1    **Fitness moderates glycemic responses to sitting and light activity breaks**

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**ABSTRACT**

**Purpose:** Regular engagement in sedentary behaviours can lead to major public health consequences. This study aimed to experimentally determine whether cardio-respiratory fitness modifies postprandial glycemia during prolonged sitting and investigated the potentially blunting influence this may have upon the benefits of interrupting postprandial sitting time with light activity breaks.

**Methods**: Thirty–four adult volunteers (18female; 16male; mean±SD age: 40±9 years, BMI: 24.5±3 kg/m$^2$) undertook two 7·5 hour experimental conditions in a randomized order: 1) Prolonged sitting; 2) Sitting interspersed with 5 minute light walking bouts every 30minutes. Blood samples were obtained while fasting and throughout the postprandial period following ingestion of two identical meals. Incremental Area Under the Curve (iAUC) was calculated for glucose and insulin throughout each experimental condition. Maximal exercise testing quantified $VO_2$ peak as a measure of cardiorespiratory fitness (CRF) prior to experimental conditions. A repeated measures ANOVA investigated whether $VO_2$ peak modified iAUC data between conditions. This trial is registered with ClinicalTrials.gov (Reg no.NCT0493309).

**Results:** Interrupting prolonged sitting time with light walking breaks reduced blood glucose iAUC from $3.89 \pm 0.7$ to $2·51 \pm 0.7$ mmol·L$^{-1}$·h (p = 0.015) and insulin iAUC from $241 \pm 46$ to $156 \pm 24$ mU·L$^{-1}$·h (p = 0.013) after adjustment for $VO_2$ peak and sex. A significant interaction between treatment response and $VO_2$ peak was observed for glucose (p = 0.035), but not insulin (p = 0.062), whereby the treatment effect reduced with higher levels of fitness. Average blood glucose iAUC responses for a man at the 25$^{th}$ centile of CRF (42.5 mL·kg$^{-1}$·min$^{-1}$) within our cohort went from 5.80 to 2.98 mmol·L$^{-1}$·h during the prolonged sitting and light walking breaks conditions respectively, whereas average responses for a man at the

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48    75th centile of CRF (60.5 mL·kg$^{-1}$·min$^{-1}$) went from 1.99 to 1.78 mmol·L$^{-1}$·h. Similar trends

49    were observed for women.

50    **Conclusions:** Individuals with low levels of CRF gained the most metabolic benefit from

51    breaking prolonged sitting with regular bouts of light walking. Future interventions aimed at

52    alleviating the deleterious impacts of sedentary behavior may be optimized by tailoring to

53    cardio-respiratory fitness levels within the general population.

**INTRODUCTION**

Adults in developed western countries typically spend 50 - 70% of their waking hours sat down (30), making sedentary behavior the new reference of modern living. Greater time spent in sedentary behaviors (defined as sitting or reclining with low energy expenditure), has been associated with an increased likelihood of; metabolic syndrome (10), diabetes, cardiovascular disease (CVD) and all-cause mortality (4, 27). The evidence of which appears to be the strongest and most consistent for the risk of type 2 diabetes (T2DM) (4).

However, recent epidemiological evidence has suggested that physical activity levels and cardiorespiratory fitness (CRF) may moderate these associations, such that the association between sedentary time and markers or outcomes of health may be weaker in those with higher fitness levels (7, 23, 25), or those undertaking greater physical activity (11). This suggests that sedentary behavior may be a less important determinant of health in those with adequate CRF or those that are physically active. While experimental evidence largely confirms that breaking prolonged bouts of sitting with light-intensity walking can significantly reduce postprandial blood glucose and insulin in healthy non-obese individuals (2, 24), in those who are overweight and obese (9, 26), and in those with dysglycemia (15), no previous experimental trials have investigated whether these responses are modified by CRF or habitual physical activity levels.

CRF in particular is an important candidate for further investigation, as it is one of the strongest predictors of morbidity and mortality (19). CRF has been shown to moderate the deleterious impacts of other exposures such as body mass index (BMI), whereby obese individuals with moderate to high CRF levels have a lower risk of morbidity and mortality outcomes compared to normal weighted individuals with low CRF levels (12). It is therefore plausible that high levels of CRF may also protect against the deleterious impacts of prolonged sedentary behavior. Therefore, we hypothesized that CRF would modify the

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82 postprandial glucose response to breaking prolonged sitting with light walking breaks with

83 lower CRF levels being associated with greater reductions to postprandial plasma glucose.

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85 **METHODS**

86 **Study Design**

87 All participants attended the Leicester Diabetes Centre on three separate occasions between

88 September 2014 and September 2015. The first visit involved consent, familiarization and a

89 fitness assessment which was followed by two experimental condition visits that were at least

90 7 days apart. This was a randomized cross-over trial whereby each participant took part in

91 two experimental treatment conditions in a random order, thereby acting as their own

92 controls. Order randomization was conducted by a statistician using an online tool. Due to the

93 nature of the trial, participants were not blinded to their randomized order, however all

94 outcomes including blood assays were analysed blinded to the experimental condition that

95 they derived from. Prior to commencing, this study received ethical approval from the

96 University of Leicester - Health Sciences department and from the local NHS Research and

97 Development committee.

98 This trial was also registered with ClinicalTrials.gov (NCT0493309).

99 **Participants**

100 Thirty six non-obese adults (BMI $<30$kg/m$^2$) aged between 25 – 55 years who worked in a

101 predominantly seated environment were recruited from the general public via study-specific

102 information distributed in the community, around the University of Leicester campus and

103 University Hospitals of Leicester NHS Trust. Two individuals were withdrawn following

104 enrollment in the study due to a change in personal circumstances (n = 2). This left 34

105    participants who went on to complete the remaining experimental conditions. This is detailed

106    in **Figure 1.**

107    Exclusion from taking part in this study came under the following circumstances; an inability

108    to communicate in spoken English, a BMI $\geq 30$kg/m$^2$, pregnancy, steroid usage, regular

109    smoking habits, diagnosed T2DM, CVD or psychotic illness. As our study was predicated on

110    having a broad range of fitness levels, and considering that most of the variance in CRF is

111    explained by habitual physical activity levels (6), we stratified recruitment by self-reported

112    leisure time physical activity. Consequently, we enrolled 12 inactive (0 minutes of

113    MVPA/week), 12 moderately active ($\geq 75$minutes - <150minutes of MVPA/week), and 12

114    highly active ($\geq 150$minutes of MVPA/week) individuals. (See **Table S-1, Supplemental**

115    **Digital Content 1,** scope of CRF levels captured).

116    **Consent, familiarisation and fitness assessment visit**

117    On arrival, a researcher described in detail all study procedures and written informed consent

118    was obtained. Participants were then shown the designated experimental area for the study.

119    A venous blood sample was taken to assess HbA1c and confirm absence of T2DM

120    (<6·5% [<47·5mmol/mol]) (29). Body weight (Tanita TBE 611: Tanita, West Drayton, U.K),

121    waist circumference (midpoint between lower costal margin and iliac crest) and height were

122    measured to the nearest 0·1kg, 0·5cm and 0·5cm, respectively.

123    In order to assess CRF, participants undertook a maximal incremental exercise test on a

124    motor driven treadmill (Technogym Excite® 700). Following a three minute warm-up at

125    4km/h (0% incline), participants would walk or jog at a constant speed that they felt

126    comfortable with (6, 8, 10, or 12km/h) while elevations in treadmill gradient occurred at a

127    rate of 0·5% every 30 seconds. All participants received encouragement to continue this

128    exercise for as long as possible. The test was terminated upon volitional exhaustion.

129    Throughout the test, gas was sampled continuously and analysed using a Metalyser 3B gas

130    analyser (Cortex 3B, Cortex Biophysik, Leipzig, Germany). Peak oxygen consumption ($VO_2$

131    peak) was calculated using the highest ten second average throughout the testing period.

132    Before each test, the gas analyser was calibrated according to the manufacturer's

133    recommendations. As a safety precaution, a 12 lead electrocardiogram was performed by a

134    cardiac nurse for each participant at rest and during the exercise test.

135    Finally, participants were issued with two activity monitors; an ActiGraph GT3X+

136    accelerometer (Pensacola, FL) worn on the right anterior axillary line, and an activPAL3

137    physical activity monitor (PAL Technologies, Glasgow, UK) worn on the midline anterior

138    portion of the right thigh. Participants were required to wear these for 7 consecutive days,

139    allowing insight into their habitual sitting and physical activity levels.

140    **Experimental procedure**

141    Participants were asked to avoid alcohol and caffeine for the 48 hours preceding experimental

142    treatment conditions. As the influence of an acute bout of physical activity on insulin

143    sensitivity can persist for 48 hours (17), avoidance of moderate and vigorous physical activity

144    for this timeframe was also instructed. Continuation in this study was subject to participants

145    being able to confirm their compliance with these restrictions. Following an ethical

146    amendment to the protocol during this study, a subset of participants were asked to wear an

147    accelerometer in the 2 days leading up to each experimental condition in order to confirm

148    adherence to the exercise restriction (See **Table S-2, Supplemental Digital Content 2**,

149    activity data leading up to experimental conditions).

150    Participants fasted from 10pm the evening before each visit and were asked to keep a record

151    of all food eaten during the day leading up to their first experimental condition. This could

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152   then be replicated prior to their second experimental condition in an attempt to eliminate the

153   potentially confounding influence of pre-experimental food intake.

154   Participants underwent two separate 7·5 hour experimental treatment conditions:

155   1) Prolonged sitting - participants sat in a designated room (occupied with a desk, books, and

156   laptop with internet services) while minimising excessive movement. Lavatory breaks were

157   permitted using a wheelchair to and from the lavatory in order to further reduce unnecessary

158   movements that could otherwise confound the study.

159   2) Light walking breaks - participants emulated the above, but interrupted sitting time with 5

160   minute bouts of walking at a light intensity of 3km·hr on the treadmill (Technogym Excite®

161   700) every 30 minutes. These bouts were performed 12 times, totalling one hour of activity

162   and 6·5 hours of sitting throughout the course of the experimental day.

163   On arrival, participants had a cannula fitted into an accessible vein from which 10mL

164   samples were obtained throughout the day. Immediately following the two fasting samples

165   (depicted at timepoints -1 and 0 in **Figure 2**), participants were given a standardized meal

166   consisting of 8 kcal per kilogram of body weight, with a macronutrient composition reflective

167   of co-ingestion in modern western diets (14% protein, 51% carbohydrate and 35% fat). Once

168   consumed (within ≤15minutes), blood sampling commenced at 30, 60, 120, and 180 minutes

169   thereafter, enabling us to capture the postprandial period. An identical meal was then issued

170   (time point 3 in **Figure 2**) and sampling continued in a similar fashion at 30, 60, 120, 180,

171   and 210 minutes following this. Participants were supervised by study staff to ensure

172   compliance with the protocol and were asked to wear an activPAL monitor to objectively

173   confirm sitting and walking times during each experimental condition (See **Table S-3,**

174   **Supplemental Digital Content 3**, sitting and walking data during experimental conditions).

175   *Ad libitum* water consumption was also noted and made consistent between conditions.

**Biochemical analysis**

Glucose was analysed on the day of collection by the University Hospitals of Leicester pathology department, using standard enzymatic techniques with commercially available kits (Beckman, High Wycombe, U.K).

Centrifuged ($4^{o}$C) plasma samples were stored in -80·C freezers and insulin was analysed from these collectively at the end of the trial using an electrochemiluminescence assay (Meso Scale Discovery, Maryland, USA). Each sample was ran in duplicate to ensure reliability of readings. Duplicate sample values with ≥20% variability were reanalysed. Ambient conditions of the laboratory were kept consistent in order to reduce variability between assays.

**Free-living activity monitor processing**

ActivPAL data were downloaded using the manufacturers software (activPAL Professional Research Edition, PAL technologies, Glasgow, UK) and 'Event' csv files were processed using a validated automated algorithm in STATA (StataCorp LP, Texas, USA) described in detail elsewhere (28).

Actigraph data (100Hz) were downloaded using the manufacturer's software (ActiLife version 6·10·4, Lite Edition), reintegrated into 60 second epoch files and processed using a bespoke tool (KineSoft, version 3·3·76; KineSoft, New Brunswick, Canada [www.kinesoft.org]). Freedson cut points were used to categorize activity intensities (13). Non-wear time was defined as a minimum of 60 minutes of continuous zero counts, and when assessing habitual activity levels, days with at least 10 hours of wear time were required to be considered valid.

The minimum amount of valid days utilised for both ActivPAL and ActiGraph data was three days.

**Statistical analysis**

Descriptive characteristics of those who completed this study are summarised overall (n = 34) and stratified by sex **(Table 1)** for descriptive purposes.

Missing glucose and insulin data during the experimental conditions accounted for roughly 2% of overall required samples (34 out of 1,496) (see Table S-4, Supplemental Digital Content 4, summary of missing glucose and insulin data). These 34 missing data points were imputed using a regression model previously developed for an acute trial investigating breaking sedentary behaviour (15). This approach uses key predictors (BMI, ethnicity, age, fasting values and treatment condition) to derive a regression equation for the glucose and insulin values at each individual time point, this regression equation is then used to impute missing values.

The iAUC of glucose and insulin was calculated for each experimental condition. Total AUC was calculated by applying the trapezium rule and further subtraction of fasting levels gave a single value of iAUC for each participant. Utilising iAUC as opposed to total AUC is common practice in acute interventions where fasting levels should be unaffected by the intervention (20). Glucose iAUC was defined *a priori* as the primary outcome.

The effect of light walking breaks compared to continuous sitting on outcomes (glucose and insulin iAUC) and whether CRF modified this response was assessed using a repeated measures ANOVA. Treatment was entered as a within-person variable, with CRF (as a continuous variable) entered as a between-subjects covariate. Sex was also entered as a between-subjects factor. 'Treatment by CRF' and 'treatment by sex' interaction terms were investigated to assess the modifying effect of fitness and sex respectively. Sex was included in the model given that it is a strong determinant of fitness and an important potential confounder. Treatment by CRF interactions were further explored by calculating the linear

224 regression coefficients within each treatment condition. To highlight the direction of

225 significant interactions, derived average glucose iAUC values for men and women at the 25th,

226 50th and 75th centile of the CRF distribution are shown in Figure 3.

227 Two-tailed $p \leq 0.05$ was considered significant. Analyses were performed with SPSS (version

228 24). Results are presented as mean $\pm$ SE or regression coefficient (95% CI) unless stated

229 otherwise.

230 **RESULTS**

231 The key characteristics of those who successfully completed all three study visits are

232 displayed in **Table 1** (n = 34). Stratification of these characteristics for both males and

233 females are also presented here.

234 **Overall treatment condition effect**

235 The average postprandial concentrations of glucose **(A)** and insulin **(B)** witnessed throughout

236 the 7·5 hour testing periods for both experimental conditions ('prolonged sitting' and 'light

237 walking breaks') are depicted in **Figure 2.** There was a significant main effect of treatment

238 for both glucose ($F_{(1, 31)} = 6.67$, $p = 0.015$) and insulin ($F_{(1, 31)} = 7.00$, $p = 0.013$) iAUC

239 after adjustment for fitness and sex. Interrupting prolonged sitting time with light walking

240 breaks reduced blood glucose iAUC by 35% (from $3.89 \pm 0.7$ to $2 \cdot 51 \pm 0.7$ mmol·L$^{-1}$·h) and

241 insulin iAUC by 35% (from $241 \pm 46$ to $156 \pm 24$ mU·L$^{-1}$·h).

242 **Impact of CRF and sex**

243 There was a significant treatment by CRF interaction for glucose iAUC ($F_{(1, 31)} = 4.89$, $p = $

244 $0.035$). The treatment by CRF interaction for insulin iAUC failed to reach significance ($F_{(1, }$

245 $_{31)} = 3.76$, $p = 0.062$). There was no treatment by sex interaction for glucose ($F_{(1, 31)} = $

246 $1.77$, $p = 0.194$) or insulin ($F_{(1, 31)} = 1.54$, $p = 0.223$) iAUC.

247   Stratified analysis revealed that each unit increment in CRF (per mL·kg$^{-1}$·min$^{-1}$) was

248   associated with a lower glucose iAUC (-0.21 mmol·L$^{-1}$·h; 95% CI -0.38, -0.05)(p = 0.013) in

249   the prolonged sitting condition, whereas there was no association between CRF and glucose

250   iAUC during the light walking breaks condition (-0.07 mmol·L$^{-1}$·h; -0.21, 0.07) (p = 0.335).

251   In contrast, each unit increment in CRF was associated with a lower insulin iAUC (-10.93

252   mU·L$^{-1}$·h; -19.48, -2.37) (p = 0.014) in the prolonged sitting condition and a lower insulin

253   iAUC (-6.35 mU·L$^{-1}$·h; -10.90, -1.83) (p = 0.007) in the light walking breaks condition.

254

255   **Figure 3** uses the derived regression coefficients to show how the predicted average

256   difference between conditions for glucose iAUC changes as CRF increases for males and

257   females. This demonstrates that average blood glucose iAUC response for a man at the 25$^{th}$

258   centile of CRF within our cohort went from 5.80 to 2.98 mmol·L$^{-1}$·h (from prolonged sitting

259   to light walking breaks, respectively), whereas average responses for a man at the 75$^{th}$ centile

260   went from 1.99 to 1.78 mmol·L$^{-1}$·h. Similar trends were observed for women.

261   **DISCUSSION**

262   This study found that interrupting prolonged sitting with regular light walking breaks reduced

263   postprandial glucose and insulin levels in a healthy cohort. However, CRF modified the

264   response for glucose such that individuals with lower levels of fitness received incrementally

265   greater reductions in postprandial glucose. For example, the average response for a man at the

266   25th centile of CRF within our population (VO$_2$ peak of 42.5 mL·kg$^{-1}$·min$^{-1}$) demonstrated

267   relatively high postprandial glucose levels during prolonged sitting (5.80 mmol·L$^{-1}$·h) but

268   was able to almost half this level through employing regular light walking breaks.  In

269   contrast, the average response for a man at the 75th centile of fitness (VO$_2$ peak of 60.5

270   mL·kg$^{-1}$·min$^{-1}$) demonstrated relatively low levels of postprandial glucose during prolonged

271 sitting (1.99 mmol·L$^{-1}$·h) but only reduced this by a further 11% through employing regular

272 light walking breaks. The same pattern was demonstrated for women. These results were

273 supported by further analysis which demonstrated that CRF was inversely associated with

274 postprandial glucose during prolonged sitting, whereby every unit increment in VO$_2$ peak

275 (per mL·kg$^{-1}$·min$^{-1}$) was associated with an average reduction of 0.21 mmol·L$^{-1}$·h in glucose

276 iAUC values. Taken together, our results suggest that having high CRF or employing regular

277 light walking breaks in those with low CRF can both reduce postprandial levels of glucose

278 during periods of prolonged sitting activity. Elevated postprandial glucose levels are

279 implicated with the development of T2DM and CVD (5) and therefore strategies to promote

280 healthy glycemic responses when sedentary are of high importance.

281 Our observation that those with higher CRF demonstrate less metabolic benefit from light

282 activity breaks is consistent with previous experimental research that has tended to show

283 relatively lower metabolic benefits of light activity breaks in healthy cohorts (1, 22)

284 compared to both those with high risk of chronic disease (9, 15). Our findings also

285 correspond to cross sectional research that has shown the influence of sedentary time on a

286 cluster of cardio-metabolic issues to be significantly less pertinent in those with higher fitness

287 levels (7, 23, 25). The concept that fitter individuals may gain less pronounced health benefits

288 from lower levels of sitting time is supported by cross-sectional research that have stratified

289 data by habitual MVPA level, finding that individuals with higher MVPA levels display

290 significantly weaker associations between sedentary time with HbA1c (3), inflammation

291 markers (16) and all-cause mortality (11).

292 In contrast, a recent meta-analysis found that the association between sedentary time and

293 health outcomes persisted in sufficiently active individuals (4). However, this pooled analysis

294 was predominantly derived from self-reported measures of sedentary time and MVPA which

295 are prone to bias and consequently may have been insensitive to detecting true interactions. It

296  should also be noted that although observational research linking sedentary behaviour to

297  health is plentiful, the vast majority have investigated the confounding rather than the

298  modifying influence of physical activity (4, 27) or fitness (25).

299  The growing observational and experimental data has supported new guidance and

300  recommendation calling for reductions in sitting time (18). However, if the findings of the

301  current study continue to be supported by further research, there may be reasonable grounds

302  to embark upon a more personalized/tailored approach to T2DM prevention. Precision

303  medicine is important given that a one size fits all recommendation is rarely effective. For

304  example, interventions to reduce sitting time may be optimized by targeting those with poor

305  CRF, whereas those with high CRF may be better served by interventions aimed at

306  maintaining CRF and physical activity levels across the lifespan. However, it should be noted

307  that median levels of CRF within our population for men and women were 50.3 and 34.0

308  $mL \cdot kg^{-1} \cdot min^{-1}$ respectively and that the average reductions in postprandial glucose at this

309  level of CRF was 41%. As the majority of the general population within the age range

310  included in this study are estimated to fall below the median levels of fitness within our

311  population (8), the importance of interrupting sitting time with light activity breaks is likely

312  to remain generalizable to the majority of the population.

313  This research also suggests that increasing CRF levels may be a viable way to protect against

314  the potential harms of prolonged sitting. Although there are genetic contributions to fitness,

315  the largest contributor to an individual's fitness is their time spent in MVPA (6).

316  Participation in regular MVPA outside of seated hours may therefore offer some protection,

317  particularly in seated occupations such as driving.

318  Our observation that fitter individuals experienced less pronounced postprandial glycemic

319  excursions during prolonged sitting may result from favourable physiological adaptations

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320   stemming from regular engagement in MVPA (one of the main determinants of fitness), such

321   as increased skeletal muscle GLUT 4 protein expression (14). This would also leave less

322   scope for further improvement, potentially explaining why the benefits of interrupting sitting

323   time with light activity breaks appear to be blunted in those with higher fitness. However,

324   given that CRF is determined by a mixture of both MVPA engagement and genetics (6), we

325   cannot distinguish between behavioral and genetic mechanisms driving the results of the

326   current study.

327   This study has some important limitations. Although this study provides an initial proof-of-

328   concept from which future research can tailor to alternative study cohorts, findings should not

329   be generalised outside the population investigated. In particular, given that the population

330   utilised in this study were healthy, the extent to which CRF modifies responses in high risk or

331   clinical population remains to be investigated.  Our second limitation is that despite

332   instructions to standardize food intake, and refrain from caffeine and alcohol consumption

333   leading up to treatment conditions, we did not objectively test participant compliance and

334   relied on self-reported adherence. In addition, fitness assessments were only conducted at one

335   time-point, thus direct causality cannot be inferred. Future interventions that actively set out

336   to manipulate fitness levels and assess prospective change in experimental data are required

337   to elucidate direct causality. Another concern was that those with higher fitness in this study

338   were predominantly men and conversely, those with lower fitness were predominantly

339   women. However, our results were adjusted for sex and it was not found to modify the

340   treatment effect for glucose which was in contrast to CRF. Therefore the correlation between

341   sex and CRF is unlikely to be confounding the results of this study.

342   In conclusion, participants with lower fitness had worse postprandial glucose and insulin

343   responses during prolonged sitting, and were able to gain greater metabolic benefit through

344   breaking their sitting time with light activities compared to individuals with higher fitness.

345 Future interventions aimed at alleviating the deleterious metabolic impacts of sedentary

346 behaviour may therefore be optimized by tailoring to cardio-respiratory fitness levels of the

347 general population.

369 interventions for individuals at high risk) Program Development Group. MJD, KK, and TY

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376 results of the present study do not constitute endorsement by the American College of Sports

377 Medicine. The results of the study are presented clearly, honestly, and without fabrication,

378 falsification, or inappropriate data manipulation.

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381 **LIST OF SUPPLEMENTAL DIGITAL CONTENT**

382 **Supplemental Digital Content 1.doc**

383 **Supplemental Digital Content 2.doc**

384 **Supplemental Digital Content 3.doc**

385 **Supplemental Digital Content 4.doc**

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479 **FIGURE CAPTIONS:**

480 **Figure 1** - Trial CONSORT Profile

481 **Figure 2** - Effect of treatment condition on average Blood Glucose and Insulin

482 **Figure 3** – The effect of treatment condition on average iAUC (95% CI) for glucose
483 categorised by fitness level by the $25^{th}$, $50^{th}$ and $75^{th}$ centile of the cohort.