The effect of dietary carbohydrate manipulation on physical activity and other energy balance components: Study protocol for a 12-week randomised controlled trial

NCT03574987

Document v3 01/09/2023

Aaron Hengist[1], Russell G. Davies[1], Francoise Koumanov[1], Dylan Thompson[1], James A. Betts[1], Jean-Philippe Walhin[1], Peter J. Rogers[2], Jeff M. Brunstrom[2], Luc J.C. van Loon[3], Javier T. Gonzalez[1,*]

[1]Department for Health, University of Bath, UK

[2]School of Experimental Psychology, University of Bristol, UK

[3]Department of Movement Sciences, Maastricht University, Netherlands

Running title: Carbohydrate, physical activity and energy balance: RCT protocol

Key words: Carbohydrate, Physical Activity, Energy Balance

Word count: (excluding refs and fig legends)

Number of references:

24

25 *Corresponding author

26 Dr Javier T Gonzalez

27 J.T.Gonzalez@bath.ac.uk

28 Department for Health, University of Bath, BA2 7AY

29

30 Abstract

31 Carbohydrates are a staple component of modern diets. When classified by structure,
32 the smallest chain carbohydrates are referred to as sugars. Free sugars comprise
33 approximately 10-20% of energy intake in European populations. Global health
34 guidelines advocate reducing free sugars intake below 5% of energy intake. It is
35 unclear what impact these guidelines will have on physical activity and energy
36 balance. Little is known about the effects of total (sugar and non-sugar) carbohydrate
37 intakes on physical activity and energy balance too, therefore this study aims to
38 measure the behavioural and metabolic responses to manipulating the type and
39 amount of dietary carbohydrate in humans. Sixty humans, age 18-65 years, with a
40 body mass index between 18.5-29.9 kg·m^{-2} will be recruited for a randomised
41 controlled trial. Participants will be randomised to a control diet (MODSUG), a low-
42 sugar diet in line with public health guidelines (LOWSUG), or a low-carbohydrate
43 ketogenic diet (LOWCHO) for 12-weeks. Self-reported dietary intake and objectively-
44 measured physical activity will be monitored throughout the intervention. Participants
45 will undergo laboratory testing at baseline, week 4, and week 12 of the diet. This will
46 comprise muscle and adipose biopsies, a submaximal incremental treadmill protocol,
47 measures of body composition, resting metabolic rate, and a mixed-meal tolerance
48 test. Faecal samples will be obtained throughout the intervention to measure changes
49 to the gut microbiome. This research will provide evidence to inform public health
50 policy on the consumption of free sugars in healthy adults and will provide insight into
51 the physiological effects of a low-carbohydrate ketogenic diet.

52

53

54
55
56
57
58

59 # Introduction

60 Carbohydrates are a staple component of modern diets, which reportedly comprise
61 nearly 50% of energy intake in the United Kingdom (roughly 224 grams per day)
62 (Roberts et al., 2018). Dietary carbohydrates are commonly classified according to
63 their size and structure as mono- or disaccharides (1-2 monomers), oligosaccharides
64 (3-9 monomers), or polysaccharides (more than 9 monomers) (SACN, 2015). The
65 smallest chain carbohydrates (e.g. glucose, fructose, sucrose) are commonly referred
66 to as 'sugars', whereas polysaccharides are comprised of many molecules (e.g.
67 amylose, amylopectin). Some of the sugars in foods can be defined as 'free sugars',
68 which are 'all monosaccharides and disaccharides added to foods by the
69 manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and
70 unsweetened fruit juices' (Swan, Powell, Knowles, Bush, & Levy, 2018). Free sugars
71 by this definition reportedly comprise ~11% of energy intake in the United Kingdom
72 (57 grams per day) (Roberts et al., 2018), and intake across developed nations has
73 been reported to vary between 8% and 22% of energy intake (Wittekind & Walton,
74 2014). Both carbohydrates and free sugars are commonly ingested by many humans
75 in developed countries, and therefore an understanding of the metabolic and energy
76 balance effects of dietary carbohydrates and sugars is relevant to a wide population.

77

78 When referring to the intake of free sugars, this can often be considered as the co-
79 ingestion of glucose and fructose (as sucrose or table sugar). Common sources of
80 sucrose intake in Europe are soft drinks (sugar sweetened beverages), along with fruit
81 juices, fruits, cakes and dairy products (Sluik, Engelen, & Feskens, 2015), therefore
82 providing rationale for the specific targeting of sugar sweetened beverages with the
83 Soft Drinks Industry Levy in the UK (Barber, 2017). Public health guidelines across the

84 world advocate that energy intake from free sugars should make up no more than 5%
85 of an individual's total energy intake (Erickson, Sadeghirad, Lytvyn, Slavin, &
86 Johnston, 2017; SACN, 2015; World Health Organisation, 2015). There are various
87 reasons these guidelines may be beneficial for public health. There is reasonably
88 strong evidence (despite a lack of randomised controlled trials) that dietary sugar
89 intake is associated with the prevalence of dental caries (Freeman, 2014; Moynihan &
90 Kelly, 2014). Data around the effects of free sugars on metabolic health and body
91 weight are less conclusive. Increasing dietary sugar intake causes an increase in
92 energy intake (SACN, 2015). However, increasing (or decreasing) dietary free sugars
93 results in modest increases (or decreases) in body weight and isoenergetic exchange
94 of free sugars with other sources of carbohydrates does not result in weight change
95 (Morenga, Mallard, & Mann, 2013). Current evidence suggests that the increased
96 weight observed with increasing free sugar intake is predominantly due to an energy
97 surplus, via increasing energy intake. One rationale for reducing sugar intake,
98 therefore, is that this would create an energy deficit which would lead to weight loss
99 over time. However, an increase in energy intake without a clear change in body mass
100 indicates that there must be interactions with other aspects of energy balance. Current
101 evidence does not consider the effect of any compensatory changes in energy
102 expenditure. On the balance of current evidence, advice to reduce total sugar intake
103 seems sensible but a more complete understanding of the role of sugar intake on the
104 complex and intricate components of energy balance is warranted.

105

106 In recent years, the notion that carbohydrates *per se* are detrimental to metabolic
107 health has become prominent. Whilst the rate of absorption is dependent on the type
108 and matrix of carbohydrate, from a physiological perspective, ingestion of glucose as
109 a monomer or a polymer can be considered physiologically similar stimuli because
110 hydrolysis of glucose polymers is not thought to be rate-limiting to intestinal absorption
111 ((Gonzalez, Fuchs, Betts, & van Loon, 2017), suggesting the overall carbohydrate
112 dose is an important consideration for general health outcomes. Furthermore, it is well-
113 established that carbohydrate availability dictates the capacity to perform physical
114 work (Krogh & Lindhard, 1920), but the role of carbohydrate in regulating physical
115 activity behaviours has only recently been considered. Participants randomised to
116 consume a carbohydrate-rich breakfast display an increase in 24-hour physical activity

117 energy expenditure (PAEE) compared with those randomised to remain fasted until
118 midday (Betts et al., 2014). The magnitude of this difference is greatest prior to midday,
119 near to when carbohydrate had been ingested and when glucose uptake to peripheral
120 tissue is increased (Betts et al., 2014). This points towards a stimulatory role of
121 carbohydrate or sugar on PAEE when carbohydrate is readily available to peripheral
122 tissue. The amount of carbohydrate present in skeletal muscle is largely dictated by
123 the amount of carbohydrate in the diet (Bergstrom, Hermansen, Hultman, & Saltin,
124 1967). As physical activity is performed by skeletal muscle, dietary carbohydrate
125 intake may be an important regulator of physical activity behaviour.

126

127 Studies in which carbohydrate has been manipulated and physical activity has been
128 measured have not been sufficient in answering whether manipulating dietary
129 carbohydrate leads to a change in PAEE (Smith, Gonzalez, Thompson, & Betts, 2017).
130 Often self-report measures of physical activity are used, which are not sufficiently
131 sensitive to discern meaningful differences. Studies which have measured physical
132 activity objectively, i.e. using pedometers (Foraker et al., 2014) or accelerometers
133 (Layman et al., 2009; Tay et al., 2014), are confounded by a lack of information about
134 actual carbohydrate intake or concurrent prescription of exercise interventions. More
135 recent evidence using doubly-labelled water suggests that carbohydrate restriction
136 increases total energy expenditure during weight maintenance after weight loss
137 (Ebbeling et al., 2018), however this effect was influenced by the methods of analysis
138 (Hall & Guo, 2018). The authors originally planned to use pre-weight loss energy
139 expenditure as a baseline, but changed this to post-weight loss energy expenditure
140 (Hall & Guo, 2018). The use of doubly-labelled water is inappropriate to measure total
141 energy expenditure when either the body water pool changes by >3% or the average
142 respiratory exchange ratio is unequal to the food quotient for the duration of the
143 measurement (Internation Atomic Energy Agency, 2009). Ebbeling et al. (2018) used
144 an assumed respiratory quotient based on the food quotient (i.e. macronutrient
145 composition) of each participant (Black, Prentice, & Coward, 1986), but this did not
146 incorporate changes which occur during weight loss. One study which investigated the
147 effects of carbohydrate availability *per se* on energy expenditure using doubly labelled
148 water in 5 participants suggested that physical activity was reduced on a low
149 carbohydrate diet (7% carbohydrate, 83% fat, 10% protein) compared with a high

150 carbohydrate diet (83% carbohydrate, 7% fat, 10% protein) (Bandini, Schoeller, &
151 Dietz, 1994), which points towards a potentially stimulatory role of carbohydrates to
152 PAEE. To address these issues, studies using an objective method of measuring
153 PAEE, which allow for incorporation of changes in substrate oxidation, are required.

154

155 Government targets to reduce sugar intake below 5% of total energy intake are not
156 aimed at overall carbohydrate intake *per se*. In the breakfast study mentioned, sugar
157 intake was significantly greater amongst individuals who ate breakfast compared with
158 individuals who fasted until midday (Betts et al., 2014). As such, it is plausible that a
159 regulatory role of carbohydrate on PAEE may be due to the type of carbohydrate rather
160 than the absolute amount. Therefore, when assessing the potential role of
161 carbohydrates on physical activity, it is important to consider the potential role of both
162 carbohydrate type and amount. Therefore, we designed a study to investigate the role
163 of carbohydrate **type** and **amount** on energy balance and physical activity, which
164 addresses some of the limitations of previous research by objectively measuring free-
165 living PAEE, incorporating changes in substrate oxidation which occur with altering
166 carbohydrate availability. The purpose of this study is to measure the behavioural and
167 metabolic responses to manipulating the type and/or amount of dietary carbohydrate
168 in humans over a 12-week period. We approach this question from an integrated
169 physiological perspective. We are interested in energy balance, metabolism,
170 endocrinology, appetite, the gut microbiome, and food preference. The primary aim is
171 to investigate the effects of dietary carbohydrate manipulation on PAEE, using
172 combined accelerometry and heart rate monitoring, calibrated to each participant at
173 each time of measurement, based on substrate oxidation and resting metabolic rate.
174 This is the first study to objectively measure free-living energy expenditure
175 incorporating changes in substrate oxidation in response to carbohydrate
176 manipulation. The intervention arms are relevant from a public health perspective, with
177 a direct investigation of the effects of implementing current public health guidelines on
178 sugar intake. This research will provide evidence to inform public health policy on the
179 consumption of free sugars in healthy adults, and will provide insight into the
180 physiological effects of a low-carbohydrate ketogenic diet.

181

Methods

Study overview

Sixty metabolically healthy men and women, age between 18-65 years, will be recruited to participate in a randomised controlled trial with three arms. All data collection will be conducted in human physiology laboratories at the University of Bath. Following free-living assessment of habitual diet and physical activity level, participants will be randomised to adhere to one of three diets for a 12-week period in which the dietary carbohydrate content and/or type is manipulated. The macronutrient composition the intervention diets will aim for is as follows:

MODSUG (CONTROL) – 50% CHO (20% SUG), 15% PRO, 35% FAT

LOWSUG – 50% CHO **(<5% SUG**), 15% PRO, 35% FAT

LOWCHO – **<8% CHO*** (**<5% SUG**), 15% PRO, **≥77% FAT**

*or ≤50 g CHO per day

A schematic of progression through the study is presented in Figure 1. Participants will undergo three laboratory testing periods in total: at baseline, at week 4, and at week 12 of the diet. Diet and physical activity will be monitored for 7 days prior to randomisation, then at week 4 and week 12 of the intervention. Each laboratory test will comprise two laboratory visits on consecutive days for measures to be taken. On the first laboratory visit, participants will undergo muscle and adipose biopsies,

followed by a submaximal, incremental treadmill protocol to measure substrate oxidation and calibrate the activity monitor. The following morning, participants will return to the laboratory to undergo measures of body composition, resting metabolic rate, and a mixed-meal tolerance test. Participants will be provided with education on macronutrients and will be provided with guidance and feedback on which types of foods will help them achieve the macronutrient composition of the prescribed diets. Participants will also receive partial reimbursement of food expenses to help them achieve desired dietary changes. Whilst macronutrient composition will be manipulated, energy intake will not be prescribed.

[INSERT FIGURE 1 HERE]

Outcome measures

The primary outcome measure is physical activity energy expenditure (expressed as kJ per day).

Secondary outcome measures include time spent and energy expenditure at different intensities, weight, steps, energy intake, macronutrient intake, body composition and bone mineral density (using dual x-ray absorptiometry), waist:hip ratio, blood pressure, substrate oxidation, resting metabolic rate, interstitial glucose concentrations, fasting and postprandial metabolite and hormone concentrations, protein glycation, gene and protein expression in muscle and adipose tissue, muscle glycogen concentrations, urinary ketone concentrations, gut microbiome measures, urinary metabolomics, subjective appetite, and preference for food types. These are outlined in Figure 2 and will be assessed subject to funda available.

[INSERT FIGURE 2 HERE]

Participant characteristics and eligibility

The following are criteria for inclusion in the study:

- Body mass index 18.5-29.9 kg·m^{-2}
- Age 18-65 years
- Able and willing to provide informed consent and safely comply with study procedures
- Females to maintain record of regular menstrual cycle phase/contraceptive use
- No anticipated changes in physical activity during the first 4 weeks of the study (e.g. holidays or training programmes)

The following are criteria for exclusion from the study:

- Any reported condition or behaviour deemed either to pose undue personal risk to the participant or introduce bias
- Any diagnosed metabolic disease (e.g. type 1 or type 2 diabetes)
- Any reported use of substances which may pose undue personal risk to the participants or introduce bias into the experiment
- Lifestyle not conforming to standard sleep-wake cycle (e.g. shift worker)
- Any reported recent (<6 months) change in body mass (± 3%) (Stevens et al., 2006)
- Use of antibiotic medication in the last 3 months
- Use of prebiotic or probiotic products in the last month

Recruitment and enrolment

Participants will be recruited from the University of Bath campus and the local community by word of mouth, by poster advertisement, and by social media. Participation will be centred on the provision of obtaining informed consent prior the study and throughout participation. Participants will be sent an information sheet after expressing interest in the study and will be asked to read thoroughly. Where possible, researchers will talk through this information sheet in person. Participants will be asked to take time to consider and ask questions about the study over the following week. Pre-enrolment, participants will undergo a consultation to affirm the information

268 provided in the information sheet and provide clarity on the procedures that the
269 participant will undergo as part of the study. Once this information has been provided
270 and consent has been clarified verbally, participants will be asked to initial and sign an
271 informed consent form. Following this, participants will undergo eligibility screening by
272 completing a health questionnaire. We will assess body mass index to make sure they
273 meet this criterion. They will be reminded that their consent is completely voluntary
274 and will be reminded of their right to withdraw from the study procedures at any point
275 without providing justification.

276

277

278 ***Preliminary testing***

279 Once participants are formally enrolled on the study, they will visit the laboratory to
280 begin their preliminary measures. Participants will be asked to fast for ~4-5 hours prior
281 to visiting the laboratory to minimise interference with respiratory measures (Compher,
282 Frankenfield, Keim, Roth-Yousey, & Evidence Analysis Working, 2006). Height will be
283 measured using a stadiometer (Seca Ltd., Birmingham, UK) in the Frankfurt plane with
284 shoes removed. Body mass and bioelectrical impedance will be measured using digital
285 scales (Tanita, Amsterdam, The Netherlands) with participants barefoot and wearing
286 light clothing. Hip and waist circumference will be measured using a handheld tape
287 measure (Seca Ltd., Birmingham, UK).

288

289 Resting metabolic rate will be measured using the Douglas bag technique. Participants
290 will rest in bed in a recumbent supine position for ~10 minutes. When rested, the
291 participant will be given a mouthpiece and nose clip to wear, and three 5-minute
292 samples of expired air will be collected into Douglas bags. Researchers will enter the
293 room to change the Douglas bag at the end of each sample and note down ambient
294 conditions. The expired fraction of oxygen and carbon dioxide will be entered into
295 equations by Frayn and Jeukendrup & Wallis (Frayn, 1983; Jeukendrup & Wallis,
296 2005) to calculate resting metabolic rate (RMR) and substrate oxidation. The fractions
297 of inspired oxygen and carbon dioxide will be measured concurrently to correct for the
298 dynamic ambient conditions (Betts & Thompson, 2012). Detailed methods for indirect

299 calorimetry are outlined below in the '***Indirect calorimetry***' section. Then participants
300 will be asked to complete a food preference task on a laptop. The details of which are
301 outlined in the '***Food preference task***' section.

302

303 Participants will then complete a 20-minute treadmill walk to calibrate the physical
304 activity monitor to their substrate oxidation and energy expenditure, based on a
305 protocol adapted from Brage et al. (Brage et al., 2007). The walk will comprise four 5-
306 minute stages at 5.2 $km \cdot h^{-1}$ with progressive inclines of 0%, 3%, 6% to 9% with
307 participants wearing a heart rate monitor (Polar, Warwick, UK). During the last minute
308 of each stage, heart rate will be recorded and expired breath will be collected into a
309 Douglas bag to measure energy expenditure and substrate oxidation.

310 Following the treadmill walk, participants will receive an Actiheart monitor (CamNtech
311 Ltd., Cambridge, UK). This is a small chest-worn device which records heart rate, inter-
312 beat-interval, and physical activity energy expenditure. Participants will be shown how
313 to wear apply the device. They will be asked to keep it on for a 7-day period. This will
314 provide a free-living measure of energy expenditure. Participants will be provided with
315 a food diary and a set of portable weighing scales. They will be asked to record all
316 food and drink they ingest for a 7-day period coinciding with the 7-days of wearing the
317 Actiheart monitor. This will be used to evaluate macronutrient composition and will
318 provide an estimate of energy intake. The 7 days of actiheart and food diary will be
319 repeated in the 7 days prior the 4-week and 12-week laboratory tests during the
320 intervention. Participants will also be provided with a pedometer (3DTriSport, Realalt,
321 UK) which will be worn throughout the week of habitual monitoring and the 12-week
322 intervention.

323

324 Once participants have completed their week of habitual lifestyle monitoring and
325 laboratory testing has been arranged, they will be provided with a faecal collection kit
326 and asked to collect a faecal sample within 24 hours of visiting the lab. The kit will
327 contain 1 pair of disposable gloves, 1 "faeces catcher" paper, 1 collection pot, 1 plastic
328 Ziploc bag, 1 icepack and 1 small opaque cool bag for discreet transportation, along
329 with a detailed instruction sheet advising them how to collect the sample. Participants
330 will be advised to place the collection pot inside the Ziploc bag, and place this inside

331 the opaque cool bag to store in their fridge at 4 degrees, and then transport the sample
332 to the lab in the cool bag together with frozen icepacks. During the week-long
333 monitoring periods before each laboratory visit.

334

335 Participants will be fitted with a research-grade continual glucose monitor (CGM)
336 (Freestyle Libre, Abbott, UK) which will collect data for ~14 days. A member of the
337 research team will fit the continual glucose monitor and this will remain attached to the
338 participant until being removed by a member of the research team. A new CGM will
339 be fitted at 10 weeks into the intervention to record data between week 10 and 12. In
340 total, interstitial glucose will be measured for 7 days of habitual lifestyle, the first 7 days
341 of the diet intervention, and the final 14 days of the diet intervention.

342 ***Randomisation***

343 Participants will be randomised to one of three diet interventions by a member of the
344 research team who is not participant-facing. Allocation will be stratified on two levels:
345 by sex (male *vs* female), and mean physical activity level (PAL) across the habitual
346 monitoring period (<1.70 *vs* ≥1.70). PAL is simple way of assessing an individual's
347 habitual physical activity, and is calculated by taking total energy expenditure and
348 dividing by basal metabolic rate. A PAL ≥1.70 is associated with lower risk of many
349 metabolic diseases including type 2 diabetes and cardiovascular disease
350 (FAO/WHO/USU, 2001). Due to the nature of the intervention, dropouts are expected.
351 As such, we will be conducting a rolling recruitment to achieve statistical power
352 whereby dropouts will be replaced by the next recruited individual. A dropout will be
353 defined as an individual who does not wish to continue with the diet intervention within
354 the first 4 weeks (i.e. initial 4 weeks will be a completer's analysis). When the first 4
355 weeks have been completed, any deviations in adherence to diets will be considered
356 as part of the research question (i.e. intention to treat analysis).

357

358 ***Laboratory tests***

359 There are three laboratory tests across the study, one at baseline, one at week 4, and
360 one at week 12. These require laboratory visits which will be split across two days.
361 Day one will take place in the afternoon ~16:30 and day two will take place the

362 following morning ~08:00. On day one, participants will be asked to arrive at the
363 laboratory following a minimum 5-hour fast and they will be asked not to perform
364 structured exercise in this period. They will undergo an abdominal adipose tissue
365 biopsy. The abdominal region will be sterilised with iodine and local anaesthetic
366 (lidocaine hydrochloride) will be administered. After 5 minutes, when the area is numb,
367 a 14 G needle attached to a 50 mL syringe will be inserted into the subcutaneous
368 abdominal adipose tissue. A vacuum will be created using the syringe and adipocytes
369 aspirated into the syringe via the needle. Pressure will be applied to stop any bleeding
370 then the wound will be dressed. Treatment and analysis of samples is outlined in the
371 section titled '***Adipose biopsy samples***'.

372

373 Participants will then be given time to rest before any other procedures are carried out.
374 When the participant is ready and willing, they will undergo a muscle biopsy from the
375 vastus lateralis. The participant's quadriceps will be sterilised with iodine before local
376 anaesthetic (lidocaine hydrochloride) is administered subcutaneously and on the
377 muscle fascia. When the area is numb, a small incision will be made to the skin and
378 muscle fascia using a sterile scalpel blade. Then a Bergstrom needle will be inserted
379 into the muscle belly and ~2-3 snips will be made with suction applied, as described
380 by Tarnopolsky et al. (Tarnopolsky, Pearce, Smith, & Lach, 2011). The cutaneous
381 incision will then be stitched up and pressure will be applied to the site. Muscle
382 sampling handling and analyses is outlined in the section titled '***Muscle biopsy
383 samples***'.

384

385 Following the muscle biopsy, participants will be allowed time to recover and rest. They
386 will then complete a graded exercise protocol on the treadmill. This will mirror the
387 Actiheart calibration protocol outlined in the '***Preliminary measures***' section, with four
388 5-minute stages where the speed of the treadmill will be fixed but the gradient will be
389 increased. Participants will be asked to walk at 5.2 $km \cdot h^{-1}$ at 0%, 3%, 6%, and 9%
390 incline. During the last minute of each stage heart rate will be measured using a heart
391 rate monitor (Polar, Warwick, UK), substrate oxidation and energy expenditure will be
392 measured by collecting expired air in Douglas bags, and ratings of perceived exertion
393 (RPE) will be measured using Borg's 6-20 scale (Borg, 1970). Participants will be

394 asked to have a relaxing evening at home ready for the continuation of the laboratory
395 testing the following morning.

396

397 On day two, participants will be asked to arrive to the laboratory at around 08:00,
398 following an overnight fast of 10-12 hours. They will be asked to drink a pint of water
399 between waking and attending the laboratory and will be asked to refrain from
400 performing physical activity during their commute (i.e. take the bus or car, instead of
401 walking or cycling). Height will be measured using a stadiometer (Seca Ltd.,
402 Birmingham, UK), with participants barefoot in the Frankfurt plane. Body mass will be
403 measured using digital scales (Tanita, Amsterdam, The Netherlands) with participants
404 barefoot and wearing light clothing. Hip and waist circumference will be measured
405 using a handheld tape measure (Seca Ltd., Birmingham, UK). Following this,
406 participants will undergo a whole-body dual x-ray absorptiometry (DXA) scan to
407 assess body composition and bone mineral density.

408

409 At ~08:45, participants will be escorted to the resting laboratory where they will rest in
410 a recumbent supine position for 15 minutes. When rested, the participant will be given
411 a mouthpiece and nose clip to wear, and three 5-minute samples of expired air will be
412 collected into Douglas bags. Researchers will enter the room to change the Douglas
413 bag at the end of each sample and note down ambient conditions. The expired fraction
414 of oxygen and carbon dioxide will be entered into equations by Frayn and Jeukendrup
415 & Wallis (Frayn, 1983; Jeukendrup & Wallis, 2005) to calculate resting metabolic rate
416 (RMR) and substrate oxidation. The fractions of inspired oxygen and carbon dioxide
417 will be measured concurrently to correct for the dynamic ambient conditions (Betts &
418 Thompson, 2012). Detailed methods for indirect calorimetry are outlined in the
419 ‘***Indirect calorimetry***’ section.

420

421 Following this, a measure of blood pressure will be taken using an automated
422 sphygmomanometer (Diagnostec EW3106, Panasonic, Japan). A cannula will then be
423 inserted into a dorsal hand or antecubital vein and the hand of the corresponding arm
424 will be placed in a box heated to 55°C to arterialise the blood (Edinburgh et al., 2017).

425 Two mL of blood will be drawn to make sure blood is flowing from the cannula and
426 then it will be flushed with sterile saline solution (B. Braun, Pennsylvania, USA) to
427 maintain patency. Whilst waiting for the vein to arterialise, the participant will complete
428 visual analogue scales (VAS) for appetite and mood; these are 100-mm scales which
429 are attached to a statement with opposing extremes (e.g. not at = 0, extremely = 100).
430 Following this, participants will complete a food preference task; details of which are
431 outlined in the section titled '***Food preference task***'. Once this is complete, a baseline
432 blood sample will be collected. For all blood samples, 2 mL blood will be drawn and
433 disposed of, 10 mL blood will be collected for analysis, and then the cannula will be
434 flushed with 5-10 mL of sterile saline solution (B. Braun, Pennsylvania, USA) to
435 maintain patency. All samples will be dispensed into 2 sterile collection tubes. One
436 containing ethylenediaminetetraacetic acid (EDTA) (Sarstedt, Nümbrecht, Germany)
437 which will be immediately centrifuged to extract blood plasma. The other containing
438 plastic beads (Sarstedt, Nümbrecht, Germany) which will be allowed to clot at room
439 temperature for 15 minutes before being centrifuged to extract blood serum. All
440 samples will be centrifuged at 4000 g for 10 minutes at 4°C. Blood serum and blood
441 plasma will be aliquoted into Eppendorf tubes (Eppendorf, Hamburg, Germany) and
442 stored on dry ice for the remainder of the visit, before being moved and stored at -
443 80°C until analysis.

444

445 Following the baseline blood sample, participants will be provided with a mixed meal
446 tolerance test (MMTT). Participants will ingest a milk chocolate milk shake (Ensure
447 Plus, Abbott, Illinois, US) equating to 30% of resting metabolic rate as determined by
448 indirect calorimetry in the first laboratory test (e.g. 600 kcal for an individual with a
449 resting metabolic rate of 2000 kcal·day^{-1}). The composition of this drink is similar in
450 composition to the control diet and is reflective of typical food intake of European
451 populations; the contribution of macronutrients to total calories are 54% CHO (23%
452 SUG), 31% FAT, 15% PRO. A timer will be started when the participant ingests the
453 first sip of the drink and they will be asked to try and finish ingesting the MMTT within
454 5 minutes. Blood will be collected at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240
455 minutes following ingestion of the MMTT. VAS measures will be collected each hour
456 minutes following ingestion of the MMTT. A 5-minute expired breath samples of will
457 be collected at each hour following ingestion of the MMTT to measure dietary induced

458 thermogenesis. Blood pressure will also be measured each hour. Participants will be
459 asked to remain in a semi-recumbent supine position for the duration of the 240
460 minutes, unless they need to use the toilet, and will be allowed to perform sedentary
461 tasks like watching television, reading, or working on a laptop. Participants will be
462 allowed to take their hand out of the heated box until 10 minutes prior each blood
463 sample to ensure the vein remains adequately arterialised. Towards the end of the
464 postprandial period, participants will be asked to complete a second food preference
465 task. Then, the cannula will be removed and pressure will be applied to prevent
466 bruising. Urine produced during the laboratory visit will be collected into a beaker for
467 measurement of urea nitrogen excretion, relevant for indirect calorimetry measures.

468

469 ***Indirect calorimetry***

470 Energy expenditure and substrate utilisation will be determined using equations from
471 Frayn and Jeukendrup & Wallis (Frayn, 1983; Jeukendrup & Wallis, 2005), with
472 adjustments for the contribution of glycogen during low-intensity exercise:

473

474 Fat oxidation at rest and during exercise ($g \cdot min^{-1}$) =
475 $(1.695 \times VO_2) - (1.701 \times VCO_2) - (1.77 \times \text{urinary nitrogen excretion})$

476

477 Carbohydrate oxidation at rest ($g \cdot min^{-1}$) =
478 $(4.55 \times VCO_2) - (3.21 \times VO_2) - (2.87 \times \text{urinary nitrogen excretion})$

479

480 Carbohydrate oxidation during exercise ($g \cdot min^{-1}$) =
481 $(4.344 \times VCO_2) - (3.061 \times VO_2) - (0.40 \times \text{urinary nitrogen excretion})$

482

483 At rest, these equations assume that glucose provides all the carbohydrate for
484 metabolism, whereas during low-intensity exercise carbohydrate metabolism is
485 achieved by an equal contribution from glucose and glycogen. These equations will
486 also be used with the assumption that the energy contents of fat, glucose, glycogen,
487 and nitrogen are 9.75, 3.74, 4.15, and 4.09 respectively (Jeukendrup & Wallis, 2005).

488

489 ### *Food preference task*

490 Participants will complete food preference tasks. These will comprise an ‘alternative
491 forced choice task’ and an ‘ideal portion size task’ (Wilkinson et al., 2012). The AFCT
492 consists of 18 plates of food which are individually photographed on a white plate or
493 transparent bowl. The participant will choose which food they would ‘choose to eat
494 right now’. Foods are distinguished into three categories: sweet high-carbohydrate
495 foods, non-sweet high-carbohydrate foods, non-sweet low-carbohydrate foods. The
496 outcomes of this test will indicate preference for different types of foods corresponding
497 to the categories above. The ideal portion size task consists of 3 test foods: chocolate
498 mousse, roasted potatoes, and vegetarian sausages (corresponding to the 3
499 categories above). These are photographed in portions ranging from 20-1000 kcal in
500 increments. Participants can increase or decrease the amount of food they desire at
501 by being instructed to imagine they are offered this food right now, using the left and
502 right arrow keys to adjust the portion to show how much of it they would eat.

503

504 ### *Adipose biopsy samples*

505 Samples will be cleaned using sterile saline solution (B. Braun, Pennsylvania, USA).
506 Cleaned adipose tissue will be aliquoted, weighed, and snap frozen in liquid nitrogen
507 before being stored at -80°C until further analyses. With the exact nature of the
508 analyses being dependent on the size of the sample, these samples will be assessed
509 primarily for gene expression (via real-time polymerase chain reaction) and protein
510 expression (via Western blotting). The sample required to perform these analyses are
511 ~50 and ~30 mg respectively.

512

513 ### *Muscle biopsy samples*

514 Muscle tissue samples will be promptly snap-frozen in liquid nitrogen and stored on
515 dry ice until being transferred to -80°C awaiting analysis. Tissue will be prioritised for
516 glycogen analysis, which will be performed similarly to previous studies (Jansson,
517 1981; van Loon, Saris, Kruijshoop, & Wagenmakers, 2000). Muscle tissue will be

518 freeze-dried before removal of non-muscle fibre material. Dried muscle tissue will be
519 heated in hydrochloric acid to hydrolyse glycogen to glycosyl units, before being
520 neutralised, and glucose concentration will be analysed. Leftover tissue will be used
521 to analyse gene expression (via real-time polymerase chain reaction) and protein
522 expression (via Western blotting), with the precise nature of the analyses to depend
523 on tissue sample size.

524

525 ***Faecal tissue handling and processing***

526 Faecal samples provided by participants will be homogenised, aliquoted and stored
527 frozen at -80°C for later processing. Total DNA will be extracted using a commercial
528 kit (QIAGEN QIAamp® Fast DNA Stool Mini Kit) and faecal water will be extracted for
529 metabolic profiling. Extracted DNA will be analysed to determine taxonomic
530 composition and functional potential via 16S rRNA sequencing and shotgun
531 metagenomic sequencing, respectively. A range of bioinformatic and statistical tools
532 will be used to determine taxonomic and functional diversity of microbes present. No
533 analyses will be conducted on the human components of the sequenced DNA. Faecal
534 water will be prepared through centrifugation and both faecal water and faecal matter
535 will be later processed using 'omic' technologies (e.g. mass spectrometry and nuclear
536 magnetic resonance) to determine presence of metabolites. Analyses of faecal and
537 urine samples collected will be conducted in Teagasc laboratories (Moorepark, Cork,
538 Ireland). On some occasions the analyses may be done in collaboration with a third
539 party including commercial companies. All samples and extracted DNA will be stored
540 at -80°C for future analysis.

541

542 ***Implementing the intervention and monitoring adherence***

543 Adherence to the study diets is a key component of the study and is one of the major
544 shortcomings of previous research into carbohydrate manipulation and energy
545 balance (Smith et al., 2017). There will be a focus on developing and maintaining a
546 friendly but professional relationship between the research team and participants –
547 easing them into the study procedures and familiarising them with the laboratories. To
548 further facilitate adherence, participants will be reimbursed up to a predetermined

549 amount of £18 per week for participants in the MODSUG or LOWSUG groups, and
550 £26 per week for participants in the LOWCHO group. This is due to the relative
551 difficulty in adhering to low carbohydrate diets and the relative expense of typically
552 suitable menus compared with the other groups. Members of the research team will
553 meet with participants weekly and will keep in touch with them via email to check how
554 they are finding the study. Participants will be asked to send a weekly measure of body
555 weight using scales they have been provided (Etekcity Digital Scales, California, USA)
556 and urinary ketone body concentrations in the fasted state (using Ketostix, Ascensia,
557 Newbury, UK). Participants will be asked to record 3-day food diaries during each
558 week. These will be analysed and feedback returned to the participant as soon as
559 possible. Feedback about what they have done well in the previous week and where
560 they might be able to improve to meet their macronutrient targets will also be provided.
561 We will focus on specific food items and tailor any substitutions to habitual dietary
562 habits.

563

564 The absolute cut-off of 50 g of CHO in the LOWCHO diet is according to
565 recommendations from ketogenic diet proponents (Volek & Phinney, 2012). This will
566 comprise 8% of energy intake if an individual's daily energy intake is 2337.5 kcal, with
567 the assumption that 1 g of carbohydrate provides 3.74 kcal of energy. It is anticipated
568 that, in most individuals, 50 g of carbohydrate will equate to ≤ 8% of energy intake.
569 However, in individuals consuming fewer kilocalories per day, the recommended
570 grams per day of carbohydrate will be decreased to equate to 8% of energy intake
571 (e.g. an individual consuming 1600 kcal per day will be asked to consume ~34 g of
572 carbohydrate per day).

573

574 We will attempt to check the accuracy of information from food diaries by rearranging
575 the energy balance equation, using measures obtained from DXA throughout the
576 study, as validated previously (Racette et al., 2012; Sanghvi, Redman, Martin,
577 Ravussin, & Hall, 2015). It is worth noting that the energy expenditure values will be
578 extrapolated from the 7 days of recording to reflect the time between measurements.
579 The energy content of the tissues may also be influenced by the change in water
580 content induced by the intervention, particularly in the ketogenic LOWCHO diet.

581 Hydration status has been shown to influence measures of non-fat mass by DXA with
582 little influence on fat mass measures (Toomey, McCormack, & Jakeman, 2017). For
583 this reason, we will interpret rearranged energy balance data in the context of these
584 limitations.

585

586 ***Diet analysis***

587 There is no consensus on the exact caloric value of each macronutrient because fibre
588 ingestion, water ingestion, energy balance, dietary macronutrient composition, dietary
589 food variety, chewing during meals, meal timing/distribution, preparation/cooking of
590 food, alcohol intake, physical activity level, sex, age, disease status, and stress are all
591 thought to influence the metabolisable energy of foods (Sanchez-Pena et al., 2017).
592 Further complicating individual variability in the energy harvested from macronutrients
593 is the gut microbiome, particularly through the production of short-chain fatty acids
594 (Canfora, Jocken, & Blaak, 2015). Despite these limitations, metabolisable caloric
595 values for each macronutrient have been estimated in the literature, suggesting that
596 the caloric values of carbohydrate, fat, protein, and alcohol are around 3.74, 9.75,
597 4.09, and 7.10 kcal·g^{-1} (Jeukendrup & Wallis, 2005; Morgan & Levine, 1988). Whilst
598 these exact values may be slightly contended amongst academics who may prefer to
599 use values from older studies (Atwater, 1910; Frayn, 1983), they have been chosen
600 because the same values have been used for oxidation of macronutrients during
601 measures of energy expenditure. Diet analysis for preliminary measures, the 3-day
602 food diaries for week 1-3 and week 5-11, and the 7-day food diaries from week 4 and
603 week 12 will be analysed using diet analysis software (Nutritics, Dublin, Ireland).
604 Grams for each macronutrient will be exported and these will be multiplied by the
605 caloric factor mentioned. This will be used to provide energy in kilocalories and as a
606 percentage of total energy intake. Total energy intake will not be fed back to
607 participants, just macronutrient proportions as a percentage of total energy intake. One
608 major limitation with current sugar guidelines is that they target 'free sugars', but there
609 is no legal requirement for manufacturers to include this information, the requirement
610 is to provide information on total sugars. Therefore, as we are unable to definitively
611 determine free sugars in commercial products, we will use all sugars other than from
612 fruits and vegetables as a proxy for 'free sugars'. We will report the following in our

613 results: carbohydrate, fat, protein, alcohol, total sugars, sugars from fruit and
614 vegetable sources, all other sugars. This means our intervention will target total sugars
615 minus fruit and vegetable sugars, as it is impossible to measure the intake of free
616 sugars *per se*.

617

618 ***Laboratory test design***

619 It is worth clarifying the rationale behind the test design. Obtaining muscle and adipose
620 biopsies in the fasted and rested state is preferable, but obtaining biopsies
621 immediately prior to the mixed meal tolerance test would risk these procedures
622 influencing metabolic measures. The treadmill walk should also be performed in the
623 fasted state, but there should be sufficient washout between the treadmill walk and the
624 mixed meal tolerance test. Furthermore, it is inappropriate to perform the treadmill
625 walk immediately before biopsies due to the potential effects this would have on gene
626 and protein expression. Therefore, it was decided to perform the treadmill walk
627 following the biopsy procedures, allowing for a rest period between the procedures
628 and the walk. This enables calibration of the Actiheart monitor to each participant's
629 individual physiology at each visit, which allows for fluctuations in substrate oxidation
630 and resting metabolic rate across the course of the intervention. The treadmill walk
631 acts to standardise physical activity levels the evening before the meal test, which
632 reduces the chance of seeing metabolic effects which are caused solely by the
633 previous evening's activity. Participants will attend the lab the morning after the biopsy
634 and treadmill walk in an overnight fasted state having eaten dinner a of their choice at
635 home. This allows assessment of body composition and the mixed meal tolerance test
636 to be performed prior to lunchtime of the second laboratory testing day.

637

638 ***Statistical analyses and power calculation***

639 A required sample size for the present study was estimated based on the Bath
640 Breakfast Project (Betts et al., 2014) using G*Power 3.1 software (Faul, Erdfelder,
641 Lang, & Buchner, 2007). The mean ± standard deviation PAEE for the fasting *vs*
642 breakfast groups during the morning (when differences in carbohydrate availability
643 between groups were present) were 311 ± 124 kcal vs 492 ± 227 kcal. Based on this

644 effect size, a between-subject design with 20 participants in each group would provide
645 an >85% chance (power) of detecting the expected effect with an α-level of 0.05.
646 Therefore, 60 participants will be recruited. As dropout with diet interventions is
647 expected to occur, a rolling recruitment model will be employed whereby dropouts are
648 replaced to achieve a complete dataset. The sample size estimations have been
649 based on the primary outcome of PAEE which, as the primary behavioural dependent
650 variable, also displays the most variance and thus the metabolic variables may require
651 fewer participants to achieve a similar power, given greater precision of measurement.

652

653 Descriptive statistics will be calculated on Microsoft Excel (Microsoft, Washington,
654 USA). Two-way ANOVAs will be used to identify significant interactions between
655 conditions and time across the study. Post hoc adjustments will be employed to
656 determine the nature of these differences using GraphPad Prism (GraphPad Software
657 Inc., California, USA). Both p-value hypothesis testing and inference-based statistics
658 will be utilised. Where p-value hypothesis testing is used, significance will be accepted
659 at $p = 0.05$.

660

661 **UPDATED STATISTICAL ANALYSIS PLAN**

662 For parallel group RCTs it is more efficient to use ANCOVA with baseline values as
663 the covariate to estimate the causal effects of an intervention (i.e., estimated
664 differences between interventions *vs.* control, see
665 https://doi.org/10.3945/ajcn.115.119768). Therefore, the statistical analysis plan was
666 adjusted accordingly in line with the following description. SPSS v25 (IBM, USA)
667 were used for statistical analyses. Total (tAUC) and incremental area under the
668 curve (iAUC) were calculated using the Time Series Response Analyser. Figures
669 were drawn using Prism v9.5.0 (GraphPad Software Inc., USA). For all outcomes
670 with quantitative units at week 4 and week 12, ANCOVA was used to assess
671 differences between groups with baseline values as the covariate. Unadjusted
672 means are presented for baseline outcomes, but ANCOVA-adjusted means (and
673 mean differences *vs* MODSUG) are reported for week 4 and week 12 unless
674 otherwise stated. Since skeletal muscle protein and adipose mRNA levels were
675 expressed as the fold-change from baseline, one-way ANOVAs were used at week 4

676 and week 12 respectively to detect differences between groups. *Post-hoc*
677 comparisons were made according to the principle of closed testing to assess the
678 effect of sugar restriction (LOWSUG vs MODSUG) or ketogenic carbohydrate
679 restriction (LOWCHO vs MODSUG). Data from Visual Analogue Scales were
680 assessed by repeated-measures ANOVA of within-group comparisons due to their
681 subjective nature. Figure legends state whether means or ANCOVA-adjusted means
682 are presented for each outcome. Simple linear regression and Pearson correlation
683 coefficients were used to assess linear associations between outcomes where
684 appropriate. Significance was accepted at $p \leq 0.05$. Data are presented as mean and
685 95% confidence intervals (CI) unless otherwise stated.

686

687

688

689

690

691

692

693

694

695

696

697

698

699

700

701

702

References

Agency, I. A. E. (2009). *Assessment of body composition and total energy expenditure in humans using stable isotope techniques*. Vienna: IAEA.

Atwater, W. O. (1910). *Principles of Nutrition and Nutritive Value of Food*. Washington DC: Government Printing Office.

Bandini, L. G., Schoeller, D. A., & Dietz, W. H. (1994). Metabolic differences in response to a high-fat vs. a high-carbohydrate diet. *Obes Res, 2*(4), 348-354. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16358395. doi:10.1002/j.1550-8528.1994.tb00074.x

Barber, S. (2017). The Soft Drinks Industry Levy. *House of Commons Library Briefing Paper, Number 7876*.

Bergstrom, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiol Scand, 71*(2), 140-150. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5584523. doi:10.1111/j.1748-1716.1967.tb03720.x

Betts, J. A., Richardson, J. D., Chowdhury, E. A., Holman, G. D., Tsintzas, K., & Thompson, D. (2014). The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults. *American Journal of Clinical Nutrition, 100*(2), 539-547. Retrieved from <Go to ISI>://WOS:000339599100007. doi:10.3945/ajcn.114.083402

Betts, J. A., & Thompson, D. (2012). Thinking outside the bag (not necessarily outside the lab). *Med Sci Sports Exerc, 44*(10), 2040; author reply 2041. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22986475. doi:10.1249/MSS.0b013e318264526f

Black, A. E., Prentice, A. M., & Coward, W. A. (1986). Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr, 40*(5), 381-391. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3771290.

Brage, S., Ekelund, U., Brage, N., Hennings, M. A., Froberg, K., Franks, P. W., & Wareham, N. J. (2007). Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity. *J Appl Physiol (1985), 103*(2), 682-692. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17463305. doi:10.1152/japplphysiol.00092.2006

Canfora, E. E., Jocken, J. W., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. In *Nat Rev Endocrinol* (Vol. 11, pp. 577-591). England.

Compher, C., Frankenfield, D., Keim, N., Roth-Yousey, L., & Evidence Analysis Working, G. (2006). Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J Am Diet Assoc, 106*(6), 881-903. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16720129. doi:10.1016/j.jada.2006.02.009

Ebbeling, C. B., Feldman, H. A., Klein, G. L., Wong, J. M. W., Bielak, L., Steltz, S. K., . . . Ludwig, D. S. (2018). Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. *BMJ, 363*, k4583. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/30429127. doi:10.1136/bmj.k4583

Edinburgh, R. M., Hengist, A., Smith, H. A., Betts, J. A., Thompson, D., Walhin, J. P., & Gonzalez, J. T. (2017). Prior exercise alters the difference between arterialised and venous glycaemia: implications for blood sampling procedures. *Br J Nutr, 117*(10), 1414-1421. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28615090. doi:10.1017/S0007114517001362

748 Erickson, J., Sadeghirad, B., Lytvyn, L., Slavin, J., & Johnston, B. C. (2017). The Scientific Basis of
749 Guideline Recommendations on Sugar Intake: A Systematic Review. *Ann Intern Med, 166*(4),
750 257-267. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/27992898.
751 doi:10.7326/M16-2020
752 FAO/WHO/USU. (2001). *Human Energy Requirements: Report of a joint FAO/WHO/UNU expert*
753 *consultation. Rome, 17-24 October 2001*. Retrieved from
754 Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis
755 program for the social, behavioral, and biomedical sciences. *Behav Res Methods, 39*(2), 175-
756 191. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17695343.
757 Foraker, R. E., Pennell, M., Sprangers, P., Vitolins, M. Z., DeGraffinreid, C., & Paskett, E. D. (2014). Effect
758 of a low-fat or low-carbohydrate weight-loss diet on markers of cardiovascular risk among
759 premenopausal women: a randomized trial. *J Womens Health (Larchmt), 23*(8), 675-680.
760 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25029619.
761 doi:10.1089/jwh.2013.4638
762 Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl*
763 *Physiol Respir Environ Exerc Physiol, 55*(2), 628-634. Retrieved from
764 http://www.ncbi.nlm.nih.gov/pubmed/6618956.
765 Freeman, R. (2014). Moderate evidence support a relationship between sugar intake and dental caries.
766 *Evid Based Dent, 15*(4), 98-99. Retrieved from
767 https://www.ncbi.nlm.nih.gov/pubmed/25522936. doi:10.1038/sj.ebd.6401055
768 Gonzalez, J. T., Fuchs, C. J., Betts, J. A., & van Loon, L. J. (2017). Glucose Plus Fructose Ingestion for
769 Post-Exercise Recovery-Greater than the Sum of Its Parts? *Nutrients, 9*(4). Retrieved from
770 https://www.ncbi.nlm.nih.gov/pubmed/28358334

771 https://res.mdpi.com/nutrients/nutrients-09-00344/article_deploy/nutrients-09-00344-
772 v2.pdf?filename=&attachment=1. doi:10.3390/nu9040344
773 Hall, K. D., & Guo, J. (2018). Rapid Response: No Significant Effect of Dietary Carbohydrate versus Fat
774 on the Reduction in Total Energy Expenditure During Maintenance of Lost Weight. *BMJ, 363*,
775 k4583.
776 Jansson, E. (1981). Acid soluble and insoluble glycogen in human skeletal muscle. *Acta Physiol Scand,*
777 *113*(3), 337-340. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6285675.
778 doi:10.1111/j.1748-1716.1981.tb06904.x
779 Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by
780 means of gas exchange measurements. *Int J Sports Med, 26 Suppl 1*, S28-37. Retrieved from
781 http://www.ncbi.nlm.nih.gov/pubmed/15702454. doi:10.1055/s-2004-830512
782 Krogh, A., & Lindhard, J. (1920). The Relative Value of Fat and Carbohydrate as Sources of Muscular
783 Energy: With Appendices on the Correlation between Standard Metabolism and the
784 Respiratory Quotient during Rest and Work. *Biochem J, 14*(3-4), 290-363. Retrieved from
785 http://www.ncbi.nlm.nih.gov/pubmed/16742941.
786 Layman, D. K., Evans, E. M., Erickson, D., Seyler, J., Weber, J., Bagshaw, D., . . . Kris-Etherton, P. (2009).
787 A moderate-protein diet produces sustained weight loss and long-term changes in body
788 composition and blood lipids in obese adults. *J Nutr, 139*(3), 514-521. Retrieved from
789 http://www.ncbi.nlm.nih.gov/pubmed/19158228. doi:10.3945/jn.108.099440
790 Morenga, L. T., Mallard, S., & Mann, J. (2013). Dietary sugars and body weight: systematic review and
791 meta-analyses of randomised controlled trials and cohort studies. *Bmj-British Medical Journal,*
792 *346*. Retrieved from <Go to ISI>://WOS:000313867100001

793 https://www.bmj.com/content/bmj/346/bmj.e7492.full.pdf. doi:ARTN e7492

794 10.1136/bmj.e7492
795 Morgan, M. Y., & Levine, J. A. (1988). Alcohol and nutrition. *Proc Nutr Soc, 47*(2), 85-98. Retrieved from
796 https://www.ncbi.nlm.nih.gov/pubmed/3054901.

797 Moynihan, P. J., & Kelly, S. A. (2014). Effect on caries of restricting sugars intake: systematic review to
798 inform WHO guidelines. *J Dent Res, 93*(1), 8-18. Retrieved from
799 https://www.ncbi.nlm.nih.gov/pubmed/24323509. doi:10.1177/0022034513508954
800 Racette, S. B., Das, S. K., Bhapkar, M., Hadley, E. C., Roberts, S. B., Ravussin, E., . . . Group, C. S. (2012).
801 Approaches for quantifying energy intake and %calorie restriction during calorie restriction
802 interventions in humans: the multicenter CALERIE study. *Am J Physiol Endocrinol Metab,*
803 *302*(4), E441-448. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22127229.
804 doi:10.1152/ajpendo.00290.2011
805 Roberts, C., Steer, T., Maplethorpe, N., Cox, L., Meadows, S., Nicholson, S., . . . Swan, G. (2018).
806 *National Diet and Nutrition Survey: Results from Years 7 and 8 (combined) of the Rolling*
807 *Programme (2014/2015 to 2015/2016)*. Retrieved from Public Health England:
808 SACN. (2015). *Carbohydrates and Health*. London: Public Health England.
809 Sanchez-Pena, M. J., Mraquez-Sandoval, F., Ramirez-Anguiano, A. C., Velasco-Ramirez, S. F., Macedo-
810 Ojeda, G., & Gonzalez-Ortiz, L. J. (2017). Calculating the metabolizable energy of
811 macronutrients: a critical review of Atwater's results. *Nutr Rev, 75*(1), 37-48. Retrieved from
812 <Go to ISI>://WOS:000397100100004. doi:10.1093/nutrit/nuw044
813 Sanghvi, A., Redman, L. M., Martin, C. K., Ravussin, E., & Hall, K. D. (2015). Validation of an inexpensive
814 and accurate mathematical method to measure long-term changes in free-living energy
815 intake. *American Journal of Clinical Nutrition, 102*(2), 353-358. Retrieved from
816 http://www.ncbi.nlm.nih.gov/pubmed/26040640. doi:10.3945/ajcn.115.111070
817 Sluik, D., Engelen, A. I., & Feskens, E. J. (2015). Fructose consumption in the Netherlands: the Dutch
818 National Food Consumption Survey 2007-2010. *Eur J Clin Nutr, 69*(4), 475-481. Retrieved from
819 https://www.ncbi.nlm.nih.gov/pubmed/25514900. doi:10.1038/ejcn.2014.267
820 Smith, H. A., Gonzalez, J. T., Thompson, D., & Betts, J. A. (2017). Dietary carbohydrates, components
821 of energy balance, and associated health outcomes. *Nutr Rev, 75*(10), 783-797. Retrieved from
822 https://www.ncbi.nlm.nih.gov/pubmed/29028272. doi:10.1093/nutrit/nux045
823 Swan, G. E., Powell, N. A., Knowles, B. L., Bush, M. T., & Levy, L. B. (2018). A definition of free sugars
824 for the UK. *Public Health Nutr, 21*(9), 1636-1638. Retrieved from
825 https://www.ncbi.nlm.nih.gov/pubmed/29587886. doi:10.1017/S136898001800085X
826 Tarnopolsky, M. A., Pearce, E., Smith, K., & Lach, B. (2011). Suction-modified Bergstrom muscle biopsy
827 technique: experience with 13,500 procedures. *Muscle Nerve, 43*(5), 717-725. Retrieved from
828 http://www.ncbi.nlm.nih.gov/pubmed/21462204. doi:10.1002/mus.21945
829 Tay, J., Luscombe-Marsh, N. D., Thompson, C. H., Noakes, M., Buckley, J. D., Wittert, G. A., . . .
830 Brinkworth, G. D. (2014). A very low-carbohydrate, low-saturated fat diet for type 2 diabetes
831 management: a randomized trial. *Diabetes Care, 37*(11), 2909-2918. Retrieved from
832 http://www.ncbi.nlm.nih.gov/pubmed/25071075. doi:10.2337/dc14-0845
833 Toomey, C. M., McCormack, W. G., & Jakeman, P. (2017). The effect of hydration status on the
834 measurement of lean tissue mass by dual-energy X-ray absorptiometry. *Eur J Appl Physiol,*
835 *117*(3), 567-574. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28204901.
836 doi:10.1007/s00421-017-3552-x
837 van Loon, L. J., Saris, W. H., Kruijshoop, M., & Wagenmakers, A. J. (2000). Maximizing postexercise
838 muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid
839 or protein hydrolysate mixtures. *American Journal of Clinical Nutrition, 72*(1), 106-111.
840 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10871568.
841 Volek, J. S., & Phinney, S. D. (2012). *The Art and Science of Low Carbohydrate Performance*. Florida,
842 US: Beyond Obesity LLC.
843 Wilkinson, L. L., Hinton, E. C., Fay, S. H., Ferriday, D., Rogers, P. J., & Brunstrom, J. M. (2012). Computer-
844 based assessments of expected satiety predict behavioural measures of portion-size selection
845 and food intake. *Appetite, 59*(3), 933-938. Retrieved from
846 http://www.ncbi.nlm.nih.gov/pubmed/22989621. doi:10.1016/j.appet.2012.09.007

847 Wittekind, A., & Walton, J. (2014). Worldwide trends in dietary sugars intake. *Nutrition Research*
848 *Reviews, 27*(2), 330-345. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25623085

849 https://www.cambridge.org/core/services/aop-cambridge-
850 core/content/view/C34DAC1F784A3C8EE10E73AA7872C4E5/S0954422414000237a.pdf/div-
851 class-title-worldwide-trends-in-dietary-sugars-intake-div.pdf.
852 doi:10.1017/S0954422414000237
853 World Health Organisation. (2015). *Sugars intake for adults and children*. Geneva, Switzerland: WHO
854 Press.

855

856

857

858

859

860

861

862

863

864

865

866

867

868

869

870

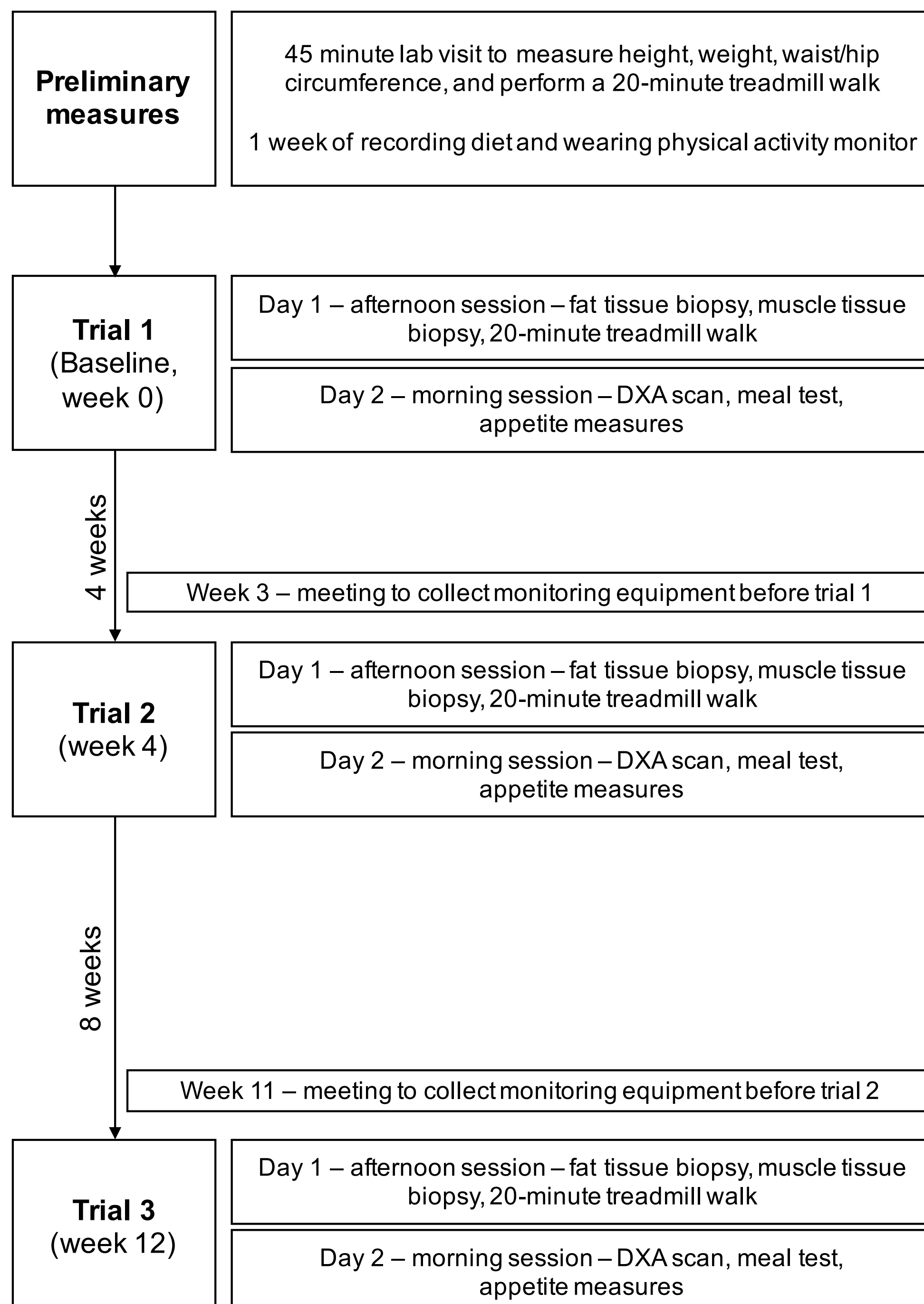
871

872

873

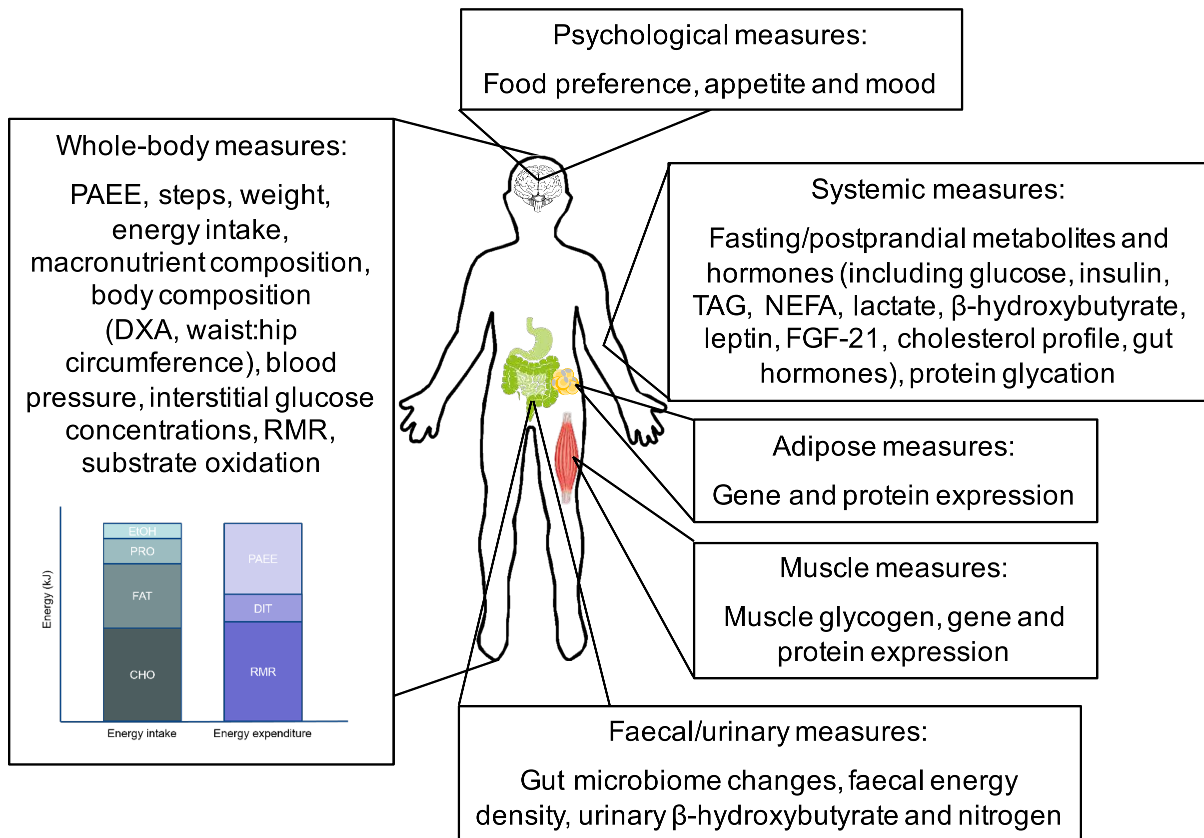
874 # Figures

875 **Figure 1. Schematic of trial progression. Participants will complete preliminary**
876 **measures and then be randomised to one of three trial arms. Laboratory visits**
877 **will comprise three trials at baseline, week 4, and week 12 of the intervention.**
878 **They will be split across two days, day one in the afternoon and day two the**
879 **following morning. Physical activity and diet will be measured for 7 days during**
880 **week 4 and during week 12. DXA = dual x-ray absorptiometry.**



881

882 **Figure 2. Schematic of outcome measures in the study. PAEE = physical activity**
883 **energy expenditure, DXA = dual x-ray absorptiometry, RMR = resting metabolic**
884 **rate, TAG = triacylglyceride, NEFA = non-esterified fatty acids, FGF-21 =**
885 **fibroblast growth factor 21.**



886