

1 **Small-sided soccer in school reduces postprandial lipaemia in adolescent boys**

2 James W Smallcombe¹, Laura A Barrett¹, John G Morris², Lauren B Sherar¹, Keith Tolfrey¹

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4 **Institutional affiliation:**

5 ¹Loughborough University, School of Sport, Exercise and Health Sciences, Loughborough,

6 UK.

7 ² Sport, Health and Performance Enhancement (SHAPE) Research Centre, Nottingham Trent

8 University, Nottingham, UK.

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10 **Corresponding author:** Dr Keith Tolfrey, Loughborough University, School of Sport
11 Exercise and Health Sciences, Epinal Way, Loughborough, LE11 3TU.

12 k.tolfrey@lboro.ac.uk, +44 (0)1509 226355

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27 **ABSTRACT**

28 **Purpose:** While laboratory based moderate- to high-intensity exercise reduces postprandial
29 lipaemia in adolescents this exercise differs to the free-living physical activities in which
30 young people typically engage. This study compared the effect of free-living afterschool
31 soccer activity and treadmill exercise on in-school postprandial lipaemia in adolescent boys.
32 **Methods:** Fifteen boys (12.6 (0.5) years) completed three, 2-day experimental trials. On Day
33 1, participants either: rested (CON); exercised for 48 min on a treadmill at 60% peak $\dot{V}O_2$
34 (TM); played 48 min of 5-a-side soccer (SOC). On Day 2, participants attended school where
35 a capillary blood sample determined fasting triacylglycerol ([TAG]) and glucose ([glucose])
36 concentrations. Participants then consumed a standardised breakfast (0 h) and lunch (4.5 h)
37 and blood samples were taken postprandially at 2.5, 5.0 and 7.0 h. **Results:** Reductions in
38 fasting [TAG] were small-moderate after TM (-16%, 95% CI = -27 to -2%, ES = 0.46), but
39 large after SOC (-30%, 95% CI = -40 to -20%, ES = 1.00) compared with CON; the
40 concentration was also lower in SOC compared with TM (-18%, 95% CI = -29 to -5%, ES =
41 0.53). Based on ratios of geometric means, the area under the TAG versus time curve was 18%
42 lower after TM (95% CI = -29 to -5%, ES = 0.51) and 25% lower after SOC (95% CI = -35 to
43 -13%, ES = 0.76,) compared with CON. In contrast, SOC and TM were not significantly
44 different (-9%, 95% CI = -21 to 5%, ES = 0.25). **Conclusion:** Compared with duration-
45 matched inactivity (CON), after-school small sided soccer (SOC) and treadmill exercise (TM)
46 resulted in a similar, moderate reduction of postprandial lipaemia in adolescent boys.

47 **KEY WORDS:** Games-activity, lipid, metabolism, triacylglycerol, cardiovascular disease
48 risk

49 **INTRODUCTION**

50 Regular exposure to elevated postprandial plasma triacylglycerol concentrations ([TAG]) is
51 associated with the development of atherosclerosis (1) and is considered an independent risk
52 factor for adverse cardiovascular events (2, 3). Although atherosclerosis manifests typically
53 in adulthood, it has been long established that atherogenesis is an insidious process initiated
54 much earlier during childhood and adolescence (4, 5). Consequently, interventions aimed at
55 reducing postprandial lipaemia may offer the greatest protection to long-term cardiovascular
56 health when commenced in early life.

57

58 Compelling evidence indicates that a single session of moderate- to high-intensity exercise
59 reduces postprandial lipaemia in young people (6). However, reliance upon laboratory-based
60 experimental protocols represents a limitation of this previous body of research. Typical
61 laboratory-based exercise protocols bear little resemblance to the activities performed by
62 children and adolescents in free-living settings. Furthermore, the tightly-controlled laboratory
63 conditions, under which experimental measures are most commonly conducted, also differ
64 considerably to the settings in which young people engage routinely.

65

66 Ergometer-based activity (e.g., treadmill running) is the most common laboratory mode of
67 exercise. In contrast, soccer (including five-a-side) has been reported to be the most popular
68 sport amongst 11 to 15 year olds in the UK (7). Given that only 20% of adolescents achieve
69 the recommended daily minimum of 60 minutes of moderate- to vigorous-intensity physical
70 activity (8), it is important to investigate activities that adolescents enjoy and which are, thus,
71 potentially more conducive to long-term adherence. Current scientific understanding remains
72 limited as to how the physiological stimuli provided by free-living modes of exercise, such as
73 soccer, compare with the laboratory-based exercise employed in the laboratory. Therefore,

74 while laboratory-derived data clearly demonstrate the potential benefits of an exercise
75 intervention, its practical benefits remain unclear until comparable responses are
76 demonstrated in real-world settings. Unlike ergometer exercise, during which exercise
77 intensity and energy expenditure can be precisely quantified and controlled, free-living
78 physical activity performed by children is far less predictable. For example, soccer is
79 characterised by bouts of intermittent high-intensity running, periods of acceleration and
80 deceleration, changes of direction, jumping, tackling as well as lower intensity ‘cruising’ and
81 standing (9). Furthermore, intrinsic motivation during game-based activity is likely to exert
82 an important influence on the total exercise ‘dose’. Although soccer *training* has been
83 recognised as a powerful stimulus for health promotion in adults (10), and has recently been
84 demonstrated to induce acute reductions in postprandial lipemia in normal and overweight
85 adult males (11), it has not yet been established if participation in school-based games
86 activity confers similar metabolic benefit during youth.

87

88 Additionally, the laboratory conditions under which the post-exercise blood samples are
89 taken routinely differ markedly from conditions in schools. Whilst typical laboratory
90 protocols require participants to spend long periods of time sedentary under tightly-controlled
91 conditions, children spend much of their free-living time at school – a setting in which they
92 face both formal and informal opportunities to accumulate physical activity and break-up
93 sedentary time throughout the school day. It is, therefore, important that steps are taken
94 towards ‘translational’ experimental designs, which incorporate representative forms of
95 exercise, coupled with ecologically valid measures of the outcome variables of interest. Such
96 advancements are required to facilitate a more representative assessment of the complex
97 interaction between exercise, free-living physical activity and postprandial metabolism,
98 enabling further elucidation of the relevance of childhood exercise to public health policy.

99

100 In light of the aforementioned shortcomings of much of the previous literature, the aim of the
101 present study was to examine the efficacy of school-based free-living 5-a-side soccer activity
102 in reducing in-school postprandial lipaemia in adolescent boys.

103

104 **METHODS**

105 *Participants*

106 After approval from the Loughborough University Ethics Approvals (Human Participants)
107 Sub-Committee, 15 healthy adolescent boys volunteered for and completed all measures (i.e.,
108 only 15 volunteers and no drop-outs). These participants were recruited from a local
109 secondary school after their attendance at a school-based presentation. Written assent was
110 obtained from each participant and written informed consent was obtained from a parent or
111 guardian. Suitability for admittance into the study was confirmed by the completion of a
112 general health screen questionnaire. Participant characteristics are presented in Table 1.

113

114 [PLEASE INSERT TABLE 1 HERE]

115

116 **Preliminary session**

117 *Anthropometry and physical maturation*

118 Anthropometry was conducted with participants wearing shorts, T-shirt and socks. Body
119 mass was measured to the nearest 0.1 kg using a digital scale and stature was measured to the
120 nearest 0.01 m using a wall-mounted stadiometer (Holtain, Crosswell, UK). Triceps and
121 subscapular skinfold thicknesses were measured on the right-hand side of the body to the
122 nearest 0.2 mm using Harpenden callipers (John Bull, St. Albans, UK). The skinfold
123 thickness was calculated as the median of three measurements. Percentage body fat (%BF)
124 was estimated using maturation, race and sex-specific equations (12). Waist circumference
125 was measured midway between the 10th rib and the iliac crest (13). Physical maturity was
126 estimated with a five-point self-assessment of secondary sexual characteristics (14).
127 Scientific photographs depicting the five stages of genital and pubic hair development,

128 ranging from 1 indicating pre-pubescence to 5 indicating full sexual maturity, were used
129 privately by the participants to provide this information.

130 *Preliminary exercise measures*

131 Before the preliminary exercise tests, participants were familiarised with exercising on the
132 treadmill ergometer (Mercury Medical, h/p/cosmos sports & medical GmbH, Germany).
133 Short-range telemetry (PE4000, Polar-Electro, Kempele, Finland) was used to monitor HR
134 continuously throughout the exercise tests. Peak heart rate (HR_{peak}) was defined as the highest
135 HR recorded during the test. Ratings of perceived exertion (RPE) were measured during the
136 final 15 s of each exercise stage using the pictorial OMNI (0 to 10) scale (15).

137

138 The steady-state relationship between treadmill speed, oxygen uptake ($\dot{V}O_2$) and heart rate
139 (HR) was ascertained via a 4 × 4 min incremental exercise protocol. The starting treadmill
140 speed was set at a speed of 5.0 km·h⁻¹ and was increased by 1.0 km·h⁻¹ at the end of each 4
141 min stage. An expired air sample was collected using the Douglas bag (Cranlea and
142 Company, Birmingham, UK) technique during the final 60 seconds of each 4 min stage.

143

144 Peak $\dot{V}O_2$ was determined using an incremental gradient-based treadmill protocol with each
145 participant running at an individual fixed speed (8.0 to 10.5 km·h⁻¹). Expired air was collected
146 into Douglas bags during each successive minute of exercise via open-circuit spirometry. The
147 treadmill belt gradient was raised by 1% every minute until volitional exhaustion was
148 attained. Due to the limited number of children (20-40%) that display a plateau in their $\dot{V}O_2$
149 when performing exercise to exhaustion, and to avoid the possible acceptance of a
150 'submaximal peak $\dot{V}O_2$ ' based on secondary criteria (16), each participant completed a
151 supramaximal verification stage to volitional exhaustion after a ten-minute recovery period

152 (17). During this verification stage, the treadmill was set at a gradient 2% greater than that
153 attained at the end of the initial incremental exercise test.

154

155 A paramagnetic oxygen (O₂) analyser and infrared carbon dioxide (CO₂) analyser (Servomex,
156 Sussex, UK) were used to determine the concentration of O₂ and CO₂ in the expired air
157 samples. The volumes of expired gas were determined using a dry gas meter (Harvard
158 Apparatus, Kent, UK) and were corrected to standard temperature and pressure (dry). For
159 each expired gas sample, oxygen uptake ($\dot{V}O_2$), expired carbon dioxide ($\dot{V}CO_2$), minute
160 ventilation (\dot{V}_E), and respiratory exchange ratio were calculated.

161

162 *Experimental design*

163 All participants completed three counter-balanced, 2-day main conditions; a resting control
164 (CON); laboratory-based, moderate-intensity treadmill exercise (TM); and participation in an
165 afterschool 5-a-side soccer tournament (SOC). All experimental conditions commenced at
166 15:45 and were completed by 17:15. Body mass was measured at the start of each
167 experimental condition to standardise the test meals provided on Day 2 of each condition. A
168 schematic representation of the study design is provided in Figure 1.

169

170 [PLEASE INSERT FIGURE 1 HERE]

171

172 **Day 1 – Intervention**

173

174 *Resting control (CON) & moderate-intensity treadmill exercise (TM)*

175 During CON participants remained at school at the end of the school day and rested for 90
176 min in a seated position. During TM, participants attended the laboratory afterschool and
177 completed 48-min of moderate-intensity exercise on a treadmill. The treadmill exercise was

178 divided into 3 × 16-min bouts of exercise, interspersed by 8-min periods of rest. Participants
179 exercised at a fixed intensity, based on a HR target set at the HR corresponding to 60% peak
180 $\dot{V}O_2$ (as determined from the previously described preliminary exercise testing protocols).
181 The treadmill speed was adjusted at the end of each minute to ensure the target heart rate was
182 maintained. As described previously, heart rate was monitored continuously and RPE
183 recorded during the final minute of each bout of treadmill exercise. Expired air was collected
184 for 60 s at two standardised time points (7 to 8 min and 15 to 16 min) during each 16 min
185 interval of treadmill exercise. Individual gas exchange data were used to verify exercise
186 intensity, retrospectively.

187

188 ***5-a-side soccer (SOC)***

189 During SOC, all participants took part in three, round-robin, 5-a-side soccer tournaments,
190 over the course of three consecutive weeks. During each tournament, each team (and thus
191 each participant) played six 8-min games with playing time totalling 48-min. All games were
192 played on an outdoor, grass pitch that complied with current English Football (Soccer)
193 Association age-specific guidelines (dimensions 44 × 22 m). Goalkeepers were rotated every
194 2 min to avoid position-specific variation in activity. Of the 15 participants, five participated
195 in competitive soccer regularly with local soccer clubs. The remainder of the participants did
196 not play competitively but reported enjoying taking part in school-based soccer activities (e.g.
197 physical education lessons). The competitive players were divided across the three teams to
198 distribute playing ability evenly.

199

200 All participants played in all three after-school soccer tournaments; however, subsequent
201 postprandial blood sampling (Day 2) was completed with each participant following only one
202 afterschool soccer tournament. Postprandial test-meal measures were completed with 5

203 participants after each of the three afterschool tournaments. The tournament game schedule
204 was standardised to ensure that all participants completed Day 2 postprandial blood sampling
205 measures after playing their allocation of games in three blocks of two consecutive 8-min
206 games, thus mirroring the pattern of treadmill exercise completed during TM.

207

208 Physical activity was assessed continuously during each 5-a-side soccer tournament.
209 Participants were equipped with individual 5-Hz Global Positioning System (GPS) devices
210 (SPI Elite, GPSport, Canberra, Australia) that were worn for the duration of each soccer
211 tournament. Heart rate was also monitored continuously (as described previously), and RPE
212 was recorded at the end of the final soccer match of each tournament.

213

214 ***GPS analysis***

215 All GPS data were analysed using Team AMS software version 1.2 (GPSports, Australia). In
216 accordance with previous research (18), movement during the soccer activity was classified
217 into five speed categories: standing (speed ≤ 0.4 km·h⁻¹); walking (speed from > 0.4 to 3.0
218 km·h⁻¹); low-intensity running (LIR, speed from > 3.0 to 8.0 km·h⁻¹); medium-intensity
219 running (MIR, speed from > 8.0 to 13.0 km·h⁻¹); high-intensity running (HIR, speed
220 from >13.0 to 18.0 km·h⁻¹); sprinting (speed > 18.0 km·h⁻¹). Total distance covered during the
221 soccer activity was quantified and distance covered in each speed category was also
222 determined. The method proposed by di Prampero and colleagues (19) was applied to the
223 GPS data to estimate energy expenditure during SOC.

224

225 **Day 2 - Post-intervention**

226 ***Postprandial test-meal measures***

227 Following the consumption of a standardised carbohydrate-rich snack (3.6 g fat, 19.7 g
228 carbohydrate, 2.0 g protein, 516 kJ energy) at 19:45 on Day 1 of each trial, participants
229 observed a 12-h overnight fast before arriving at school at 07:40. After 10 min seated rest, a
230 capillary blood sample was taken. At 08:10 a standardised breakfast was started, marking the
231 start of the postprandial period, and consumed within 25 min. Participants then attended their
232 normal timetabled school lessons with blood samples and meals provided during scheduled
233 breaks in the school day (see Figure 1). Once blood samples had been collected during the
234 breaks in the school day, participants were able to continue with their habitual break-time
235 activities.

236

237 ***Standardisation of diet and physical activity***

238 Physical activity and dietary intake were recorded during the 48-h period (pre-intervention
239 and intervention days) preceding Day 2 of each experimental condition. Participants were
240 asked to replicate dietary intake and physical activity patterns from the first condition before
241 each subsequent experimental condition.

242

243 Participants completed weighed food diaries using digital kitchen scales (Andrew James UK
244 Ltd., Bowburn, UK) and the CompEat Pro 5.8.0 computerised food tables (Nutrition Systems,
245 London, UK) were used to analyse dietary intake subsequently. Physical activity was
246 quantified via accelerometry (ActiGraph GT1M, ActiGraph, Pensacola, Florida, USA). The
247 accelerometer was worn on the right hip during waking hours (removed for water-based
248 activities). Raw ActiGraph data files were analysed using custom made data reduction
249 software (KineSoft Software, version 3.3.76, Loughborough University, UK;
250 <http://www.kinesoft.org>). During data processing, 5 s epoch data were re-integrated to 60 s
251 epochs; 60 min of consecutive zeros, allowing for 2 min of non-zero interruptions, was used

252 to remove non-wear, and a minimum of 8-h of valid wear time was required for a valid day.
253 Physical activity was expressed as average counts per minute (CPM) and interpreted using
254 age-specific intensity cut points (20). Participants were asked to minimise physical activity
255 during this 48-h period.

256

257 *Test meals*

258 Participants were provided with standardised meals on Day 2 of each trial. Breakfast
259 consisted of croissants, chocolate spread, whole milk, double cream and milkshake powder.
260 Meals were standardised to body mass and provided 1.6 g fat, 1.8 g carbohydrate, 0.4 g
261 protein and 95 kJ energy per kilogram of body mass. The test lunch comprised of white bread,
262 mild cheddar cheese, butter, potato crisps, whole milk and milkshake powder and provided
263 1.1 g fat, 1.9 g carbohydrate, 0.6 g protein and 83 kJ energy per kilogram of body mass. The
264 time taken for individual participants to consume the test meals during the first condition was
265 recorded and replicated during each subsequent experimental condition.

266

267 *Analytical methods*

268 The whole hand was submerged in 40°C water for 5 min and then dried thoroughly before the
269 fingertip was pierced (Unistick 3 Extra, Owen Mumford, UK) to provide a capillary blood
270 sample. The first drop of blood was discarded before 300 to 600 µL of blood was collected in
271 potassium-EDTA-coated microvette tubes (Sarstedt Ltd., Leicester, UK). The whole blood
272 was centrifuged immediately at 12,800g for 15 min (Eppendorf 5415c, Hamburg, Germany).
273 The resulting plasma sample was stored at -20°C for subsequent analysis. Plasma [TAG] and
274 [glucose] were determined by a benchtop analyser (Pentra 400; HORIBA ABX Diagnostics,
275 Montpellier, France) using enzymatic, colorimetric methods (Randox Laboratories Ltd.,
276 Crumlin, UK). The within-batch coefficients of variation for [TAG] and [glucose] were 1.4%

277 and 0.5%, respectively. Acute changes in plasma volume were estimated from haemoglobin
278 concentration and haematocrit ascertained from the fasting and final blood samples.
279 Haemoglobin concentration was determined via the cyanmethemoglobin method; 20 μ L of
280 whole blood was added to 5 mL of Drabkin's reagent and the absorbance was quantified via
281 photometry at a wavelength of 546 nm (Cecil CE1011; Cecil instruments, Cambridge, UK).
282 A microhaematocrit centrifuge and reader (Haematospin 1300 Microcentrifuge; Hawksley
283 and Sons Ltd., Sussex, UK) were used to quantify haematocrit.

284

285 *Statistical analyses*

286 The Statistical Package for Social Sciences (SPSS) software version 23.0 for Windows (SPSS
287 Inc., Chicago, IL, USA) was used for all data analyses. The trapezium rule was used to
288 calculate total 7 h area under the plasma concentration versus time curve for TAG (TAUC-
289 TAG) and glucose (TAUC-glucose) for all experimental conditions. The same method was
290 used to calculate incremental area under the variable versus time curve (iAUC) after
291 correcting for fasting concentrations. Normality of the data was checked using Shapiro Wilk
292 tests. Normally distributed data are presented as mean (SD). Student's paired *t*-tests were
293 used to determine differences between responses to exercise during TM and SOC. Data for
294 free-living physical activity and sedentary time, and concentrations of plasma TAG and
295 glucose were natural log transformed before analyses. These data are presented as geometric
296 mean (95% confidence interval) and analyses are based on ratios of geometric means and 95%
297 confidence intervals (CI) for ratios. Linear mixed models repeated for condition were used to
298 examine differences in dietary intake, free living physical activity and sedentary time (wear
299 time included as a covariate), plasma volume changes, fasting concentrations and TAUC
300 responses. Differences in postprandial [TAG] and [glucose] were examined using linear
301 mixed models repeated for condition and time. Where appropriate, to supplement key

302 findings, absolute standardised effect sizes (ES) were calculated for within-measures
303 comparisons as follows:

304
$$ES = \frac{\text{mean } v2 - \text{mean } v1}{\text{CON SD}}$$

305

306 Where *v1 and v2* represent the two variable mean values being compared and the CON SD
307 is the control condition standard deviation. In the absence of a clinical anchor, an ES of 0.2
308 was considered to be the minimum important difference, 0.5 moderate and 0.8 large (21).

309

310

311 **RESULTS**

312 *Dietary intake*

313 Average energy intake did not differ significantly during the 48 h prior to day 2 of CON, TM,
314 and SOC (8.7 (2.1), 8.7 (2.4), and 8.2 (2.1) MJ·day⁻¹, respectively, $P = 0.686$). Macronutrient
315 intake did not differ between CON, TM, and SOC for carbohydrate (297.1 (94.2), 293.4
316 (118.8), and 275.5 (87.9) g·day⁻¹, $P = 0.729$), protein 71.6 (22.0), 73.3 (18.2) and 66.2 (20.6)
317 g·day⁻¹, $P = 0.212$) and fat (66.6 (20.3), 68.1 (14.3), and 66.8 (21.1) g·day⁻¹, $P = 0.934$),
318 respectively.

319

320 *Free-living physical activity and sedentary time*

321 After adjusting for accelerometer wear time, no significant differences were observed for
322 counts per minute ($P = 0.294$), sedentary time ($P = 0.342$), light activity ($P = 0.146$),
323 moderate activity ($P = 0.089$) or vigorous activity ($P = 0.843$) during the 48 hours preceding
324 Day 2 of the experimental model. Data for this 48-hour period are presented in Table 2.

325

326 [PLEASE INSERT TABLE 2 HERE]

327

328 *Exercise responses to TM and SOC*

329 Mean exercise-intensity during TM was 61 (6)% peak $\dot{V}O_2$ and gross energy expenditure was
330 1.4 (0.3) MJ. Average heart rate was higher during SOC compared with TM (175 (8) vs 157
331 (7) beats·min⁻¹, 95% CI = 11 to 24, $P < 0.001$). Participants covered a shorter total distance
332 during SOC compared with TM (3.6 (0.4) vs 5.9 (0.5) km, 95% CI = -2.7 to -2.1, $P < 0.001$)
333 at a lower average speed (4.4 (0.5) vs 7.4 (0.7) km·h⁻¹, 95% CI = -3.4 to -2.6, $P < 0.001$).

334 Rating of perceived exertion (0 to 10 OMNI) did not differ between SOC and TM (5 (2) vs 5
335 (1), 95% CI = -1 to 1, $P = 0.883$).

336

337 During SOC, the following proportions of game time were spent exercising within the
338 progressive absolute heart rate intensities shown (beats·min⁻¹): 21% <160; 12% 160 to 169;
339 18% 170 to 179; 24% 180 to 189; 21% 190 to 199 and 4% ≥ 200 beats·min⁻¹. The times spent
340 during SOC in each of the 6 identified speed zone classifications are presented in Table 3.

341

342 [PLEASE INSERT TABLE 3 HERE]

343

344 *Plasma volume changes*

345 The small changes in plasma volume between fasting and 7 h blood samples did not vary
346 significantly between the three experimental conditions (CON = 1.1 (2.3)%, TM = 1.4 (2.2)%,
347 SOC = 1.5 (2.2)%, $P = 0.901$). Therefore, further analyses were completed without
348 adjustment to the raw plasma [TAG] and [glucose].

349

350 *Fasting [TAG] and [glucose]*

351 Fasting [TAG] and [glucose] for each condition are presented in Table 4. Fasting plasma
352 [TAG] was 30% lower in SOC compared with CON (95% CI = -40 to -20%, ES = 1.00, $P \leq$
353 0.001) and 18% lower than TM (95% CI = -29 to -5%, ES = 0.53, $P = 0.011$). Fasting [TAG]
354 was also 16% lower in TM compared with CON (95% CI = -27 to -2%, ES = 0.46, $P =$
355 0.025). Compared with CON, fasting [glucose] was 3% lower in TM (95% CI = -5 to -1%,
356 ES = 0.52, $P = 0.009$) and 4% lower in SOC (95% CI = -5 to -2%, ES = 0.67, $P = 0.001$). No
357 meaningful difference was observed for fasting glucose between TM and SOC (95% CI = -3
358 to 1%, ES = 0.15 $P = 0.368$).

359

360

[PLEASE INSERT TABLE 4 HERE]

361

362 ***Plasma [TAG] and [glucose] in the postprandial period***

363 Plasma [TAG] responses over time and across conditions are shown in Figure 2. Differences
364 in postprandial plasma [TAG] were observed across conditions (main effect condition, P
365 <0.001 ; main effect time, $P < 0.001$) but no condition–time interaction was observed ($P =$
366 0.469). Simple pairwise comparison indicated that mean postprandial [TAG] was 16% lower
367 after TM (95% CI = -22 to -9, ES = 0.46, $P < 0.001$) and 25% lower after SOC (95% CI = -
368 31 to -19, ES = 0.76, $P = 0.006$) compared with CON. An 11% reduction was observed after
369 SOC compared with TM (95% CI = -17 to -3, ES = 0.30, $P < 0.001$).

370

371 The TAUC-TAG (Table 4) was 18% lower after TM (95% CI = -29 to -5%, ES = 0.51, $P =$
372 0.009) and 25% lower after SOC (95% CI = -35 to -13%, ES = 0.76, $P < 0.001$) compared
373 with CON. The TAUC-TAG was 9% lower after SOC compared with TM but this difference
374 was small and non-significant (95% CI = -21 to 5%, ES = 0.25, $P = 0.191$). Individual
375 responses to exercise were similar after both exercise conditions with a reduction of TAUC-
376 TAG exhibited by 13 (87%) and 14 (93%) of the fifteen participants after TM and SOC,
377 respectively.

378

379 When accounting for the differences in fasting [TAG], the incremental area under the TAG
380 versus time curve (iAUC-TAG) was 19% lower after TM (95% CI = -35 to 2%, ES = 0.41, P
381 $= 0.078$) and 16% lower after SOC (95% CI = -33 to 5%, ES = 0.35, $P = 0.109$) compared
382 with CON, however, these differences did not reach statistical significance. The 6%

383 difference in iAUC-TAG between TM and SOC was trivial and non-significant (95% CI -18
384 to 30%, ES = 0.06, $P = 0.793$).

385

386 [PLEASE INSERT FIGURE 2 HERE]

387

388 There were no significant differences in postprandial plasma [glucose] across the conditions
389 (main effect condition, $P = 0.876$; condition–time interaction, $P = 0.905$). Similarly, no
390 meaningful differences were observed in TAUC-glucose (ES = 0.07 to 0.15, $P = 0.770$)
391 between conditions.

392

393 **DISCUSSION**

394 The main finding of the present study was that the reduction in postprandial lipaemia
395 following after-school 5-a-side soccer activity was similar to that observed after time-
396 matched, moderate-intensity treadmill exercise despite participants covering a lower total
397 distance at a lower average speed. This is encouraging as team game activities reflect more
398 accurately the habitual intermittent activity preferences of British adolescents compared with
399 the continuous laboratory-based ergometer exercise employed typically in research settings.
400 The present study provides empirical evidence supporting the efficacy of an acute bout of
401 soccer activity to reduce postprandial lipaemia during adolescence and represents an
402 important step towards the translation of previous laboratory research into ecologically valid
403 settings.

404

405 To our knowledge, this is the first study to examine the effect of school-based soccer activity
406 on in-school postprandial lipaemia in adolescent boys. This is highly relevant when
407 considering that soccer continues to represent the most likely form of sports participation for

408 young males in the UK with 53% of 5 to 10-year-old boys and 78% of 11 to 15-year-old boys
409 reporting recent soccer participation (7). In agreement with the existing body of literature (6),
410 a moderate reduction of circulating [TAG] was observed after 48 minutes of both SOC and
411 continuous TM exercise, compared with duration-matched inactivity. The magnitude of the
412 reduction observed following free-living SOC was similar to the effects reported in young
413 males previously after laboratory-based continuous moderate intensity-exercise (22 – 25) and
414 high-intensity running (26) and sprint cycling (27). Furthermore, the present study yielded
415 findings remarkably similar to those reported by Barrett and colleagues (28) after participants
416 completed a modified version of the Loughborough Intermittent Shuttle Test (LIST) which
417 was designed to simulate games activity. In this previous study, 72 min of intermittent
418 exercise resulted in a 26% (ES = 0.78) reduction in [TAG] compared with a smaller 14% (ES
419 = 0.46) reduction after 60 min of continuous moderate-intensity treadmill exercise.
420 Importantly, whilst the LIST protocol was strictly standardised and dictated by an audio
421 signal, exercise volume during our free-living soccer activity was self-regulated and largely
422 dependent on intrinsic motivation. In addition, in the present study the 48 minute durations
423 of SOC and TM were considerably shorter than the 72-minute LIST exercise, however,
424 similar reductions in [TAG] were still observed.

425

426 The similar reductions in postprandial [TAG] observed after SOC and TM were somewhat
427 surprising given the extent to which the two exercise stimuli differed. During SOC,
428 participants covered a shorter total distance (3.6 vs 5.9 km) at a lower average speed (4.4 vs
429 7.4 km·h⁻¹) compared with TM. The physiological response to the two exercise conditions
430 also differed considerably. During SOC, participants exercised at 87% of individual peak
431 heart rate compared with an average of 78% in TM. This is in agreement with reports that
432 soccer players typically exercise in excess of 80% peak heart rate irrespective of playing level

433 or age (29). Furthermore, a large proportion of time (~25%) was spent exercising at a heart
434 rate exceeding 190 beats per minute (~92% peak heart rate). Although there may be a
435 mismatch in the HR- $\dot{V}O_2$ relationship during intermittent, non-steady state exercise, the heart
436 rate data still provide valuable evidence of the high relative intensity at which participants
437 exercised during the soccer games. Supplementary GPS data strengthen this evidence and
438 revealed that participants covered, on average, 433 m in high-intensity running whilst a
439 further 72 m was covered at a speed associated with maximal sprinting (Table 3). These
440 periods of high-intensity effort were likely sufficient to offset the periods of lower intensity
441 work performed, during which participants spent approximately 20 minutes of the total game
442 time (48 min) walking and standing. Indeed, explorative analysis of the GPS data – using the
443 method proposed by di Prampero and colleagues (19) – indicated that the energy cost of SOC
444 was likely very similar to that of TM (~1.4 MJ). Although this estimate was derived using
445 methods validated in adults, it is corroborated by metabolic intensity estimates reported in the
446 Youth Compendium of Physical Activities (30). However, future efforts to determine more
447 accurately the metabolic demands of small-sided soccer during youth are recommended.
448 Despite the repeated bouts of high-intensity effort that characterised the soccer activity, rating
449 of perceived exertion (OMNI 0 to 10 pictorial scale) did not differ between SOC and TM;
450 this finding is of importance when exercise tolerance is considered.

451

452 Unfortunately, the minimally invasive procedures employed in the current study, precluded
453 the elucidation of the mechanisms underpinning the exercise-induced reductions of
454 postprandial lipaemia observed after SOC and TM. However, the available evidence suggests
455 that both enhanced clearance of circulating TAG and altered hepatic VLDL kinetics –
456 specifically the secretion of fewer TAG-rich VLDL particles (31) – likely contributed to the
457 reduction in [TAG] after both exercise stimuli. Furthermore, total carbohydrate oxidation

458 during exercise is known to increase exponentially with exercise intensity (32) resulting in
459 increased exercise-induced glycogen depletion. Intramuscular glycogen concentration is
460 inversely associated with resting fat oxidation after exercise (33, 34), which, in turn, has been
461 highlighted as a potentially important mediator of postprandial lipaemia (35). Although the
462 field-based study design precluded estimation of substrate utilisation, it is likely that the
463 higher relative exercise intensity during SOC resulted in a shift towards carbohydrate
464 oxidation during exercise and thus an elevated fat oxidation rate post-exercise. Additionally,
465 high-intensity sprinting, as performed during SOC, is associated with elevated catecholamine
466 and growth hormone concentrations (36, 37), which may also mediate the lipoprotein lipase –
467 the rate limiting enzyme central to the hydrolysis of circulating TAG – response to exercise
468 (38, 39). It is, therefore, likely that intensity-driven mechanisms contributed, at least partially,
469 to the attenuated fasting TAG concentrations observed after SOC only, as well as the subtle
470 differences in postprandial [TAG] observed after SOC compared with TM. Although
471 reductions in the incremental areas under the [TAG] versus time curve after SOC and TM did
472 not reach statistical significance, this finding is in line with previous research in adolescents
473 (26) and may be of physiological relevance. Furthermore, analysis of the total area under the
474 curve offers greater insight into the holistic metabolic benefit conferred by exercise as lower
475 postabsorptive [TAG] – as indicated by reduced fasting [TAG] – contributes to the promotion
476 of a healthy lipid profile during adolescence and, thus, represents an important response to
477 the exercise performed during the current study.

478

479

480 A novel feature of the current study was the use of in-school measures of postprandial
481 metabolism. This represents an important step towards the translation of laboratory-derived
482 findings into representative, free-living settings. Highly-controlled laboratory conditions limit

483 free-living physical activity artificially and are unrepresentative of daily variation. This is
484 particularly relevant to school; a setting in which adolescents are presented with both formal
485 (e.g., physical education lessons) and informal (e.g., walking between lessons and recess
486 activities) opportunities to accrue physical activity throughout the day. The effect of this
487 additional free-living physical activity on postprandial metabolism has received little
488 scientific attention. Preliminary data do, however, suggest that subtle yet potentially
489 important differences in postprandial metabolism are observed when blood sampling
490 measures are conducted in the free-living school setting as opposed to in the laboratory (40).
491 The free-living measures, employed during the natural breaks during a normal school day,
492 facilitated an ecologically valid assessment of the complex interaction between prior exercise,
493 free-living physical activity and postprandial metabolism and represents a major strength of
494 the current experimental design.

495

496 Despite considerable attempts to standardise prior free-living physical activity between
497 experimental conditions, subtle differences were observed in light and moderate free-living
498 physical activity during the 48 hours preceding Day 2 of the experimental model (day of
499 postprandial blood measures). An average daily discrepancy of 17 minutes of light physical
500 activity was observed during this period between CON and TM, whilst participants
501 performed, on average, 9 minutes more moderate activity in SOC compared with TM (Table
502 2). Whilst it is unlikely that such small amounts of additional free-living physical activity,
503 performed so far in advance (up to 48 hours prior) of the post-intervention blood measures,
504 exerted a meaningful influence on either TAG or glucose concentrations, this cannot be
505 dismissed entirely. Although between-condition variation in free-living activity is difficult to
506 avoid when studying paediatric populations in representative settings, we recognise that this

507 is a potential limitation of the study, but also the reality of working with free-living
508 adolescents.

509

510 **CONCLUSION**

511 The present study is the first to demonstrate the efficacy of after school small-sided soccer
512 games to reduce postprandial lipaemia in adolescent boys. Furthermore, the self-regulated
513 soccer activity resulted in a similar reduction in postprandial lipaemia compared with that
514 elicited by time-matched, moderate intensity treadmill exercise, despite participants covering
515 a shorter total distance at a lower average speed. These findings highlight the benefits in
516 metabolic health that can be gained by adolescents when games activity or similar sporting
517 activities are offered in a school setting.

518

519 **ACKNOWLEDGEMENTS**

520 The authors acknowledge the support of the North American Society for Pediatric Exercise
521 Medicine (NASPEM) and their awarding of the Marco Cabrera Student Research Award to
522 support this research.

523

524 This research was supported by the National Institute for Health Research (NIHR) Leicester
525 Biomedical Research Centre. The views expressed are those of the authors and not
526 necessarily those of the NHS, the NIHR or the Department of Health.

527

528 **CONFLICT OF INTEREST**

529 The authors declare no conflict of interest. The results of the present study do not constitute
530 endorsement by ACSM. The results of the study are presented clearly, honestly, and without
531 fabrication, falsification, or inappropriate data manipulation.

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677

678 **FIGURE LEGENDS**

679

680 **Figure 1.** Diagram of the 2-day study protocol. TAG, triacylglycerol. All food and drink
681 consumption was standardised and replicated across conditions.

682

683 **Figure 2.** Fasting (0 hours) and postprandial TAG concentrations for the three experimental
684 conditions. Black rectangles represent the consumption of breakfast and lunch,
685 respectively. TAUC-TAG was significantly reduced after SOC and TM compared
686 with CON ($P \leq 0.009$) but iAUC-TAG was not ($P \geq 0.078$).

687

Table 1 Participant characteristics ($n = 15$)

	Mean (SD)	Range		
Age (y)	12.6 (0.5)	11.7	to	13.3
Body mass (kg)	45.1 (6.8)	33.1	to	56.8
Stature (m)	1.56 (0.08)	1.44	to	1.68
Body mass index ($\text{kg}\cdot\text{m}^{-2}$)	18.5 (2.5)	14.7	to	23.1
Waist circumference (cm)	68.2 (8.1)	56.6	to	81.1
Body fat (%)	19.7 (7.4)	8.9	to	35.1
Genital development*	3 (1)	1	to	4
Pubic hair development*	3 (1)	1	to	4
Peak $\dot{V}\text{O}_2$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	54 (8)	37	to	66
Peak heart rate ($\text{beats}\cdot\text{min}^{-1}$)	201 (7)	190	to	215

* Self-assessment – median (interquartile range)

Table 2 Accelerometer data for free-living physical activity and sedentary time during the 48 hours preceding Day 2 of the experimental model across the three experimental conditions.

	CON	TM	SOC
Daily wear time (min)	735 (694 to 780)	764 (721 to 810)	780 (736 to 827)
Counts per minute	486 (402 to 586)	479 (397 to 579)	562 (466 to 680)
Sedentary time (min) †	519 (494 to 545)	535 (511 to 562)	512 (488 to 538)
Light activity (min) †	159 (146 to 174)	142 (130 to 155)	157 (144 to 172)
Moderate activity (min) †	42 (34 to 50)	38 (32 to 46)	47 (39 to 57)
Vigorous activity (min) †	19 (13 to 27)	17 (12 to 24)	18 (12 to 25)

Values are geometric means for $n = 13$. Statistical analyses were based on natural log transformed data. † Data adjusted for wear time in statistical analysis. No significant differences were observed across the experimental conditions ($P \geq 0.089$).

Table 3 Absolute and percentage of total game time spent in each speed zone classification. Also, absolute and percentage of total distance covered in each speed zone classification during the 48 min of 5-a-side soccer (SOC).

Speed Classification ($\text{km}\cdot\text{h}^{-1}$)		Time		Distance	
		min	%	m	%
Standing	(0 to 0.4)	4.9	10.1	4	0.1
Walking	(>0.4 to 3.0)	14.8	30.8	409	11.7
Low-intensity run	(>3.0 to 8.0)	20.7	43.2	1655	46.5
Medium-intensity run	(>8.0 to 13.0)	5.8	12.0	999	27.8
High-intensity run	(>13.0 to 18.0)	1.7	3.5	433	11.9
Maximal sprint	(>18.0)	0.2	0.4	71	2.0

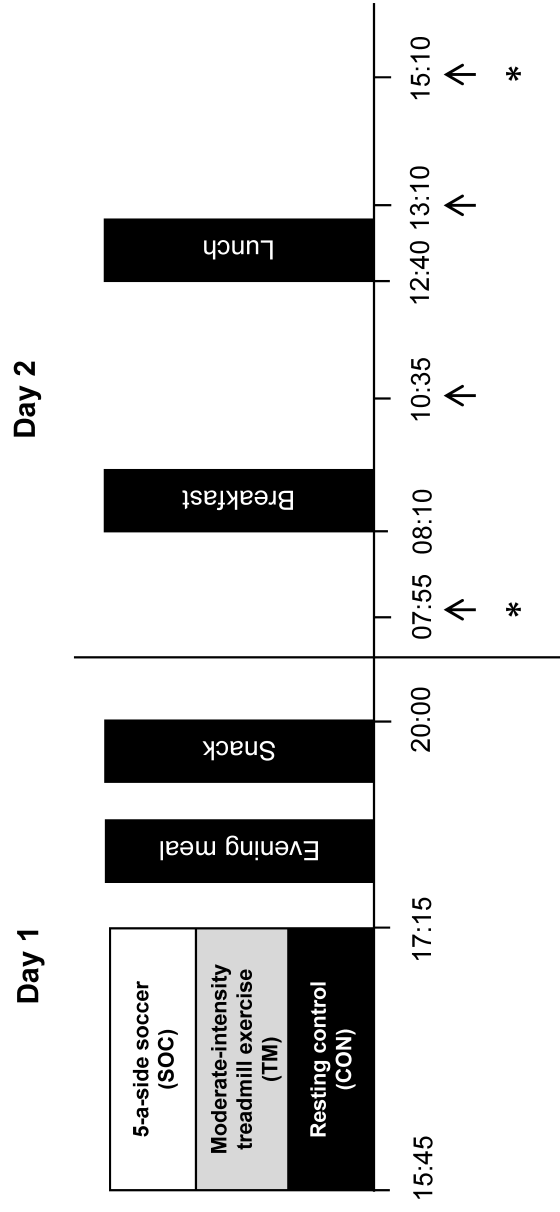
Values are mean (SD) for $n = 15$. The percentages represent the proportions of total time spent playing soccer and total distance moved during soccer.

Table 4 Fasting and total area under the curve (TAUC) for TAG and glucose in the CON, TM and SOC experimental conditions.

	Ratio Difference % (95% CI)					
	CON	TM	SOC	TM vs. CON	SOC vs. CON	SOC vs. TM
Fasting TAG (mmol·L ⁻¹)	0.80 (0.67 to 0.97)	0.68 (0.56 to 0.82)	0.56 (0.46 to 0.67)	-16 (-27 to -2) [†]	-30 (-40 to -20) [†]	-18 (-29 to -5) [†]
Fasting [glucose] (mmol·L ⁻¹)	5.08 (4.92 to 5.25)	4.95 (4.79 to 5.11)	4.91 (4.75 to 5.07)	-3 (-4 to -1) [†]	-4 (-5 to -2) [†]	-1 (-3 to 1)
TAUC-TAG (mmol·L ⁻¹)*	1.33 (1.08 to 1.65)	1.10 (0.89 to 1.36)	1.00 (0.81 to 1.24)	-18 (-29 to -5) [†]	-25 (-35 to -13) [†]	-9 (-21 to 5)
TAUC-glucose (mmol·L ⁻¹)*	6.10 (5.89 to 6.31)	5.87 (6.79 to 6.28)	6.04 (5.84 to 6.25)	0 (-3 to 2)	-1 (-3 to 2)	0 (-3 to 2)

Values are geometric means and corresponding 95% CI for $n = 15$. Pairwise comparisons are percentage difference (%) based on ratios of geometric means and corresponding 95% CI (%). Statistical analyses are based on natural log transformed data. † Statistically significant difference ($P < 0.05$). * TAUC values have been converted from mmol·L⁻¹ 7 h to mmol·L⁻¹ for clearer interpretation.

Figure 1



Key: ↑ Capillary blood sample for [TAG] and [glucose]
* Capillary blood sample for [haemoglobin] and haematocrit

Figure 2

