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ABSTRACT

Background: According to cross-sectional and acute experimental evidence, reducing sitting time should improve cardio-metabolic health risk biomarkers. Furthermore, the improvements obtained may depend on whether sitting is replaced with standing or ambulatory activities. Based on data from the *Stand Up Victoria* multi-component workplace intervention, we examined this issue using compositional data analysis — a method that can examine and compare all activity changes simultaneously.

Methods: Participants receiving the intervention (n=136 ≥0.6 full-time equivalent desk-based workers, 65% women, mean±SD age=44.6 ±9.1 years from seven worksites) were asked to improve whole-of-day activity by standing up, sitting less and moving more. Their changes in the composition of daily waking hours (activPAL-assessed sitting, standing, stepping) were quantified, then tested for associations with concurrent changes in cardio-metabolic risk (CMR) scores and 14 biomarkers concerning body composition, glucose, insulin and lipid metabolism. Analyses were by mixed models, accounting for clustering (3 months, n=105–120; 12 months, n=80–97).

Results: Sitting reduction was significantly (p<0.05) associated only with lower systolic blood pressure at three months, and with CMR scores, weight, body fat, waist circumference, diastolic blood pressure, and fasting triglycerides, total/HDL cholesterol and insulin at 12 months. Significant differences between standing and stepping were only observed for systolic blood pressure and insulin; both favored stepping. However, replacing sitting with standing was significantly associated only with improvements in CMR scores, while replacing sitting with stepping was significantly associated with CMR scores and six biomarkers.

Conclusions: Improvements in several cardio-metabolic health risk biomarkers were significantly associated with sitting reductions that occurred in a workplace intervention. The greatest degree and/or widest range of cardio-metabolic benefits appeared to occur with long-term changes, and when increasing ambulatory activities.

Keywords: sedentary; compositional data analysis (CoDA); ambulation; intervention; biomarkers

TRIAL REGISTRATION: ACTRN1211000742976

1   **INTRODUCTION**

2   Increased risk of developing cardiovascular disease and diabetes (1), and elevated biomarkers of

3   risk for these chronic diseases (2), have been observed with high volumes of sitting time, and

4   especially sitting time accrued in a prolonged, continuous manner. Supporting the

5   epidemiological evidence, laboratory studies have shown acute benefits to glucose, insulin, and

6   lipid metabolism of interspersing long periods of sitting with even small amounts of activity (3-

7   5). Accordingly, interventions to reduce sitting, especially in the workplace — a key setting for

8   addressing prolonged sitting time — have been advocated as a public-health strategy (6, 7). In

9   particular, sit-stand workstations have emerged as effective tools in multi-component workplace

10 sitting interventions (8) as their usage reduces sitting time by large volumes.

11

12 By contrast with the clear evidence that such interventions can reduce sitting time, the evidence

13 concerning whether they are likely to impart non-acute benefits to cardio-metabolic health is less

14 clear, especially when sitting is primarily replaced with standing. Workplace sitting-reduction

15 interventions that primarily increase standing (e.g., through installation of sit-stand desks) have

16 shown benefits concerning lipid and glucose biomarkers, but inconsistently (9-11). Notably, thus

17 far, only the sitting-reduction interventions that have increased stepping (e.g., by use of treadmill

18 desks) have shown significant benefits to body weight or body composition (12, 13). The short-

19 term evaluations and insufficient sample sizes of most studies may explain the mixed findings.

20 However, it is also possible that the potential cardio-metabolic benefits of reducing sitting in an

21 intervention are inherently variable because participants can make a plethora of different

22 behavior changes when reducing sitting. Potentially relevant considerations include the volume

23   of sitting reduction, the activities replacing sitting (e.g., standing versus ambulatory activities),

24   and any compensatory activity changes that may or may not occur (14).

25

26   Recently, compositional data analysis (CoDA) has been used to simultaneously examine all

27   activities occupying a 24-hour day and test them in relation to cardio-metabolic biomarkers (15).

28   The study findings revealed that some biomarkers, notably those pertaining to glucose

29   metabolism, improve significantly when increasing light activity at the expense of sedentary time

30   (15). Importantly, CoDA is a valid method for examining data that sum to a fixed total, such as

31   24 hours (15) and it can be applied to evaluate all of the changes in activity that occur during an

32   intervention simultaneously, and test these in relation to changes in cardio-metabolic biomarkers.

33   To our knowledge, CoDA has not been applied in this context, nor to the examination of

34   standing as a separate component from ambulatory light activities. Using CoDA, we therefore

35   examined the associations of short- and long-term (3- and 12-month) changes in daily time use

36   with concurrent changes in cardio-metabolic biomarkers, within participants receiving the *Stand*

37   *Up Victoria* intervention.

38

39   **METHODS**

40   The *Stand Up Victoria* cluster-randomized trial was registered with the Australian New Zealand

41   Clinical Trials register (ACTRN12611000742976). The Alfred Health Human Ethics Committee

42   (Melbourne, Australia) granted ethical approval. Participants provided written consent. The

43   study was conducted in accordance with the CONSORT guidelines for cluster-randomized trials

44   (http://www.consort-statement.org/). Details are published elsewhere concerning the study

45   protocol (16), the measures used, development and pilot testing (10, 17), evaluation of the main

46   activity outcomes (18) and the secondary cardio-metabolic biomarker outcomes(19).

47

48   **Setting and participants**

49   Teams from study worksites that were at least one kilometre apart were identified and recruited

50   from a single organization, then were randomized to the intervention (n=7 sites, n=136 workers)

51   or control (n=7 sites, n=95 workers) condition. Eligibility criteria for individual participants in

52   the selected teams were: aged 18–65 years; not pregnant; ambulatory; speaks English; capable of

53   standing or sitting for ≥10 minutes continuously; and, working ≥0.6 full time equivalent with

54   designated access to a telephone, internet, and desk. Participants and study staff were not blinded

55   to group allocation. The present study evaluates only the intervention participants.

56

57   **Intervention**

58   The *Stand Up Victoria* intervention consisted of organizational support (senior management

59   support, a team champion who sent emails containing the intervention messages); environmental

60   modification (sit-stand workstations); and, individual health coaching (including goal setting and

61   tracking). The intervention was tapered over 12 months with intensive components (e.g., health

62   coaching, team champion intervention) ceasing after 3 months. It primarily targeted reductions in

63   workplace sitting time, especially sitting accrued for ≥30 minutes at a time continuously. The

64   main message was to "Stand Up, Sit Less, Move More". The intervention encouraged

65   participants to replace part of their sitting across the entire day with standing and stepping, by

66   standing at their workstation for at least an hour a day, and by using a variety of self-selected

67   strategies, which might target standing, stepping or both. Evaluation of the study's activity

68 outcomes previously revealed that, net of control, the intervention on average produced

69 moderately large effects on reduced sitting and increased standing ($\approx$ 80 min/day at 3-months

70 and $\approx$ 40 min/day at 12-months) with no significant effect on stepping (-6 min/day at 12-months)

71 (18). These effects were established across the entire waking day (i.e., at work and outside of

72 work, considering the entire week rather than just workdays). Cardio-metabolic biomarker

73 outcomes, net of control, showed a significant improvement in overall cardio-metabolic risk and

74 fasting glucose at 12 months, and non-significant (but typically favorable) effects on the other

75 biomarkers (19).

76

77 **Data collection and measures**

78 Measurements were at baseline, three months into the intervention (upon completion of the

79 individual-level health coaching and champion emails) and at 12 months, and included an onsite

80 assessment of biomarkers and an activity monitoring assessment. Further participant

81 characteristics were assessed using Oonline questionnaires (LimeService:

82 www.limeservice.com) assessed most other participant characteristics.

83

84 *Cardio-metabolic biomarker outcomes*

85 The collection of these biomarkers is described in detail elsewhere(19), along with their changes

86 over the course of the intervention. The cardio-metabolic biomarkers examined were: systolic

87 blood pressure, diastolic blood pressure, weight, fat mass (kg, % of bodyweight), waist

88 circumference, fasting triglycerides, high-density lipoprotein (HDL)- and low density lipoprotein

89 (LDL)- cholesterol, total/HDL cholesterol ratio, glucose, insulin, insulin sensitivity (%S) and

90 steady state beta cell function (%B) as calculated using the homeostatic model assessment

91 (HOMA2) online calculator (https://www.dtu.ox.ac.uk/homacalculator/) version 2.2.3 and an

92 overall cardio-metabolic risk (CMR) score. CMR scores (20) were calculated by first log10

93 transforming and normalizing (mean/SD) the relevant biomarkers, then by taking a weighted

94 average of their values:1/5·waist circumference + 1/5·triglycerides -+ 1/5·HDL-cholesterol +

95 1/5·fasting glucose + 1/5· mean of systolic and diastolic blood pressure. Changes in the

96 biomarkers were calculated as follow up score minus baseline score.

97

98 *Activity measures*

99 Activity was measured by the highly accurate (21) and responsive (22) activPAL3[TM] activity

100 monitor (PAL Technologies Limited, Glasgow, UK; minimum version 6.3.0). The waterproofed

101 monitor was secured onto the right anterior thigh with a hypoallergenic patch at the onsite

102 assessment. Each participant was asked to wear the monitor continuously (24 h/day) for the

103 following seven days, and to record the following times daily in a diary: starting and finishing

104 work; waking up; going to sleep ("lights out"); removing and re-attaching the monitor. Monitor

105 data were processed as reported in the primary outcomes paper (18). Though daily activities can

106 be classified in many ways, we subdivided time use by activity classifications consistent with the

107 intervention and measurement tool: sitting, standing, and stepping (during waking hours, while

108 wearing the monitor) and "other" time (non-wear time and time in bed).

109

110 **Statistical analyses**

111 Analyses were performed in STATA version 13 (STATACorp, College Station, Texas, US) and

112 R version 3.3.0, using the packages "compositions" ("acomp" framework) "nlme" and

113 "lsmeans". Statistical significance was set at p<0.05, two-tailed. Missing data were excluded.

8

115 *Quantifying activity and activity change compositionally*

116

117 We used compositional methods, which have been outlined as applied to cross-sectional physical

118 activity and sedentary behavior data by Chastin et al (15). The total 24-hour day was divided

119 across four activities (stepping, standing, sitting, "other"). Sleep, other time in bed and non-wear

120 time comprised "other" time (i.e., 24 hours minus monitored waking hours). CoDA's property of

121 "sub-compositional coherence" means that the exclusion of irrelevant activities does not

122 adversely affect results (23). The analysis includes only the sub-composition of activities that

123 comprise waking hours (stepping, standing, sitting); i.e., the composition of waking hours.

124 "Other" time was excluded in order to reduce the number of dimensions and provide efficient

125 estimates. This decision seemed to be reasonable since the "other" time was not targeted by the

126 intervention and did not change much over time at the group level or for individuals. At baseline,

127 three months, and 12 months, compositions were calculated using the R function "acomp". No

128 method was required to address the problem of zero time use, as all participants spent some time

129 in every time-use category at each assessment. Compositional changes $[\text{Step}_\Delta, \text{Stand}_\Delta, \text{Sit}_\Delta]$ were

130 then measured by Aitchison's perturbation method (23, 24). The ratios of each component in the

131 composition or sub-composition, such as $\left[\frac{\text{Step}_{12M}}{\text{Step}_{BL}}, \frac{\text{Stand}_{12M}}{\text{Stand}_{BL}}, \frac{\text{Sit}_{12M}}{\text{Sit}_{BL}}\right]$ for 12-month changes from

132 baseline, were calculated and were then divided by the sum total of these ratios. An equal

133 composition of these three activities at baseline and follow up would result in a compositional

134 change of [1/3,1/3,1/3]. Compositional changes were plotted as ternary diagrams (Figure 1), with

135 key some guide values marked: no change; average sitting reduces by 1 h/16h day replaced with

9

136   either all stepping, all standing, or half of each; and, the average sitting reduces by 2 h/16h day

137   replaced entirely with standing.

138

139   *Quantifying associations of activity changes with biomarker changes*

140   The associations of activity changes with biomarker changes were examined as mixed models

141   ("lme" function), with a random intercept for cluster, and fixed effects for changes in the activity

142   composition [$Step_\Delta, Stand_\Delta, Sit_\Delta$]. Short- and long-term changes were examined separately.

143   Briefly, we used an isometric log-ratio transformation (i.e., "ilr" function) to measure the

144   compositional change as two parameters (z1 and z2). These parameters are orthogonal and can

145   therefore be safely included together as independent variables in the mixed models (15, 23). The

146   isometric log-ratio transformation can be performed from a number of perspectives. The primary

147   perspective we used allows for the effect of a decrease in the parameter z1 on biomarkers to

148   indicate the effects of making sitting a smaller proportion of the waking day. These effects are

149   estimated while controlling for shifts in the remaining non-sitting time between standing and

150   stepping, the effect of which is measured as the parameter z2. The transformation was as

151   follows:

$$z1_{Sit\ vs\ stand\ \&\ step} = \sqrt{\frac{2}{3}} \ln\left(\frac{Sit_\Delta}{\sqrt{Stand_\Delta \times Step_\Delta}}\right)\ [Eq.\ 1]$$

$$z2_{Stand\ vs\ step} = \sqrt{\frac{1}{2}} \ln\left(\frac{Stand_\Delta}{Step_\Delta}\right)\ [Eq.\ 2]$$

152

153   In addition, we presented selected estimates for the z2 parameter calculated from different

154   perspectives that indicate the effects of shifts in non-stepping time between sitting and standing

10

155 (more standing less sitting), and the shifts in non-standing time between sitting and stepping

156 (more stepping less sitting). Although the direction and significance of the parameters can be

157 used to understand the findings, the clinical relevance of the coefficients is not straightforward.

158 Estimates were presented partially standardized, with biomarker changes all expressed as a

159 number of baseline standard deviations, so that the relative effects on the different biomarkers

160 can be compared. To better understand the results, tertiles of predicted improvement (most

161 improved/least worsened to least improved/most worsened) were plotted across changes in the

162 composition that participants made (as presented in Figure 2). Also, to better indicate effect

163 sizes, the predicted mean improvement was calculated across a range of standing and stepping

164 changes in the composition that culminate in reducing sitting to recommended levels of 50%

165 (25). Consistent with the use of CoDA methods, our analyses did not adjust for total waking

166 hours (or wear time). Instead, a sensitivity analysis using the composition of all ~~waking~~ 24 hours

167 was conducted to verify that excluding changes in "other" time was reasonable (and by

168 implication that ignoring the total amount of waking hours was reasonable).

169

170 **RESULTS**

171 Baseline characteristics of intervention participants are shown in Supplemental Table 1. Relevant

172 data on short- and long-term changes were available from 105–120 participants (77–88%) and

173 80–97 (59–71%), respectively. Generally, those who provided data were similar to those who

174 dropped out, with the exception being that more women than men dropped out during the

175 intervention, which shifted anthropometric biomarkers in directions expected for a group

176 containing more males.

177

178   **Activity composition**

179   Activity outcomes have been reported previously (18). Considering activity as a composition of

180   daily time use, the intervention group's daily activity was very high in sitting, low in standing

181   and very low in stepping both at baseline [65.4%, 24.1%, 10.5%] and to a lesser extent at 12

182   months [60.4%, 29.5%, 10.1%] (Supplemental Figure 1), corresponding to a mean 12-month

183   change of [0.30, 0.39, 0.31]. Figure 1 is a ternary plot of the 12-month changes, with each corner

184   indicating a complete change towards that activity (from 0% to 100% of waking hours) and with

185   the centre indicating no change. Individual changes made by participants were highly variable.

186   The mean change in the composition was statistically significant (with the 95% confidence

187   region excluding no change) and was very close to the point indicating a drop in mean baseline

188   sitting of 1 hour/16 hours awake, when sitting is replaced exclusively with standing.

189

190   **Changes in the activity composition with changes in biomarkers**

191   Three-month sitting reductions were significantly associated only with changes in systolic blood

192   pressure (p=0.039), with the direction of associations indicating sitting reduction to be beneficial

193   (Tables 1–2). Long-term (12-month) sitting reductions were significantly associated with

194   improvements in CMR, triglycerides, total/HDL cholesterol ratio, diastolic blood pressure,

195   weight and body fat, waist circumference and insulin, and had a borderline significant (p=0.063)

196   association with improved insulin sensitivity (Tables 3–4).

197

198   In terms of the forms of sitting reductions associated with biomarker changes, overall CMR

199   scores improved significantly with sitting-standing substitutions (p=0.031) and with sitting-

200   stepping substitutions (p=0.028) without a statistically significant difference between standing

201 and stepping (p=0.240). By contrast, for fasting insulin and insulin sensitivity (HOMA-S),

202 stepping was significantly better than standing as a sitting replacement (p=0.006 and 0.032). No

203 significant effect on these biomarkers was seen of replacing sitting with standing (p=0.889 and

204 0.943) whereas replacing sitting with stepping was associated with significant benefit (p=0.006

205 and 0.029). Figure 2 displays the results graphically. CMR improvements were seen when

206 reducing the contribution of sitting to the overall waking day. At some levels of sitting change,

207 there was patterning whereby more CMR improvement was seen when the remaining time use

208 was shifted more towards stepping rather than standing (i.e., from left to right across the graph),

209 but this was not evident with the largest sitting reductions. All of the participants in the most

210 improved tertile of CMR had made sitting reductions. Figure 2b shows that the degree of

211 improvement that occurred at all levels of sitting change appeared dependent on how much of

212 the remaining (non-sitting) time use shifted towards stepping (most beneficial) versus standing.

213

214 For the other outcomes that had significantly improved with long-term sitting reduction (i.e.,

215 triglycerides, total/HDL cholesterol, diastolic blood pressure, weight, body fat (kg and %) and

216 waist circumference), it was not clear whether or not these improvements depended on sitting

217 being replaced with ambulatory activities. Suggestive that either standing or ambulation can

218 improve these outcomes, there was no significant difference whether sitting was replaced with

219 standing or stepping. However, the effects on these outcomes observed for replacing sitting with

220 standing did not reach statistical significance, while replacing sitting with stepping was

221 significantly associated with improved total/HDL cholesterol ratio (p=0.045), diastolic blood

222 pressure (p=0.027), and fat mass (kg and %, p=0.034 and 0.022). In addition to statistical

223 significance, the direction of the results, and the patterning of biomarker changes across activity

224 as plotted in Supplemental Figures 2–5, are informative. These were consistent with these

225 biomarkers improving somewhat by substituting sitting with standing and improving slightly

226 more by substituting sitting with stepping. Supplemental Table 2 shows the estimated mean 12-

227 month changes in cardio-metabolic outcomes when reducing baseline mean sitting (65.4%) to

228 desirable levels (50%) via various replacement strategies. Moderate to strong improvements

229 (0.5–0.8 SD) were seen for many outcomes but only with substantial increases in ambulation. In

230 order to see a small improvement in mean biomarkers (0.2 SD), only a small percentage of the

231 sitting reduction needed be achieved by increasing ambulatory activities for lipids and blood

232 pressure (20% or less), for insulin (21%) and for some of the adiposity indicators (waist

233 circumference and body fat percentage). The requirement for ambulation was higher for the other

234 outcomes, ranging from 30% to 68% of the sitting replacement.

235

236 Changes in the amount of "other" time relative to sitting standing and stepping were only

237 significantly associated with systolic blood pressure at 12 months, and triglycerides, HDL

238 cholesterol and HOMA-S at three months (Supplemental Table 3). For all these outcomes, the

239 conclusions concerning reducing sitting relative to standing and stepping, and shifts between

240 standing and stepping were no different whether examining all hours or only waking hours.

241

242 **DISCUSSION**

243 Previously, we showed the *Stand Up Victoria* workplace sitting-reduction intervention

244 predominantly reduced sitting by increasing standing (18), and was effective in the long term for

245 improving fasting glucose and an overall CMR score, net of control (19). The present study

246 extends from these findings to understand how the various activity changes that intervention

247 participants made were associated with concurrent biomarker changes, using a novel application

248 of compositional analysis. We found that sitting reduction was associated with significant

249 improvements in the biomarkers of cardiovascular and metabolic health across all of the areas

250 examined (glucose and insulin metabolism, lipid metabolism, blood pressure, body composition).

251 To varying degrees, the various benefits appeared to depend on the type of sitting reduction (i.e.,

252 whether sitting was replaced with standing or with stepping).

253

254 Both the previously reported outcomes of the workplace sitting intervention (19) and the present

255 findings may indicate the need for long-term intervention to improve biomarkers via sitting

256 reduction. We saw many significant associations of activity changes with biomarker changes

257 over a 12-month timeframe, and very few over a three-month period. While this could be a

258 chance finding, it could also reflect a physiological requirement for long-term behavior change in

259 order to improve these biomarkers. Either way, it appears prudent to investigate long-term

260 effects rather than infer them from short-term interventions, where benefits may be missed.

261

262 Our CMR findings showed that cardio-metabolic biomarker improvement can occur when

263 replacing sitting time with non-ambulatory activities. However, findings for the individual

264 biomarkers suggested the degree and/or range of cardio-metabolic biomarker improvements may

265 be greater when replacing sitting with ambulation than with standing. Fasting insulin and

266 HOMA-S improved significantly more by replacing sitting with stepping than with standing.

267 Some of the findings showed seemingly conflicting results whereby standing was neither

268 significantly beneficial, nor significantly inferior to stepping. This apparent conflict is potentially

269 explained by the study's sample size providing insufficient precision to distinguish standing from

270 either sitting or stepping, with standing having an impact that was more beneficial than sitting

271 but less beneficial than stepping. Larger RCTs or meta-analyses may yield further insights as to

272 potential benefits of replacing sitting with standing within field-based sitting-reduction

273 interventions. Cross-sectionally, in isotemporal analyses, reallocating time use away from sitting

274 towards additional standing has shown significant beneficial associations with triglycerides,

275 HDL cholesterol, total/HDL cholesterol ratio and fasting glucose though not with weight or waist

276 circumference (26). In addition to the outcomes that appear important from the existing

277 literature, our findings suggest that key biomarkers that might be important to collect when

278 evaluating interventions similar to Stand Up Victoria are: those comprising CMR scores; those

279 showing the greatest response to substituting sitting specifically with standing (i.e., waist

280 circumference, fasting glucose, triglycerides and diastolic blood pressure, whose coefficients for

281 sitting versus standing were largest at ≈0.3 to 0.6 SD); and, the biomarkers that showed the most

282 predicted improvement when reducing sitting to desirable levels (25) without large changes to

283 stepping (i.e., lipids, blood pressure, insulin, waist circumference and body fat).

284

285 Consistent with our findings, the underlying biological mechanisms would also tend to suggest

286 that both standing and stepping should be beneficial, but with the greatest benefit for stepping.

287 The added benefit for glycemic control associated with transitions to stepping compared with

288 transitions to standing may reflect greater muscle and/or metabolic activity in general (27, 28), or

289 the comparatively higher energy demand associated with activation of fast-twitch glycolytic

290 fibres (29, 30). This contrasts with the lesser glycemic benefit of transitions to standing which

291 involve a comparatively lower energy requirement and engagement of oxidative fibres, favoring

292 fat metabolism (29, 30). Broadly, the findings aligned with recent acute experimental studies in

293   overweight adults that have sometimes indicated greater improvements in postprandial glucose

294   and insulin responses (4, 31, 32) by interrupting sitting with intermittent ambulation compared

295   with standing breaks. Similarly, cross-sectional isotemporal analyses have also showed stronger

296   effects on a range of cardio-metabolic biomarkers when sitting time is reallocated to additional

297   stepping rather than standing (26). Notably "stepping" is an amalgamation of various

298   ambulatory activities, and the stepping findings are therefore reflective of the "~~average~~typical

299   mix" of the various ambulatory activities that were performed by the participants of the *Stand Up*

300   *Victoria* intervention, which had a predominant focus on light-intensity activity. Within the

301   stepping category, effects of running are likely greater than walking slowly, for example.

302   Similarly, effects of sitting are reflective of the "typical mix" of sitting for this population; it is

303   possible that certain types of sitting (e.g., sitting in long bouts, sitting after lunch) are more

304   deleterious than others.

305

306   Strengths of the study include the evaluation of the short- and long-term effects on objectively

307   assessed biomarkers alongside accurately and objectively measured behaviors, with good study

308   retention especially in the short term. A novel element was that this intervention that targeted

309   whole-of-day behavior changes was examined with analytic methods suited to such data. A key

310   limitation was that the study was not powered a priori for this secondary analysis and showed

311   evidence of limited power and precision (e.g., the wide margins of error around predicted mean

312   values). We did not adjust for co-occurring changes in the intervention (e.g., in dietary intake) as

313   these are potentially attributable to the intervention; however, the changes may have been

314   coincidental, and therefore our results may be subject to confounding. It appeared unlikely that

315   the findings were strongly affected by unexamined activities or variation in total waking hours.

316   However, this is impossible to verify without accurate and detailed measures (e.g., high-quality

317   sleep, time in bed unable to sleep etc.) or knowledge of activity during unobserved time. Another

318   limitation was that the study took neither measures of post-prandial metabolism nor continuous

319   biomarker measurements in the behavior setting (e.g., by continuous glucose monitoring or 24-

320   hour ambulatory blood pressure monitoring). A focus on the postprandial state may be especially

321   important for interventions targeting not only whole-of-day changes but also workplace changes,

322   since the postprandial periods after lunch and other meals are often spent at work.

323   Generalizability is limited, as participants were recruited non-randomly from a single

324   organization and there was some evidence of a tendency to disproportionately lose women to

325   follow-up. Also, our sample was a general population of workers; effects may also differ within

326   clinical populations.

327

328   In conclusion, our study provides further insights into the heterogeneous findings of studies

329   examining the cardio-metabolic benefits of reducing sitting time. Firstly, long-term intervention

330   seems necessary to identify relevant changes. Secondly, if using primarily sitting-standing

331   substitutions, these seemingly need to be large volume, and achieved without adversely

332   impacting stepping. Finally, sitting should be replaced with ambulatory activity if benefits to

333   fasting insulin levels are desired and for potentially greater benefits to other biomarkers as well.

334

REFERENCES

346

347 1.   Wilmot EG, Edwardson CL, Achana FA et al. Sedentary time in adults and the

348 association with diabetes, cardiovascular disease and death: systematic review and meta-analysis.

349 *Diabetologia*. 2012;55(11):2895-905.

350 2.   Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and

351 cardio-metabolic biomarkers in US adults: NHANES 2003-06. *European heart journal*.

352 2011;32(5):590-7.

353 3.   Pulsford RM, Blackwell J, Hillsdon M, Kos K. Intermittent walking, but not standing,

354 improves postprandial insulin and glucose relative to sustained sitting: A randomised cross-over

355 study in inactive middle-aged men. *Journal of Science and Medicine in Sport*. 2017;20(3):278-

356 83.

357 4.   Dempsey PC, Owen N, Yates TE, Kingwell BA, Dunstan DW. Sitting Less and Moving

358 More: Improved Glycaemic Control for Type 2 Diabetes Prevention and Management. *Current*

359 *Diabetes Reports*. 2016;16(11):114.

360 5.   Grace MS, Dempsey PC, Sethi P et al. Breaking Up Prolonged Sitting Alters the

361 Postprandial Plasma Lipidomic Profile of Adults With Type 2 Diabetes. *The Journal of clinical*

362 *endocrinology and metabolism*. 2017;102(6):1991-9.

363 6.   Healy GN, Lawler SP, Thorp A et al. *Reducing prolonged sitting in the workplace (An*

364 *evidence review: full report)*. Melbourne, Australia: Victorian Health Promotion

365 Foundation2012. Available from: Victorian Health Promotion Foundation.

366 7.   Plotnikoff R, Healy G, Morgan P, Gilson N, Kennedy S. Action area 2 - Workplaces:

367 Promote physical activity before, during and after work In. *Blueprint for an active Australia:*

368 *Government and community actions to increase population levels of physical activity and reduce*

20

369 *sedentary behaviour in Australia, 2014–2017* Melbourne, VIC Australia National Heart

370 Foundation of Australia 2014, pp. 20-5.

371 8.   Neuhaus M, Eakin EG, Straker L et al. Reducing occupational sedentary time: a

372 systematic review and meta-analysis of evidence on activity-permissive workstations. *Obesity*

373 *reviews : an official journal of the International Association for the Study of Obesity*.

374 2014;15(10):822-38.

375 9.   Alkhajah TA, Reeves MM, Eakin EG, Winkler EA, Owen N, Healy GN. Sit-stand

376 workstations: a pilot intervention to reduce office sitting time. *Am J Prev Med*. 2012;43(3):298-

377 303.

378 10.   Healy GN, Eakin EG, Lamontagne AD et al. Reducing sitting time in office workers:

379 short-term efficacy of a multicomponent intervention. *Prev Med*. 2013;57(1):43-8.

380 11.   Danquah IH, Kloster S, Holtermann A et al. Take a Stand!-a multi-component

381 intervention aimed at reducing sitting time among office workers-a cluster randomized trial.

382 *International journal of epidemiology*. 2016.

383 12.   Koepp GA, Manohar CU, McCrady-Spitzer SK et al. Treadmill desks: A 1-year

384 prospective trial. *Obesity*. 2013;21(4):705-11.

385 13.   John D, Thompson DL, Raynor H, Bielak K, Rider B, Bassett DR. Treadmill

386 workstations: a worksite physical activity intervention in overweight and obese office workers. *J*

387 *Phys Act Health*. 2011;8(8):1034-43.

388 14.   Gomersall SR, Maher C, English C et al. Testing the activitystat hypothesis: a

389 randomised controlled trial. *BMC Public Health*. 2016;16(1):900.

390 15.   Chastin SFM, Palarea-Albaladejo J, Dontje ML, Skelton DA. Combined Effects of Time

391 Spent in Physical Activity, Sedentary Behaviors and Sleep on Obesity and Cardio-Metabolic

392   Health Markers: A Novel Compositional Data Analysis Approach. *PLoS One*.

393   2015;10(10):e0139984.

394   16.   Dunstan DW, Wiesner G, Eakin EG et al. Reducing office workers' sitting time: rationale

395   and study design for the Stand Up Victoria cluster randomized trial. *BMC Public Health*.

396   2013;13(1):1057.

397   17.   Neuhaus M, Healy GN, Fjeldsoe BS et al. Iterative development of Stand Up Australia: a

398   multi-component intervention to reduce workplace sitting. *The international journal of*

399   *behavioral nutrition and physical activity*. 2014;11:21.

400   18.   Healy GN, Eakin EG, Owen N et al. A Cluster RCT to Reduce Office Workers' Sitting

401   Time: Impact on Activity Outcomes. *Med Sci Sports Exerc*. 2016.

402   19.   Healy GN, Winkler EA, Eakin EG et al. A Cluster RCT to Reduce Workers' Sitting

403   Time: Impact on Cardiometabolic Biomarkers. *Med Sci Sports Exerc*. 2017.

404   20.   Wijndaele K, Healy GN, Dunstan DW et al. Increased cardiometabolic risk is associated

405   with increased TV viewing time. *Medicine and Science in Sports and Exercise*. 2010;42(8):1511-

406   8.

407   21.   Grant PM, Ryan CG, Tigbe WW, Granat MH. The validation of a novel activity monitor

408   in the measurement of posture and motion during everyday activities. *British journal of sports*

409   *medicine*. 2006;40(12):992-7.

410   22.   Chastin SFM, Winkler EAH, Eakin EG et al. Sensitivity to Change of Objectively-

411   Derived Measures of Sedentary Behavior. *Measurement in Physical Education and Exercise*

412   *Science*. 2015;19(3):138-47.

413   23.   van den Boogaart KG, Tolosana-Delgado R. *Analyzing Compositional Data with R*.

414   Berlin: Springer Berlin Heidelberg; 2013.

415   24.   Aitchison J, Ng KW. The role of perturbation in compositional data analysis. *Statistical*

416   *Modelling*. 2005;5(2):173-85.

417   25.   Buckley JP, Hedge A, Yates T et al. The sedentary office: a growing case for change

418   towards better health and productivity. Expert statement commissioned by Public Health

419   England and the Active Working Community Interest Company. *British Journal of Sports*

420   *Medicine*. 2015;49(21):1357.

421   26.   Healy GN, Winkler EA, Owen N, Anuradha S, Dunstan DW. Replacing sitting time with

422   standing or stepping: associations with cardio-metabolic risk biomarkers. *European heart*

423   *journal*. 2015;36(39):2643-9.

424   27.   Judice PB, Hamilton MT, Sardinha LB, Zderic TW, Silva AM. What is the metabolic and

425   energy cost of sitting, standing and sit/stand transitions? *European journal of applied physiology*.

426   2016;116(2):263-73.

427   28.   Tikkanen O, Haakana P, Pesola AJ et al. Muscle activity and inactivity periods during

428   normal daily life. *PLoS One*. 2013;8(1):e52228.

429   29.   Sale DG. Influence of exercise and training on motor unit activation. *Exercise and sport*

430   *sciences reviews*. 1987;15:95-151.

431   30.   Coyle EF. Physical activity as a metabolic stressor. *The American journal of clinical*

432   *nutrition*. 2000;72(2 Suppl):512s-20s.

433   31.   Bailey DP, Locke CD. Breaking up prolonged sitting with light-intensity walking

434   improves postprandial glycemia, but breaking up sitting with standing does not. *Journal of*

435   *science and medicine in sport / Sports Medicine Australia*. 2015;18(3):294-8.

436   32.   Pulsford RM, Blackwell J, Hillsdon M, Kos K. Intermittent walking, but not standing,

437   improves postprandial insulin and glucose relative to sustained sitting: A randomised cross-over

438   study in inactive middle-aged men. *Journal of science and medicine in sport / Sports Medicine*

439   *Australia*. 2016.

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List of Figures: Figure Captions

Figure 1: Change in the composition of the waking day between baseline and 12 months. The centre shows no change, and each corner is a complete change in the activity (from 0% to 100% of the waking day).

Figure 2: Predicted improvement in overall cardio-metabolic risk score (a, left) and insulin (b, right) by changes in the waking day's composition (12 months).

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