardiner PA.** Reallocating time to sleep, sedentary behaviors, or active  
 513 behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005-2006.  
 514 *American journal of epidemiology* 179: 323-334, 2014.
- 515 8. **De Jong NP, Debache I, Pan Z, Garnotel M, Lyden K, Sueur C, Simon C, Bessesen**  
 516 **DH, and Bergouignan A.** Breaking up Sedentary Time in Overweight/Obese Adults on Work  
 517 Days and Non-Work Days: Results from a Feasibility Study. *International journal of*  
 518 *environmental research and public health* 15: 2018.
- 519 9. **Dempsey PC, Blankenship JM, Larsen RN, Sacre JW, Sethi P, Straznicky NE,**  
 520 **Cohen ND, Cerin E, Lambert GW, Owen N, Kingwell BA, and Dunstan DW.** Interrupting  
 521 prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control.  
 522 *Diabetologia* 60: 499-507, 2017.
- 523 10. **Dempsey PC, Larsen RN, Sethi P, Sacre JW, Straznicky NE, Cohen ND, Cerin E,**  
 524 **Lambert GW, Owen N, Kingwell BA, and Dunstan DW.** Benefits for Type 2 Diabetes of  
 525 Interrupting Prolonged Sitting With Brief Bouts of Light Walking or Simple Resistance Activities.  
 526 *Diabetes Care* 39: 964-972, 2016.
- 527 11. **Dunstan DW, Kingwell BA, Larsen R, Healy GN, Cerin E, Hamilton MT, Shaw JE,**  
 528 **Bertovic DA, Zimmet PZ, Salmon J, and Owen N.** Breaking Up Prolonged Sitting Reduces  
 529 Postprandial Glucose and Insulin Responses. *Diabetes Care* 35: 976-983, 2012.
- 530 12. **Duvivier BM, Schaper NC, Bremers MA, van Crombrugge G, Menheere PP, Kars M,**  
 531 **and Savelberg HH.** Minimal intensity physical activity (standing and walking) of longer duration  
 532 improves insulin action and plasma lipids more than shorter periods of moderate to vigorous  
 533 exercise (cycling) in sedentary subjects when energy expenditure is comparable. *PLoS one* 8:  
 534 e55542, 2013.
- 535 13. **Frayn KN.** Calculation of substrate oxidation rates in vivo from gaseous exchange.  
 536 *Journal of applied physiology: respiratory, environmental and exercise physiology* 55: 628-634,  
 537 1983.
- 538 14. **Galgani J, and Ravussin E.** Energy metabolism, fuel selection and body weight  
 539 regulation. *International journal of obesity (2005)* 32 Suppl 7: S109-119, 2008.
- 540 15. **Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, and Owen N.**  
 541 Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care* 31: 661-  
 542 666, 2008.
- 543 16. **Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, and Owen N.**  
 544 Objectively measured light-intensity physical activity is independently associated with 2-h  
 545 plasma glucose. *Diabetes Care* 30: 1384-1389, 2007.
- 546 17. **Healy GN, Matthews CE, Dunstan DW, Winkler EA, and Owen N.** Sedentary time and  
 547 cardio-metabolic biomarkers in US adults: NHANES 2003-06. *European heart journal* 32: 590-  
 548 597, 2011.

- 549 18. **Holmstrup M, Fairchild T, Keslacy S, Weinstock R, and Kanaley J.** Multiple short  
550 bouts of exercise over 12-h period reduce glucose excursions more than an energy-matched  
551 single bout of exercise. *Metabolism - Clinical and Experimental* 63: 510-519.
- 552 19. **Jakicic JM, Winters C, Lang W, and Wing RR.** Effects of intermittent exercise and use  
553 of home exercise equipment on adherence, weight loss, and fitness in overweight women: a  
554 randomized trial. *Jama* 282: 1554-1560, 1999.
- 555 20. **Jankowski CM, Gozansky WS, Van Pelt RE, Wolfe P, Schwartz RS, and Kohrt WM.**  
556 Oral dehydroepiandrosterone replacement in older adults: effects on central adiposity, glucose  
557 metabolism and blood lipids. *Clinical endocrinology* 75: 456-463, 2011.
- 558 21. **Larsen RN, Kingwell BA, Robinson C, Hammond L, Cerin E, Shaw JE, Healy GN,  
559 Hamilton MT, Owen N, and Dunstan DW.** Breaking up of prolonged sitting over three days  
560 sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in  
561 overweight and obese adults. *Clinical science (London, England : 1979)* 129: 117-127, 2015.
- 562 22. **Lunde MSH, Hjellset VT, Holmboe-Ottesen G, and Høstmark AT.** Variations in  
563 postprandial blood glucose responses and satiety after intake of three types of bread. *Journal of  
564 nutrition and metabolism* 2011: 437587-437587, 2011.
- 565 23. **Matthews CE, George SM, Moore SC, Bowles HR, Blair A, Park Y, Troiano RP,  
566 Hollenbeck A, and Schatzkin A.** Amount of time spent in sedentary behaviors and cause-  
567 specific mortality in US adults. *The American journal of clinical nutrition* 95: 437-445, 2012.
- 568 24. **Matthews CE, Keadle SK, Troiano RP, Kahle L, Koster A, Brychta R, Van Domelen  
569 D, Caserotti P, Chen KY, Harris TB, and Berrigan D.** Accelerometer-measured dose-  
570 response for physical activity, sedentary time, and mortality in US adults. *The American journal  
571 of clinical nutrition* 104: 1424-1432, 2016.
- 572 25. **Melanson EL, Ingebrigtsen JP, Bergouignan A, Ohkawara K, Kohrt WM, and  
573 Lighton JR.** A new approach for flow-through respirometry measurements in humans.  
574 *American journal of physiology Regulatory, integrative and comparative physiology* 298: R1571-  
575 1579, 2010.
- 576 26. **Mummery WK, Schofield GM, Steele R, Eakin EG, and Brown WJ.** Occupational  
577 sitting time and overweight and obesity in Australian workers. *American journal of preventive  
578 medicine* 29: 91-97, 2005.
- 579 27. **Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, and Perry TL.** Breaking  
580 prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized  
581 crossover trial. *The American journal of clinical nutrition* 98: 358-366, 2013.
- 582 28. **Randle PJ.** Regulatory interactions between lipids and carbohydrates: the glucose fatty  
583 acid cycle after 35 years. *Diabetes/metabolism reviews* 14: 263-283, 1998.
- 584 29. **Reutrakul S, and Van Cauter E.** Sleep influences on obesity, insulin resistance, and  
585 risk of type 2 diabetes. *Metabolism: clinical and experimental* 2018.
- 586 30. **Rising R, Harper IT, Fontvielle AM, Ferraro RT, Spraul M, and Ravussin E.**  
587 Determinants of total daily energy expenditure: variability in physical activity. *The American  
588 journal of clinical nutrition* 59: 800-804, 1994.
- 589 31. **Rynders CA, Bergouignan A, Kealey E, and Bessesen DH.** Ability to adjust nocturnal  
590 fat oxidation in response to overfeeding predicts 5-year weight gain in adults. *Obesity* 25: 873-  
591 880, 2017.
- 592 32. **Sadakiyo T, Ishida Y, Inoue S-I, Taniguchi Y, Sakurai T, Takagaki R, Kurose M,  
593 Mori T, Yasuda-Yamashita A, Mitsuzumi H, Kubota M, Watanabe H, and Fukuda S.**  
594 Attenuation of postprandial blood glucose in humans consuming isomaltodextrin: carbohydrate  
595 loading studies. *Food & nutrition research* 61: 1325306-1325306, 2017.
- 596 33. **Schrauwen P, Lichtenbelt WD, Saris WH, and Westerterp KR.** Fat balance in obese  
597 subjects: role of glycogen stores. *The American journal of physiology* 274: E1027-1033, 1998.

- 598 34. van Dijk JW, Venema M, van Mechelen W, Stehouwer CD, Hartgens F, and van  
 599 Loon LJ. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood  
 600 glucose homeostasis in male patients with type 2 diabetes. *Diabetes Care* 36: 3448-3453, 2013.  
 601 35. Villalon KL, Gozansky WS, Van Pelt RE, Wolfe P, Jankowski CM, Schwartz RS, and  
 602 Kohrt WM. A losing battle: weight regain does not restore weight loss-induced bone loss in  
 603 postmenopausal women. *Obesity (Silver Spring, Md)* 19: 2345-2350, 2011.

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606

607 **Figure legends**608 **Figure 1: Trial CONSORT diagram**609 **Figure 2: Protocol of the study day in the whole-room calorimeter**

610 SED, sedentary condition in which participants remained sedentary (no exercise); ONE,  
 611 one bout condition in which participants remained sedentary for the day except for  
 612 completing one bout of moderate intensity treadmill walking for 45-minutes; MICRO,  
 613 microbouts condition in which participants remained sedentary for the day except for  
 614 completing 9 bouts of 5-min moderate intensity treadmill walking once an hour for 9  
 615 consecutive hours. ■ indicates 45-minute bout of walking. ▮ indicates 5-minute walking  
 616 bout. ↑ indicates blood collection. ▲ indicates meals.

617

618 **Figure 3: Absolute and relative twenty-four hour nutrient oxidation, balance and dietary**  
 619 **fat oxidation**

620 (A) Energy intake (MJ/d), energy expenditure (MJ/d) and energy balance (MJ/d) during  
 621 the study day in the whole room calorimeter. (B) Relative contribution (%) of  
 622 carbohydrate (CHO), fat (FAT) and protein (PRO) oxidation to total 24-h energy



623 expenditure. (C) Absolute nutrient oxidation (grams/day) for carbohydrate (CHO), fat  
 624 (FAT) and protein (PRO). (D) 24-h non-protein respiratory quotient for each study  
 625 condition: sedentary (SED), one bout (ONE) and microbouts (MICRO). (E) 24-h  
 626 dietary fat oxidation (percent dose recovery from 1-<sup>13</sup>C oleic acid stable isotope  
 627 tracer) for each study condition: sedentary (SED), one bout (ONE) and microbouts  
 628 (MICRO). Data are presented as mean ± SEM. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

629

#### 630 **Figure 4: Waking and sleeping substrate oxidation**

631 Absolute and relative substrate oxidation during waking (0800h-2230h) and sleeping  
 632 (2230h-0630h) time. (A) Relative contribution (%) of carbohydrate (CHO), fat (FAT) and  
 633 protein oxidations to waking energy expenditure. (B) Waking absolute nutrient oxidation  
 634 (g) of carbohydrate (CHO), fat (FAT) and protein oxidations for waking energy  
 635 expenditure. (C) Waking energy expenditure. (D) Relative contribution (%) of  
 636 carbohydrate (CHO), fat (FAT) and protein oxidations to sleeping energy expenditure.  
 637 (E) Sleeping absolute nutrient oxidation (g) of carbohydrate (CHO), fat (FAT) and protein  
 638 oxidations for sleeping energy expenditure. (F) Sleeping energy expenditure. %EE,  
 639 percent contribution to energy expenditure; g, grams; MJ, mega Joules. Data are  
 640 presented as mean ± SEM. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

641

#### 642 **Figure 5: Twenty-four hour and active period iAUCs for plasma metabolites**

643 iAUCs for plasma glucose, TG, insulin and FFAs measured during the inpatient study  
 644 day. (A) 24-h and active period plasma glucose iAUCs. (B) 24-h and active period  
 645 plasma TG iAUCs. (C) 24-h and active period plasma insulin iAUCs. (D) 24-h and active  
 646 period plasma FFA iAUCs. Active period iAUC measured from the first to the last  
 647 microbout of activity (1000h – 1800h). TG, triglyceride; FFA, free fatty acids; SED,  
 648 sedentary exposure; ONE, one bout (45-min moderate intensity at 1000h) walking

649 exposure; MICRO, microbouts exposure (5-min walking per hour for 9 consecutive hours  
 650 at moderate intensity from 1000h-1800h). Data are presented as mean  $\pm$  SEM. \*  $p < 0.05$ ,  
 651 \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

652

653 **Supplemental Figures**654 **Supplemental Figure 1: <https://doi.org/10.5281/zenodo.2399298>**655 **Supplemental Figure 2: <https://doi.org/10.5281/zenodo.2399364>**

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658

659 **Tables**660 **Table 1**

	<b>Males</b>	<b>Females</b>	<b>Total</b>
<b>n</b>	10	10	20
<b>Age (years)</b>	31.5 $\pm$ 7.4	33.2 $\pm$ 5.1	32.4 $\pm$ 6.3
<b>BMI (kg/m<sup>2</sup>)</b>	28.9 $\pm$ 3.0	31.9 $\pm$ 2.0	30.6 $\pm$ 2.9
<b>Body Mass (kg)</b>	88.0 $\pm$ 13.6 **	83.5 $\pm$ 7.2	85.7 $\pm$ 10.8
<b>FFM (kg)</b>	63.2 $\pm$ 10.0 ***	48.3 $\pm$ 3.6	55.8 $\pm$ 10.5
<b>FM (kg)</b>	24.6 $\pm$ 4.4 ***	35.2 $\pm$ 4.4	29.9 $\pm$ 6.9
<b>Fat mass (%)</b>	28.1 $\pm$ 2.5 ***	42.0 $\pm$ 2.3	35.0 $\pm$ 7.5
<b>Fasting Glucose (mg/dL)</b>	88.0 $\pm$ 4.8	87.6 $\pm$ 4.3	87.8 $\pm$ 4.4
<b>Fasting Triglyceride (mg/dL)</b>	154.6 $\pm$ 107.7 *	83.5 $\pm$ 14.7	117.2 $\pm$ 81.2
<b>Fasting Insulin (<math>\mu</math>U/mL)</b>	5.4 $\pm$ 2.7	5.15 $\pm$ 1.3	5.2 $\pm$ 2.1
<b>HOMA-IR (SED day fasting)</b>	1.5 $\pm$ 0.8	1.4 $\pm$ 0.4	1.4 $\pm$ 0.6
<b>IPAQ self-reported sitting time (hrs/day)</b>	9.6 $\pm$ 2.9	11.3 $\pm$ 3.5	10.5 $\pm$ 3.3

661

662 Data are presented as the mean  $\pm$  SD663 \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$  vs. Female

664 n, number of subjects; BMI, body mass index; FFM, fat-free mass; FM, fat mass; HOMA-IR,  
 665 homeostatic model assessment of insulin resistance; IPAQ, international physical activity  
 666 questionnaire

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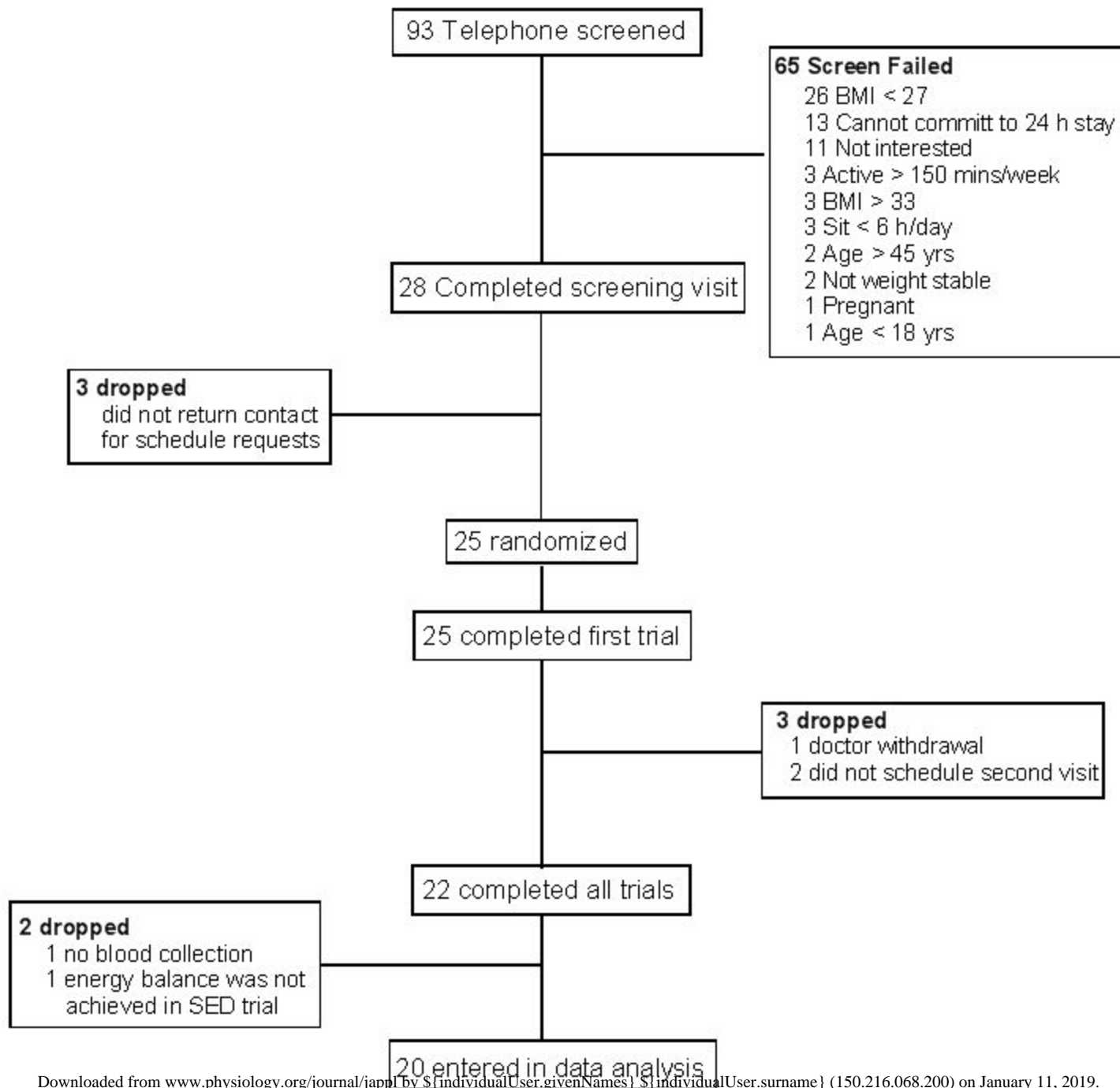
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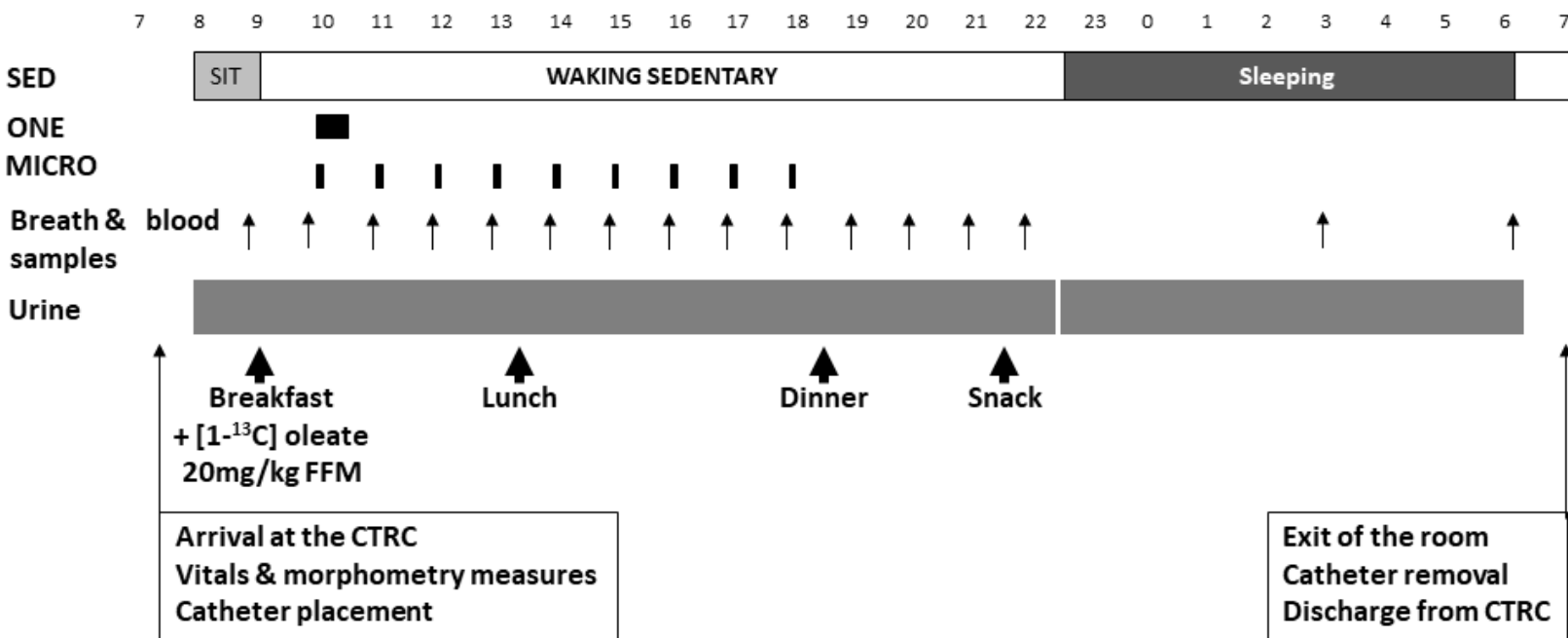
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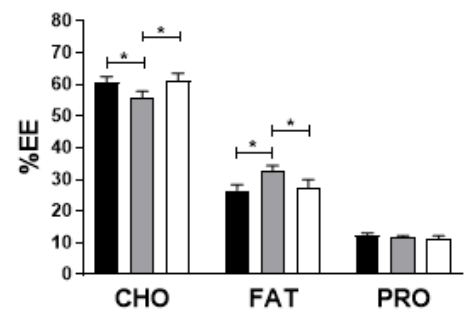




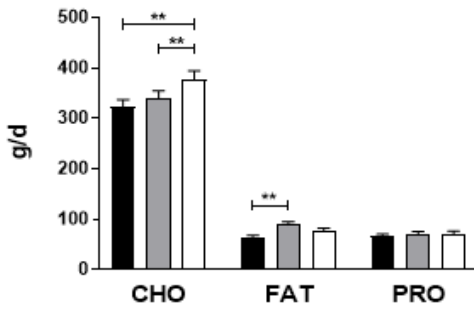
**A. 24h Energy Intake, Expenditure and Balance**



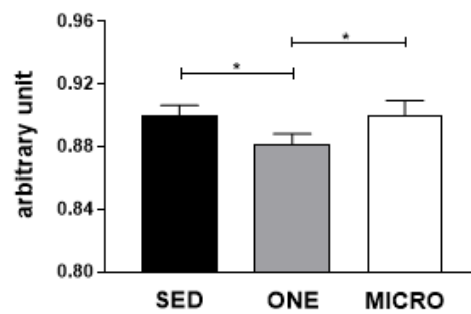
**B. Contribution of substrate oxidation to 24h total energy expenditure**



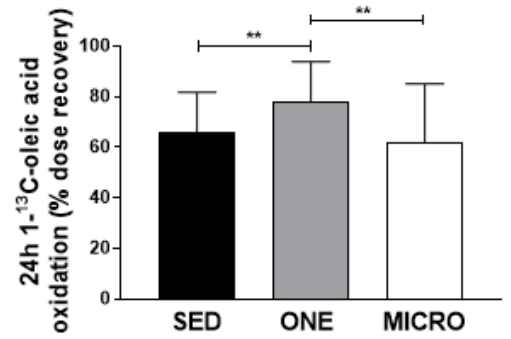
**C. 24h Nutrient Oxidation**



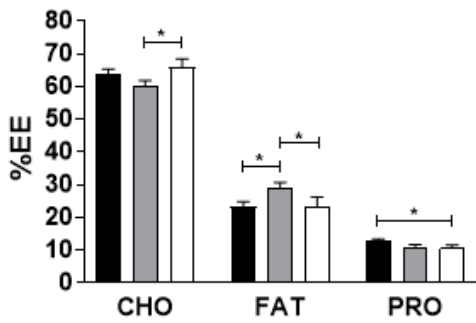
**D. 24h NPRQ**



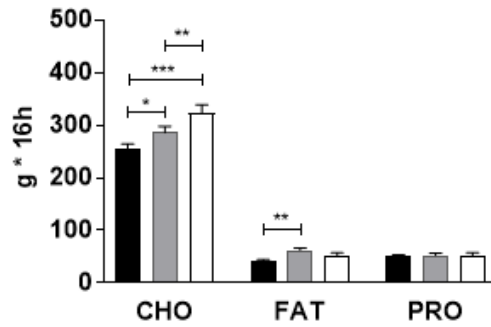
**E. 24h Dietary Fat Oxidation**



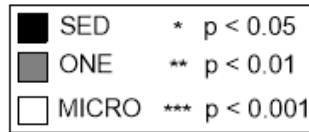
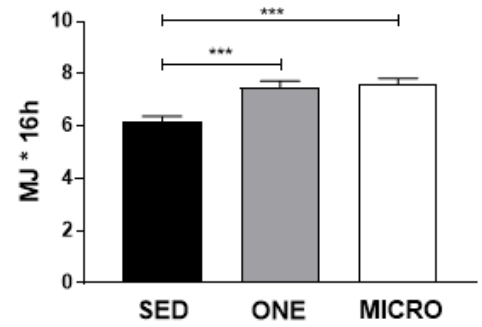
**A. Contribution of substrate oxidation to waking total energy expenditure**



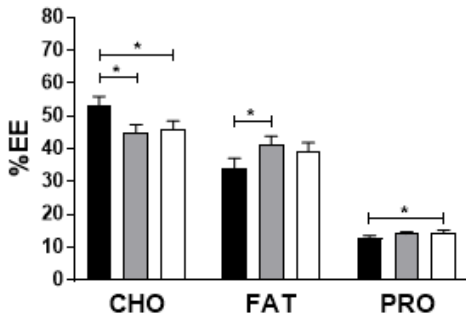
**B. Waking nutrient oxidation**



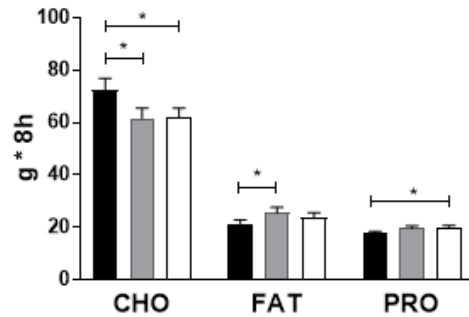
**C. Waking energy expenditure**



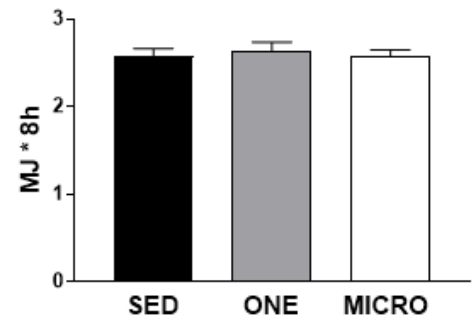
**D. Contribution of substrate oxidation to sleeping total energy expenditure**



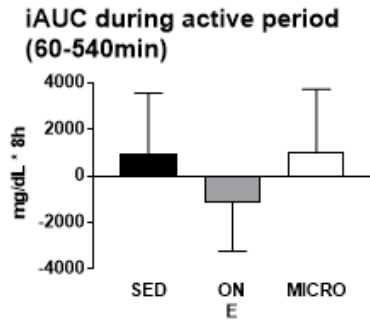
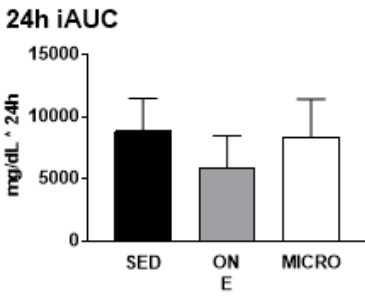
**E. Sleeping nutrient oxidation**



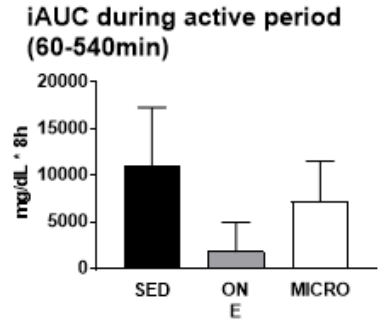
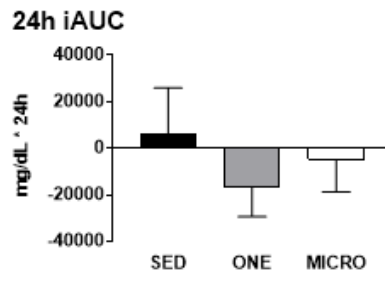
**F. Sleeping energy expenditure**



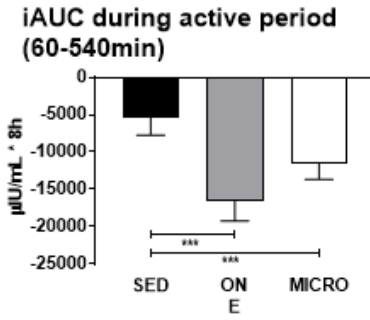
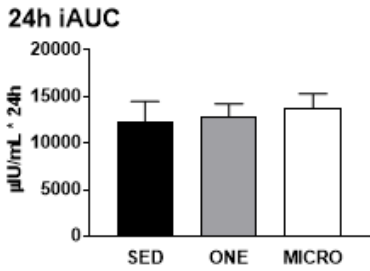
## A. Glucose



## B. TG



## C. Insulin



## D. FFA

