

RESEARCH ARTICLE

Preexercise breakfast ingestion versus extended overnight fasting increases postprandial glucose flux after exercise in healthy men

Robert M. Edinburgh,¹ Aaron Hengist,¹ Harry A. Smith,¹ Rebecca L. Travers,¹ Francoise Koumanov,¹ James A. Betts,¹ Dylan Thompson,¹ Jean-Philippe Walhin,¹ Gareth A. Wallis,² D. Lee Hamilton,^{3,5} Emma J. Stevenson,⁴ Kevin D. Tipton,³ and Javier T. Gonzalez¹

¹Department for Health, University of Bath, Bath, United Kingdom; ²School of Sport, Exercise and Rehabilitation, University of Birmingham, Birmingham, United Kingdom; ³Physiology, Exercise and Nutrition Research Group, University of Stirling, Stirling, United Kingdom; ⁴Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, United Kingdom; and ⁵School of Exercise and Nutrition Sciences, Deakin University, Victoria, Australia

Submitted 23 April 2018; accepted in final form 2 August 2018

Edinburgh RM, Hengist A, Smith HA, Travers RL, Koumanov F, Betts JA, Thompson D, Walhin JP, Wallis GA, Hamilton DL, Stevenson EJ, Tipton KD, Gonzalez JT. Preexercise breakfast ingestion versus extended overnight fasting increases postprandial glucose flux after exercise in healthy men. *Am J Physiol Endocrinol Metab* 315: E1062–E1074, 2018. First published August 14, 2018; doi:10.1152/ajpendo.00163.2018.—The aim of this study was to characterize postprandial glucose flux after exercise in the fed versus overnight fasted state and to investigate the potential underlying mechanisms. In a randomized order, twelve men underwent breakfast-rest [(BR) 3 h semirecumbent], breakfast-exercise [(BE) 2 h semirecumbent before 60 min of cycling (50% peak power output)], and overnight fasted exercise [(FE) as per BE omitting breakfast] trials. An oral glucose tolerance test (OGTT) was completed after exercise (after rest on BR). Dual stable isotope tracers ([U-¹³C] glucose ingestion and [6,6-²H₂] glucose infusion) and muscle biopsies were combined to assess postprandial plasma glucose kinetics and intramuscular signaling, respectively. Plasma intestinal fatty acid binding (I-FABP) concentrations were determined as a marker of intestinal damage. Breakfast before exercise increased postexercise plasma glucose disposal rates during the OGTT, from 44 g/120 min in FE [35 to 53 g/120 min [mean (normalized 95% confidence interval)] to 73 g/120 min in BE [55 to 90 g/120 min; $P = 0.01$]]. This higher plasma glucose disposal rate was, however, offset by increased plasma glucose appearance rates (principally OGTT-derived), resulting in a glycemic response that did not differ between BE and FE ($P = 0.11$). Plasma I-FABP concentrations during exercise were 264 pg/ml (196 to 332 pg/ml) lower in BE versus FE ($P = 0.01$). Breakfast before exercise increases postexercise postprandial plasma glucose disposal, which is offset (primarily) by increased appearance rates of orally ingested glucose. Therefore, metabolic responses to fed-state exercise cannot be readily inferred from studies conducted in a fasted state.

breakfast; exercise; glycemia; insulin sensitivity; metabolism

INTRODUCTION

Postprandial glycemia is a strong predictor of future mortality and morbidity. Even in people without diabetes, those with greater blood glucose excursions after feeding are at an increased risk of cardiovascular disease (47, 48). This glycemic

response to food ingestion is dictated by blood glucose kinetics (i.e., the balance between the rates of glucose appearance into blood and glucose disposal from blood into peripheral tissues). Exercise potentially increases glucose disposal from the blood into skeletal muscle (52), and regular exercise is therefore recommended as a lifestyle strategy to improve glycemic control.

Habitual responses to exercise and nutrition are, however, the culmination of not only chronic adaptations but also the acute effects of each exposure to these daily behaviors (5, 6, 22). For example, each bout of exercise potentially stimulates postexercise insulin sensitivity and muscle glucose uptake (52). However, despite increases in blood glucose disposal rates, endurance-type exercise does not always reduce postprandial glucose excursions in the postexercise period (20, 54). The finding that postprandial blood glucose concentrations are not lowered after exercise is because when exercise is performed (at least in the fasted state), the increase in postprandial blood glucose disposal after exercise can be offset—and even superseded—by increases in both endogenous and meal-derived blood glucose appearance rates (34, 54).

Although fasting before laboratory trials is common to control for baseline metabolic status, these conditions may preclude the application of findings to situations most representative of daily living. For example, most people living in developed countries spend the majority of a typical day in the postprandial state (13, 55). Therefore, most eating occasions and exercise sessions will take place in the context of this postprandial situation (23). It has previously been shown by others that plasma glucose fluxes during exercise (16), and by us that plasma glucose concentrations after exercise (24), are elevated by preexercise feeding. However, the effect of prior feeding on postexercise plasma glucose flux has never, to our knowledge, been assessed. Therefore, there is a distinct lack of understanding regarding postprandial glucose kinetics under scenarios that are most representative of daily living, and it may not be valid to generalize existing observations of exercise in the fasted state. Moreover, an understanding of the underlying mechanisms responsible for any differences in postprandial glucose flux after exercise, with prior feeding versus fasting, is still required.

Address for reprint requests and other correspondence: J. T. Gonzalez, Dept. for Health, Univ. of Bath, Bath, BA2 7AY, United Kingdom (e-mail: J.T.Gonzalez@bath.ac.uk).

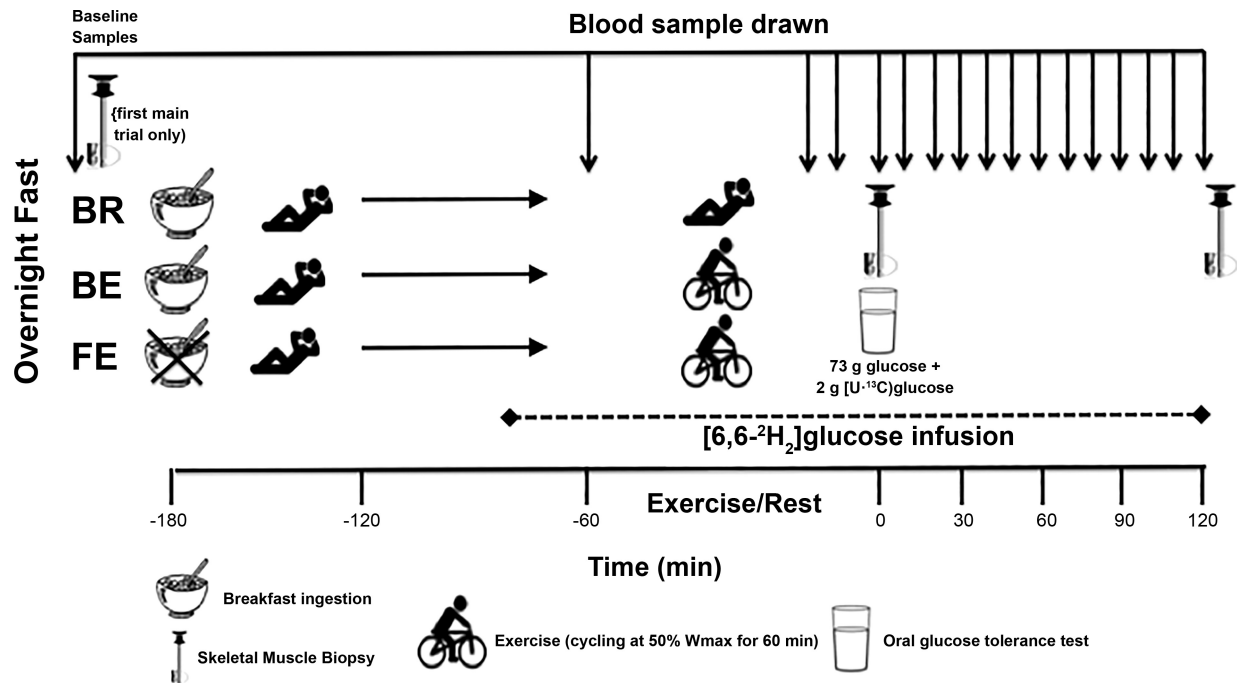


Fig. 1. Protocol schematic. An oral glucose tolerance test was conducted after breakfast followed by rest (BR), breakfast followed by exercise (BE), or extended overnight fasting followed by exercise (FE). Dual stable isotope tracers ($[U-^{13}C]$ glucose ingestion and $[6,6-^2H_2]$ glucose infusion) and muscle biopsies were combined to assess postprandial plasma glucose kinetics and intramuscular signaling, respectively. Wmax, maximum power output.

This study therefore aimed to characterize postprandial plasma glucose kinetics after 1) breakfast and rest (BR), 2) breakfast and exercise (BE), and 3) overnight fasted-state exercise (FE), while also exploring potential mechanisms (intramuscular signaling and markers of intestinal damage) to explain any differences in glucose flux between these conditions.

MATERIALS AND METHODS

Ethical approval. All trials were undertaken at the University of Bath (Bath, UK) in accordance with the Declaration of Helsinki. The study was approved by the National Health Service South-West Research Ethics Committee (reference no. 15/SW/0006) and registered at clinicaltrials.gov as no. NCT02258399. Written, informed consent was obtained from all participants before their participation.

Study design. This study was a randomized crossover design (randomization performed by J.T.G with Research Randomizer version 3.0, <https://www.randomizer.org/>). Preliminary testing was followed by three trials (separated by >7 days), namely, BR, BE, and FE. A schematic for the study protocol is shown in Fig. 1. For all trials, participants arrived at the laboratory after a 12- to 14-h overnight fast. In BR, a porridge breakfast was consumed, followed by 3 h of rest, and then a 2-h oral glucose tolerance test (OGTT). In BE, the same breakfast was consumed before 2 h of rest and 60 min of cycling before the OGTT. In FE, breakfast was omitted but the trial otherwise replicated BE. By necessity of design (food intake/exercise) the intervention was open label. Within-laboratory testing conditions were not different across the trials [(mean \pm SD) ambient temperature ($23.7 \pm 0.5^\circ\text{C}$ on BR, $23.7 \pm 0.6^\circ\text{C}$ on BE, $23.6 \pm 0.7^\circ\text{C}$ on FE) and barometric pressure (734 ± 5 mmHg on BR, 736 ± 6 mmHg on BE, 736 ± 5 mmHg on FE); all $P > 0.05$].

Participants. Twelve healthy and physically active men (self-reported as regular exercisers engaging in at least 30 min of exercise a minimum of 3 times per week) were recruited from Bath and North East Somerset between May and November 2015. Participant char-

acteristics are shown in Table 1. Exclusion criteria included any history of metabolic disease or conditions that may have posed undue personal risk to the participant or introduced bias to the study.

Preliminary testing. Participants were asked to refrain from strenuous physical activity for 24 h before preliminary testing but were asked to otherwise maintain their normal physical activity behaviors. They abstained from alcoholic and caffeinated drinks for 24 h before this visit. Food intake ceased at 2000 on the evening before testing, and participants fasted overnight (minimum 12 h), consuming only water (ad libitum) during this period. In addition, they were asked to consume 568 ml of water at least 1 h before testing and to void immediately before arriving at the laboratory. Upon arrival, the participant's stature was measured (Frankfurt plane) to the nearest 0.1 cm using a stadiometer (Seca Ltd., Birmingham, UK). Body mass was recorded to the nearest 0.1 kg (only light clothing permitted) using electronic weighing scales (BC543 Monitor, Tanita, Tokyo, Japan). A

Table 1. Participant characteristics

Characteristic	Value
Age (years)	23 (3)
Stature (cm)	179.8 (4.4)
Body mass (kg)	76.3 (7.9)
Body mass index (kg/m^2)	23.6 (2.90)
Fat mass (kg)†	10.6 (3.7)
Fat Mass Index (kg/m^2)†	3.26 (1.12)
Body fat (%)†	14 (4)
Fat free mass (kg)†	65.5 (6.4)
$\dot{V}O_{2\text{peak}}$ (l/min)*	4.00 (0.72)
$\dot{V}O_{2\text{peak}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)*	53 (10)
Peak power output (W)	317 (67)
HR_{MAX} (beats/min)	189 (10)

Data are presented as means and (standard deviation). $n = 12$. HR_{MAX} , max heart rate; $\dot{V}O_{2\text{peak}}$, peak oxygen uptake. * $n = 11$, because of technical difficulties with the breath-by-breath analysis during one participant's preliminary testing; †derived by dual-energy X-ray absorptiometry.

whole-body dual energy X-ray absorptiometry scan was completed to quantify fat and fat-free mass (Discovery, Hologic, Bedford, UK).

Participants then performed an incremental cycling exercise test at a self-selected cadence on an electronically braked ergometer (Excalibur Sport, Lode Lode Groningen, Netherlands). They were permitted to adjust the saddle and handlebar heights to their preferred position, which were replicated for cycling during the exercise trials. The initial exercise intensity was 50 W, and this was increased by 50 W every 4 min for 4 stages. Thereafter, the intensity was increased by 20 W every min until volitional exhaustion. Heart rate (Polar Electro Oy, Kempele, Finland) and continuous breath-by-breath measurements were recorded throughout (TrueOne2400, ParvoMedics, Sandy). Volume and gas analyzers were calibrated with a 3-l calibration syringe (Hans Rudolph, Kansas City) and a calibration gas (balance nitrogen mix; 16.04% O₂, 5.06% CO₂; BOC Industrial Gases, Linde AG, Munich, Germany), respectively. Peak power output was calculated as the work rate of the last completed stage, plus the fraction of time spent in the final noncompleted stage, multiplied by the work rate increment. Peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) was calculated as the highest average $\dot{V}O_2$ over a rolling 30-s period.

Main trials. Participants refrained from strenuous physical activity, alcohol, and caffeine for 24 h before all trials. They recorded the composition of their evening meal on the day before the first main trial and replicated this meal for subsequent trials in accordance with procedures for standardizing postprandial glucose tolerance testing (10). This pretrial standardization protocol has been previously shown to be effective at producing overnight fasted muscle glycogen concentrations, liver glycogen concentrations, and intramyocellular lipids, which are standardized across multiple trial days in a similar population (21). To help ensure physical activity standardization, participants completed a physical activity diary and wore a physical activity monitor (Actiheart, Cambridge Neurotechnology, Papworth, UK) for 24 h before all trials (pretrial 24-h physical activity energy expenditure [(mean \pm SD) 988 \pm 500 kcal on BR, 1,022 \pm 521 kcal on BE, 992 \pm 313 kcal on FE; all $P > 0.05$; $n = 9$].

Participants arrived at the laboratory at 0800 \pm 1 h following a 12- to 14-h overnight fast, and this arrival time was replicated for the subsequent trials. They were asked to void, and all further urine samples were collected for the remainder of the trial to allow for urinary nitrogen excretion to be estimated from urine urea concentrations. Participants then placed their dominant hand into a heated-air box (Mass Spectrometry Facility; The University of Vermont & University of Vermont Medical Center, Burlington, VT) set to 55°C. After 20 min of rest, an intravenous catheter (BD Venflon Pro, BD, Helsingborg, Sweden) was fitted into a heated dorsal hand vein (retrograde), and a 10-ml baseline blood sample was drawn before a 5-min expired gas sample was collected. On the first main trial for each participant (see Fig. 1), a baseline muscle sample was taken from the vastus lateralis to allow for an assessment of the pathways involved in exercise and insulin signaling in the muscle [5' AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), protein kinase B (herein referred to as Akt2), and Akt substrate of 160 kDa (AS160)].

In BE and BR, a porridge breakfast was then consumed within 10 min (3 h pre-OGTT) and in FE, participants were allowed water only. The breakfast was 72 g of instant refined oats (Oatso Simple Golden Syrup, Quaker Oats) and 360 ml of semiskimmed milk (Tesco), providing 431 kcal of energy [(1,803 kJ) 65 g carbohydrate, 11 g fat, 19 g protein]. The breakfast was high carbohydrate (57% of energy intake) and had a high glycemic index [oatmeal, made from mix (Quaker Oats) has a glycemic index of 69 (17)], as is commonly consumed in developed countries. Because of the coingestion of milk, this breakfast would produce a high insulinemic response (38, 46). Breakfast consumption (or omission on FE) was followed by 2 h of rest, where participants remained in a semirecumbent position, completing resting activities (e.g., watching television), with expired gas samples collected every 60 min. After 1 h and 40 min of rest (1 h and

20 min pre-OGTT), a catheter was inserted into an antecubital vein (the contralateral arm to the one used for blood sampling). A primed infusion of [6,6-²H₂]glucose was initiated and continued for the remaining within-laboratory component of the trial (Cambridge Isotope Laboratories, MA; prime: 13.5 $\mu\text{mol/kg}$; infusion: 0.35 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). After 20 min (60 min pre-OGTT) and on BE and FE only participants began 60 min of cycling at 50% peak power output on an ergometer (Lode Corival, Lode B.V., Groningen, Netherlands). The cadence was self-selected (replicated for both exercise trials), and the power output was monitored via a computerized system. In BR, participants remained rested in the semirecumbent position during this period. Expired gas samples were collected every 15 min, and blood samples were collected at 40 and 50 min of exercise (20 and 10 min pre-OGTT). Immediately after exercise (or after rest in BR) a muscle sample was taken from the vastus lateralis. Then a 2-h OGTT was completed, with arterialized blood sampled at 10-min intervals and expired gas sampled every 60 min. The OGTT was 73 g of glucose (81 g of dextrose monohydrate when corrected for water content; Myprotein, Northwich, UK) and 2 g of [U-¹³C]glucose (99%; Cambridge Isotope Laboratories, MA) to allow the rate of appearance of the orally ingested glucose (R_{OGTT}) to be assessed. A final muscle sample was taken post-OGTT (OGTT 120 min).

Tracer approach, blood sampling, and analysis. A dual-tracer approach was employed, where the tracer infusion rate was doubled during exercise (on BE and FE) to account for an expected increase in endogenous glucose production (1) and reduced to 80% of baseline at OGTT 20 min (all trials) to account for an expected suppression of endogenous glucose production after oral glucose ingestion (9). This approach reduces changes in the tracer-to-tracee ratio, thereby permitting more accurate estimations of glucose kinetics (4). Arterialized blood was sampled from a heated dorsal hand vein at baseline at 60-min intervals during the initial 2-h rest period, at 40 and 50 min of the exercise period (or after rest in BR), and at 10-min intervals during the OGTT. Whole blood was dispensed into ethylenediaminetetraacetic acid-coated tubes (BD, Oxford, UK), which were first centrifuged (4°C and 3,500 g) for 10 min (Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., UK) to obtain plasma. The plasma was then dispensed into 0.5-ml aliquots and immediately frozen at -20°C before longer-term storage at -80°C .

Plasma glucose [intra-assay coefficient of variation (CV) 3.2%; interassay CV 3.8%], lactate (intra-assay CV 1.0%; interassay CV 4.8%), and triglyceride (intra-assay CV 1.4%; interassay CV 4.0%) concentrations were measured using an automated analyzer (Daytona, Randox Laboratory, Crumlin, UK) as per the manufacturer's instructions. Plasma insulin concentrations were measured using a commercially available ELISA kit (Merckodia AB, Uppsala, Sweden; intra-assay CV 5.7%; interassay CV 9.9%). Plasma intestinal fatty acid binding protein (I-FABP) concentrations were measured as a marker of intestinal cell damage using a commercially available ELISA kit (Hycult Biotech; intra-assay CV 6.0%). Plasma nonesterified fatty acid (NEFA) concentrations were determined using an enzymatic colorimetric assay (WAKO Diagnostics; intra-assay CV 8.9%; interassay CV 10.4%). For all of these analyses, all plasma samples were analyzed in batch after all sample collection was completed and for a given participant all samples (from the three trials) were run on the same plate.

Plasma [U-¹³C]glucose and [2H₂]glucose enrichments were determined by gas chromatography-mass spectrometry (GC, Agilent 6890N; MS, Agilent 5973N; Agilent Technologies, Stockport, UK). Plasma glucose was extracted using methanol-chloroform and hydrochloric acid, dried under nitrogen gas, and then derivatised using the heptafluorobutyric acid method as previously described (30). The glucose derivative was acquired by selected ion monitoring at mass-to-charge ratios 519, 521, and 525 for [¹²C]-, [6,6-²H₂]-, and [U-¹³C]glucose, respectively. Glucose enrichments of [¹³C] and [2H₂] in plasma were determined using standard curves for [¹³C] and [2H₂] glucose, and enrichments were expressed relative to those at 519

(M + 0). The baseline sample was used for every trial to account for background isotopic plasma enrichments. To reduce any impact of analytical variability on calculations of glucose kinetics, glucose and enrichment data were curve fitted as previously described (63).

Muscle sampling and analysis. Muscle samples were collected from the vastus lateralis under local anesthesia (~5 ml of 1% lidocaine, Hameln Pharmaceuticals Ltd., Brockworth, UK). Samples were taken from a 3–5-mm incision at the anterior aspect of the thigh with a 5-mm Bergstrom biopsy needle technique adapted for suction (57). Samples were immediately extracted from the needle and frozen in liquid nitrogen before longer-term storage at –80°C. The order of dominant or nondominant leg was counterbalanced across trials for the OGTT 0- and 120-min samples. Samples were taken from separate skin incision sites, with these >2 cm proximal to any previous incision on the same leg (59). For the OGTT 0-min sample [after exercise (or after rest in BR)] the incision was made before cycling (BE and FE) and closed with Steristrips to allow for an immediate sample to be taken after exercise.

Frozen wet tissue (20–30 mg) was freeze-dried, powdered, and dissected free of visible blood and connective tissue and added to ice-cold lysis buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 0.5% sodium deoxycholate; 0.1% SDS and 0.1% Nonidet P-40] with a protease (Thermo Scientific) and phosphatase inhibitor cocktail (Millipore). Samples were homogenized with a dounce homogenizer (~40 passes), incubated for 60 min at 4°C with rotation, and centrifuged for 10 min (4°C and 20,000 g). The protein content of the resultant supernatant was measured via a bicinchoninic acid protein assay kit (Thermo Scientific). For Western blots, an equal amount of protein (40 µg) was loaded per lane for each sample and separated using SDS-PAGE on Tris-glycine SDS-polyacrylamide gels (7% for phosphorylated (p)-AMPK^{Thr172}, total AMPK, p-ACC^{Ser79}, and total ACC, 10% for p-Akt^{Ser473} and total Akt2, and 8% for p-Akt^{Thr308}, p-AS160^{Thr642}, and total AS160). Gels were electro-blotted using a semidry transfer onto a nitrocellulose membrane. Membranes were washed in Tris-buffered saline (0.09% NaCl, 100 mM Tris-HCl pH 7.4) with 0.1% Tween 20 (TBS-T) and incubated for 30 min in a blocking solution (5% nonfat dry milk in TBS-T; Marvel, Premier International Foods Ltd., UK). The membranes were incubated overnight at 4°C with primary antibodies against p-AMPK^{Thr172}, p-ACC^{Ser79}, p-Akt^{Ser473}, p-Akt^{Thr308}, and p-AS160^{Thr642} (Cell Signaling Technologies). In the morning, membranes were washed in TBS-T and incubated with a 1:4,000 dilution of antispecies IgG horseradish peroxidase-conjugated secondary antibodies made up in the aforementioned blocking solution. After further washes, membranes were incubated in an enhanced chemiluminescence reagent and visualized using a chemiluminescent imager (EpiChem II Darkroom, UVP, Upland). Nitrocellulose membranes were then incubated for 30 min at 50°C in a stripping solution (62.5 mM Tris pH 6.7, 2% SDS, 100 mM 2-mercaptoethanol) before reblotting for total AMPK, total ACC, total Akt2 (Cell Signaling Technologies), and total AS160 (Merck-Millipore, UK). For these analyses, all samples from each participant (all three trials) were run on the same gel. Band densities were quantified using VisionWorksLS Image Acquisition and Analysis Software for Windows (UVP, Upland). For all of the signaling molecules reported in this experiment, a ratio of phosphorylated to total protein was calculated, and the results were expressed relative to the baseline sample.

Substrate utilization. Expired gas samples were collected at baseline, during the initial 2-h rest period and the OGTT at 60-min intervals (for 5 min), and at 15-min intervals (for 1 min) during the exercise period (or rest in BR). For all samples, participants were provided with the mouthpiece 1 min before gas collections for a stabilization period. Samples were collected in 200-liter Douglas bags (Hans Rudolph, Kansas City) through falconia tubing (Baxter, Woodhouse and Taylor Ltd., Macclesfield, UK). Concurrent measures of inspired air were made to correct for changes in ambient O₂ and CO₂ concentrations (7). Expired O₂ and CO₂ concentrations were mea-

sured in a known volume of each sample, using paramagnetic and infrared transducers, respectively (Mini HF 5200, Servomex Group Ltd., Crowborough, East Sussex, UK). The sensor was calibrated using concentrations of low (99.998% N, 0% O₂ and CO₂) and high (balance nitrogen mix, 16.04% O₂, 5.06% CO₂) calibration gases (both BOC Industrial Gases, Linde AG, Munich, Germany). Urinary nitrogen excretion was estimated from urine urea concentrations, which were measured on an automated analyzer (Daytona; Randox Laboratory, Crumlin, UK) to allow for protein oxidation to be accounted for in calculations of substrate utilization rates.

Calculations and statistical analysis. A sample size estimation was completed a priori with the total rate of plasma glucose appearance as the primary outcome measure. Rose et al. (54) reported a difference in the total plasma glucose appearance of (mean ± SD) 1,600 ± 1,300 µmol/kg during an OGTT after rest versus after fasted exercise. Using this effect size, and an alpha level of 0.05, we calculated that 12 participants were required for an 80% probability of statistically detecting an effect in the total rate of plasma glucose appearance (Ra_{TOTAL}) using a crossover design with three trials and a two-tailed, one-way ANOVA.

The total and incremental area underneath the concentration-time curve (AUC or iAUC, respectively) for each plasma, metabolite or hormone was calculated using the trapezoid rule. The AUC or iAUC for each plasma metabolite or hormone was then divided by either the duration of the total within-laboratory period (300 min) or the OGTT observation period (120 min), as appropriate, to provide a time-averaged value (mmol/l), which are used as summary measures. Plasma glucose and insulin concentrations during the OGTT were used to estimate insulin sensitivity (ISI) according to the equation of Matsuda (41): [FPG and FPI are fasting plasma glucose and insulin concentrations, and MPG and MPI are mean plasma glucose and insulin concentrations in the OGTT (41)]:

$$ISI_{\text{MATSUDA}}(\text{au}) = \frac{10,000}{\sqrt{\text{FPG}(\text{mgdL}^{-1}) \times \text{FPI}(\text{mIU mL}^{-1}) \times \text{MPG}(\text{mgdL}^{-1}) \times \text{MPI}(\text{mIU mL}^{-1})}}$$

Plasma glucose kinetics were determined using Radziuk's two-compartment nonsteady-state model (50, 51) and SAMM II software (SAAM II v.2.3, The Epsilon Group, Charlottesville, VA). This model reduces errors in estimations of glucose kinetics that are apparent when using Steele's (56) one-compartment model (53). The Ra_{TOTAL} and glucose disappearance (Rd) were calculated as follows:

$$Ra_{\text{TOTAL}}(t) = \frac{F}{E_1(t)} - \frac{V_1 \times G(t)}{E_1(t)} \times \dot{E}_1(t) + k_{12} \left(\frac{q_2^{\text{iv}}(t)}{E_1(t)} - Q_2(t) \right) \quad (1)$$

$$Rd(t) = Ra_{\text{TOTAL}} - V_1 \times \dot{G}(t) - k_{21} \times V_1 \times G(t) + k_{12} \times sQ_2(t) \quad (2)$$

where F is the [6,6-²H₂] infusion, V₁ is the glucose volume of distribution [4% of body mass (kg)], E₁(t) is the [²H₂] plasma glucose enrichment (mole percent excess) at time t, $\dot{E}_1(t)$ is the change in E over time (derivate of E), G(t) is the plasma glucose concentrations at time t, $\dot{G}(t)$ the change in G over time (derivate of G), k₁₂ and k₂₁ are fixed rate constants between the peripheral and the accessible compartments (0.05/min and 0.07/min, respectively), and q₂^{iv} and Q₂ are the amounts of the tracer [²H₂] and tracee in the peripheral compartment, respectively, evaluated by integrating the two-compartment model.

The [U-¹³C] enrichment of the orally ingested glucose and the Ra_{TOTAL} (from Eqn. 1) were used to calculate the plasma rate of appearance of glucose from the OGTT (Ra_{OGTT}). In these equations, r₁ is the ratio of the infusion [²H₂] and oral [U-¹³C] glucose tracer concentrations in plasma, $\dot{r}(t)$ is the change in r over time (derivate of r), g is the [U-¹³C] glucose tracer in plasma, q_o^{iv} is the amount

of the $[U-^{13}C]$ tracer in the peripheral compartment (by integrating the two-compartment model), and E_{OGTT} is the $[U-^{13}C]$ enrichment of the OGTT.

$$ra^0(t) = Ra_{TOTAL}(t) \frac{F}{r_1(t)} - \frac{V_1 \times g(t)}{r_1(t)} \times \dot{r}_1(t) + k_{12} \left[\frac{q_2^{iv}(t)}{r_1(t)} - q_2^0(t) \right] \quad (3)$$

$$Ra_{OGTT}(t) = ra^0(t) \left[\frac{1}{E_{OGTT}} \right] \quad (4)$$

The metabolic clearance rate was calculated as the Rd divided by the plasma glucose concentrations for a given time point (G_1)

$$\text{Metabolic Clearance Rate} = Rd(t)/G_1 \quad (5)$$

Rates of whole-body fat and carbohydrate utilization were calculated using the expired gas samples and stoichiometric equations (31). Adjustments were made to account for the contribution made by the oxidation of protein (estimated via urinary urea nitrogen). Plasma glucose utilization was assumed to be equivalent to the plasma glucose Rd as has been confirmed previously (32). Muscle glycogen utilization during exercise (BE and FE only) was calculated as total

carbohydrate utilization during exercise minus plasma glucose utilization during exercise. Because of these methods, this estimate of muscle glycogen utilization will include the utilization of other nonglucose carbohydrates (e.g., lactate). Both the production and utilization of ketone bodies can influence the respiratory exchange ratio and therefore, theoretically complicate the estimates of carbohydrate oxidation during exercise. However, during short-duration, moderate-intensity exercise this effect is negligible (31). Within-laboratory energy expenditure was determined assuming that lipids, glucose, and glycogen give 40.81, 15.64, and 17.36 kJ/g respectively (31).

One-way, repeated measures ANOVAs were used to assess differences between trials at baseline and for summary measures (e.g., AUCs). If multiple comparisons were necessary, two-way repeated measures ANOVAs (time \times trial) were used to identify differences between trials. Degrees of freedom for F values were Greenhouse-Geisser-corrected for $\epsilon < 0.75$, with Huynh-Feldt corrections used for less severe asphericity. If time \times trial interaction effects were identified, multiple paired t -tests were used to locate variance, with Holm-Bonferroni step-wise adjustments to control for inflated type I errors. Pearson r and Spearman R were used to explore correlations between variables that display normal and nonnormal distribution, respectively. Unless otherwise stated, data in text, figures, and tables

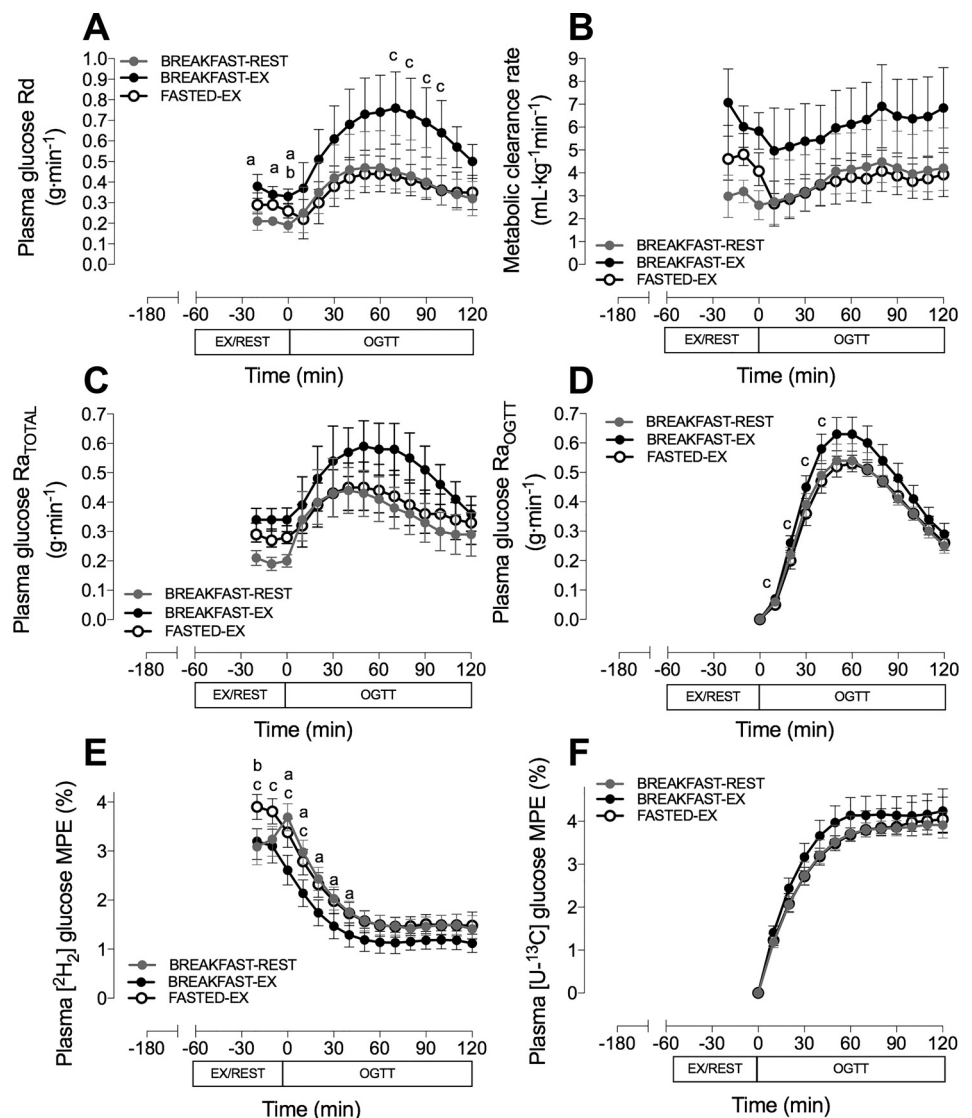


Fig. 2. The plasma glucose disposal rate (Rd) (A), metabolic clearance rate (B), the total rate of plasma glucose appearance (Ra_{TOTAL}) (C), the rate of appearance of glucose in plasma from the oral glucose tolerance test (Ra_{OGTT}) (D), and the plasma enrichments of $[^2H_2]$ -glucose (E) and $[^{13}C]$ -glucose (F) before and during an oral glucose tolerance test (OGTT) that was conducted after breakfast followed by rest (BREAKFAST-REST), breakfast followed by exercise (BREAKFAST-EX), or extended overnight fasting followed by exercise (FASTED-EX). Data are means \pm normalized 95% confidence intervals. $n = 12$ healthy men. ^aDifference between breakfast rest vs. breakfast exercise, ^bbreakfast rest vs. fasted exercise, and ^cbreakfast exercise vs. fasted exercise with $P < 0.05$. MPE, mole percent excess.

are means \pm 95% confidence intervals, which were normalized by removing between-subject variance (presented as 95% nCI) (39). All statistical analyses were completed using IBM SPSS statistics version 22 for windows (IBM, New York), with the exception of the Holm-Bonferroni step-wise adjustments and the calculation of normalized confidence intervals, which were completed using Microsoft Excel (2013). Graph Pad Prism 7 software (La Jolla, CA) was used for preparation of the manuscript figures. A complete set of muscle samples was only obtained from nine participants. Because of cannulation difficulties, for one participant's BR trial the last blood sample obtained was at OGTT 70 min, and for a different participant's BE trial the last sample was at OGTT 60 min. For these trials (2 of 36) the group average was used for the missing data. Sensitivity analysis was completed for all measures involving blood samples and including/excluding these two participants did not influence any of the primary outcome measures. For clarity, the n is presented in all figure and table legends.

RESULTS

Plasma glucose kinetics. The plasma glucose Rd displayed a time \times trial interaction ($F = 3.123$, $P = 0.05$), whereby

plasma glucose Rd was higher during exercise versus rest (Fig. 2A). Compared with extended overnight fasting, breakfast ingestion before exercise further increased the plasma glucose Rd during and after exercise (i.e., during the OGTT; Fig. 2A). A main effect of the trial was detected for the plasma glucose Rd during the OGTT ($F = 7.079$, $P = 0.01$), whereby the Rd was 45 g/120 min in BR (95% nCI: 36 to 62 g/120 min) versus 73 g/120 min in BE [(95% nCI: 55 to 90 g/120 min; $P = 0.09$ vs. BR) and 44 g/120 min in FE (95% nCI: 35 to 53 g/120 min); $P = 0.01$ vs. BE]. Metabolic clearance rates showed a main effect of trial, with the highest rates also apparent in BE (Fig. 2B; $F = 7.849$, $P < 0.01$ vs. BR and FE).

A main effect of trial was detected for Ra_{TOTAL} during the OGTT [(g/120 min); $F = 3.915$, $P = 0.05$], which was highest in BE (Fig. 2C). However, after post hoc adjustment the difference between trials was less apparent ($P = 0.19$ for BE vs. BR and $P = 0.09$ for BE vs. FE). A similar pattern was observed for the rate of appearance of glucose from the OGTT in plasma (Ra_{OGTT}) and a trial \times time interaction was detected (Fig. 2D; $F = 3.134$, $P = 0.04$). A main effect of trial was

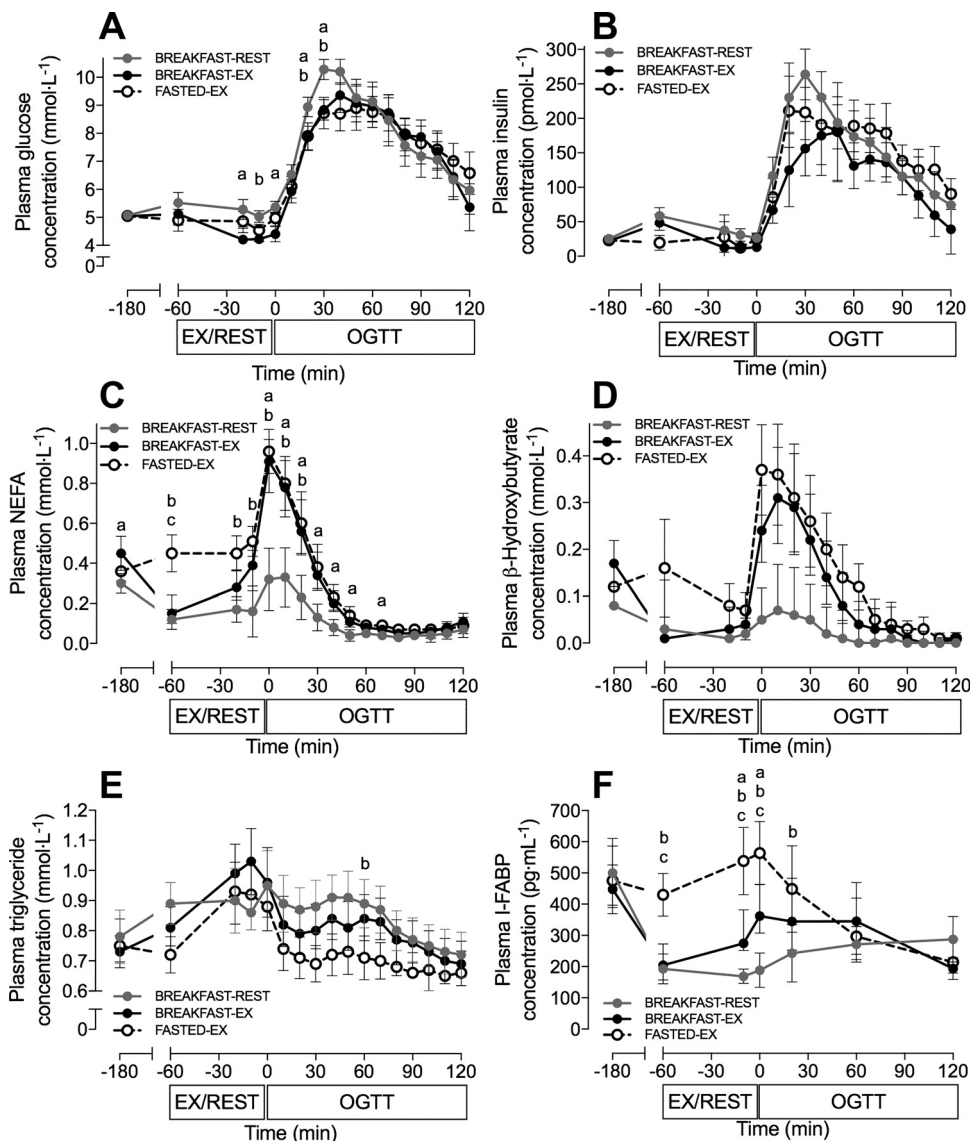


Fig. 3. Plasma glucose (A), plasma insulin (B), plasma nonesterified fatty acids (C), plasma β -hydroxybutyrate (D), plasma triglyceride (E), and plasma intestinal fatty acid binding protein (F) concentrations before and during an oral glucose tolerance test (OGTT) that was conducted after breakfast followed by rest (BREAKFAST-REST), breakfast followed by exercise (BREAKFAST-EX), or extended overnight fasting followed by exercise (FASTED-EX). Data are means \pm normalized 95% confidence intervals. $n = 12$ healthy men. ^aDifference between breakfast rest vs. breakfast exercise, ^bbreakfast rest vs. fasted exercise, and ^cbreakfast exercise vs. fasted exercise with $P < 0.05$. I-FABP, intestinal fatty acid binding protein; NEFA, nonesterified fatty acid.

detected for the total Ra_{OGTT} ($F = 5.915$, $P = 0.02$), which was 49 g/120 min in BE (95% nCI: 44 to 53 g/120 min) [65% of the OGTT (59% to 71%)] vs. 42 g/120 min in BR [95% nCI: 36 to 46 g/120 min] [56% of the OGTT (50% to 62%); $P = 0.11$ vs. BE], and 41 g/120 min in FE [95% nCI: 35 to 47 g] [55% of the OGTT (49% to 61%)] $P = 0.06$ vs. BE]. The plasma enrichment of [2H_2]- and [^{13}C]glucose is shown in Fig. 2, E and F, respectively.

Plasma glucose concentrations. No difference between trials was detected for plasma glucose concentrations at baseline (Fig. 3A; $P > 0.05$). Thereafter, a trial \times time interaction was apparent ($F = 2.957$, $P = 0.01$). During the exercise period (rest in BR), plasma glucose concentrations were higher in BR versus BE at 40 min and in BR versus FE at 50 min (both $P < 0.05$). At OGTT 0 min, glucose concentrations were higher in BR versus BE, and during the OGTT they were initially higher in BR versus BE and in BR versus FE (all $P < 0.05$), but no further differences were then detected (Fig. 3A). Peak plasma glucose concentrations were higher in BR versus BE ($P = 0.03$) but not different in BE versus FE (Table 2; $P > 0.05$). A main effect of the trial was detected for the within-laboratory (300 min) glucose AUC, which was higher in BR versus BE (Table 2; $P = 0.05$). However, no main effect of the trial was detected for the OGTT (120 min) iAUC (Fig. 4A; $F = 2.524$, $P = 0.11$).

Plasma insulin concentrations. At baseline, there was no difference between trials for plasma insulin concentrations (Fig. 3B; $P > 0.05$). Main effects of time ($F = 4.351$, $P < 0.01$) and trial ($F = 7.986$, $P < 0.01$) were detected, but there was no trial \times time interaction effect ($F = 2.395$, $P = 0.07$). Peak (and time to peak) plasma insulin concentrations are shown in Table 2. A main effect of the trial was detected for the within-laboratory (300 min) insulin AUC, which was higher in BR versus BE ($P < 0.01$) but not different in BE versus FE (Table 2; $P = 0.10$). A main effect of the trial was apparent for the insulin OGTT iAUC [Fig. 4B; (120 min)

$F = 5.132$, $P = 0.02$], which was lower in BE versus BR [by 27.34 pmol/l (95% nCI: 12.10 to 45.80 pmol/l); $P = 0.02$] and lower in BE versus FE [by 28.67 pmol/l (95% nCI: 10.21 to 47.12 pmol/l); $P = 0.04$]. There was a main effect of trial for the Matsuda ISI (Fig. 4C; $F = 22.790$, $P < 0.01$), which was higher in BE versus BR [by 8.45 arbitrary units (au), (95% nCI: 6.42 to 10.47 au); $P < 0.01$] and in BE versus FE [by 6.49 au (95% nCI: 2.93 to 8.51 au); $P < 0.01$].

Plasma NEFA concentrations. A main effect of the trial ($F = 4.314$, $P = 0.04$) was detected for plasma NEFA at baseline, with concentrations of 0.30 mmol/l in BR (95% nCI: 0.25 to 0.35 mmol/l), 0.45 mmol/l in BE (95% nCI: 0.36 to 0.53 mmol/l; $P = 0.03$ BE vs. BR), and 0.36 mmol/l in FE (95% nCI: 0.31 to 0.44 mmol/l; $P = 0.12$ FE vs. BR and BE). Thereafter, a time \times trial interaction effect was apparent (Fig. 3C; $F = 11.438$, $P < 0.01$), where plasma NEFA concentrations were lowered by breakfast consumption in BR and BE and remained lower during the exercise in BE versus FE before increasing during the initial OGTT period in BE and FE versus BR. A main effect of trial was detected for the total within-laboratory plasma NEFA (300 min) AUC and the NEFA OGTT (120 min) AUC, which in both instances was lower in BR versus BE and FE (Table 2; all $P < 0.01$).

Other plasma metabolites. No differences were detected between trials at baseline for plasma β -hydroxybutyrate concentrations (Fig. 3D; $P > 0.05$). Thereafter, a time \times trial interaction effect was apparent ($F = 6.310$, $P < 0.01$) where concentrations were lowered by breakfast in BR and BE. Plasma β -hydroxybutyrate concentrations remained lower during exercise in BE versus FE but increased during the OGTT with BE and FE versus BR. However, with post hoc adjustment the differences between trials for plasma β -hydroxybutyrate concentrations became less clear (all $P > 0.05$). The within-laboratory (300 min) β -hydroxybutyrate AUC was lower with BR versus BE ($P = 0.03$) but did not differ in BE and FE (Table 2; $P = 0.35$). No baseline differences were detected for

Table 2. Peak plasma concentrations, time to peak concentrations, and AUC for various metabolites and hormones measured during the total within-laboratory period and/or during an oral glucose tolerance test observation that was conducted after Breakfast-Rest, Breakfast-Exercise, or Fasted-Exercise

	Breakfast-Rest	Breakfast-Exercise	Fasted-Exercise	
Peak glucose conc., mmol/l	10.62 (9.98, 11.25)	9.65 (9.27, 10.03) ^a	9.82 (9.44, 10.45)	$F = 4.895$, $P = 0.03$
Time to peak glucose, min	36 (24, 47)	49 (36, 62)	49 (36, 61)	$F = 2.588$, $P = 0.12$
Glucose AUC _{TOTAL} , mmol/l	6.41 (6.21, 6.60)	6.05 (5.90, 6.15) ^a	6.07 (5.93, 6.27)	$F = 6.126$, $P = 0.01$
Peak insulin conc., pmol/l	286 (231, 341)	209 (148, 269)	282 (222, 337)	$F = 3.900$, $P = 0.07$
Time to peak insulin, min	38 (26, 49)	56 (47, 65) ^a	43 (33, 54)	$F = 4.579$, $P = 0.03$
Insulin AUC _{TOTAL} , pmol/l	88 (79, 97)	62 (54, 71) ^c	76 (67, 85)	$F = 9.280$, $P < 0.01$
NEFA AUC _{TOTAL} , mmol/l	0.16 (0.12, 0.19)	0.28 (0.24, 0.33) ^c	0.37 (0.31, 0.42) ^d	$F = 24.980$, $P < 0.01$
NEFA AUC _{OGTT} , mmol/l	0.10 (0.06, 0.14)	0.24 (0.20, 0.29) ^c	0.26 (0.22, 0.31) ^d	$F = 15.510$, $P < 0.01$
Triglyceride AUC _{TOTAL} , mmol/l	0.85 (0.79, 0.91)	0.81 (0.75, 0.86)	0.75 (0.70, 0.79)	$F = 4.319$, $P = 0.03$
Triglyceride AUC _{OGTT} , mmol/l	0.84 (0.77, 0.92)	0.79 (0.72, 0.86)	0.70 (0.64, 0.77)	$F = 4.677$, $P = 0.02$
Lactate AUC _{TOTAL} , mmol/l	1.05 (0.96, 1.13)	1.19 (1.08, 1.30)	1.16 (1.07, 1.27)	$F = 3.645$, $P = 0.06$
Lactate AUC _{OGTT} , mmol/l	1.12 (1.04, 1.20)	0.97 (0.89, 1.04) ^c	1.03 (0.96, 1.11)	$F = 6.479$, $P = 0.02$
β -hydroxybutyrate AUC _{TOTAL} , mmol/l	0.03 (0.00, 0.06)	0.08 (0.06, 0.13) ^a	0.14 (0.09, 0.19) ^b	$F = 5.936$, $P = 0.03$
I-FABP AUC _{TOTAL} , pg/ml	279 (242, 317)	304 (267, 366)	415 (353, 476) ^{b,c}	$F = 10.870$, $P < 0.01$

Data are means and (normalized 95% confidence intervals); $n = 12$ healthy men. Within-laboratory time, 300 min; oral glucose tolerance test observation time, 120 min. AUC, area under the curve; AUC_{TOTAL}, time-averaged area underneath the concentration-time curve for the total within-laboratory period (300 min); AUC_{OGTT}, time-averaged area underneath the concentration-time curve for the oral glucose tolerance test (120-min); Breakfast-Rest, breakfast followed by rest; Breakfast-Exercise, breakfast followed by exercise; conc., concentration; Fasted-Exercise, or extended overnight fasting followed by exercise; I-FABP, intestinal fatty acid binding protein; NEFA, non-esterified fatty acid. ^a $P < 0.05$, difference between breakfast rest and breakfast exercise; ^b $P < 0.05$, difference between breakfast rest and fasted exercise; ^c $P < 0.01$, difference between breakfast rest and breakfast exercise; ^d $P < 0.01$, difference between breakfast rest and fasted exercise; ^e $P < 0.01$, difference between breakfast exercise and fasted exercise.

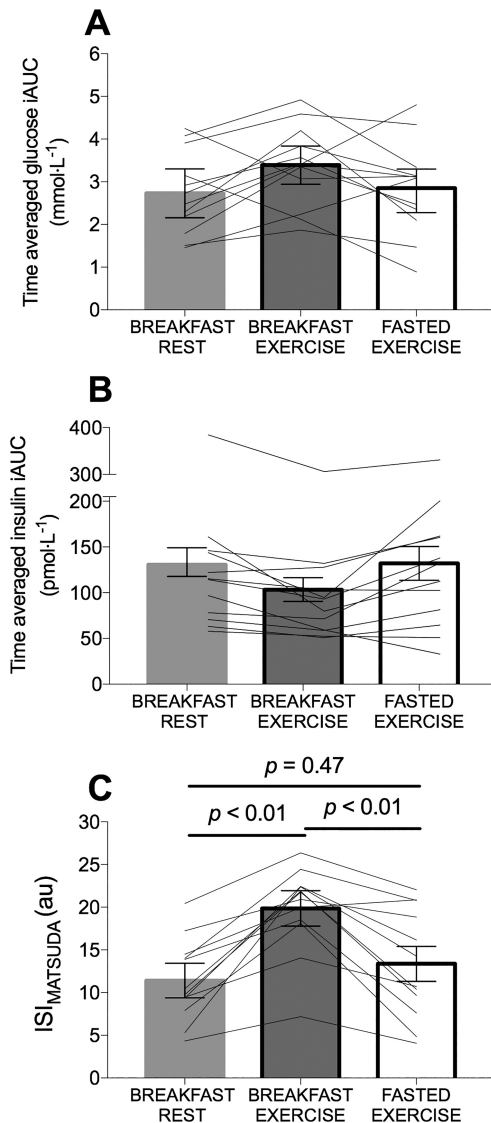


Fig. 4. The time-averaged (120 min) plasma glucose (A) and plasma insulin (B) incremental area under the curves (iAUC) and the Matsuda insulin sensitivity index (C) for an oral glucose tolerance test conducted after breakfast followed by rest (BREAKFAST REST), breakfast followed by exercise (BREAKFAST EXERCISE), or extended overnight fasting followed by exercise (FASTED EXERCISE). Data are means \pm normalized 95% confidence intervals, with individual data shown as black lines. $n = 12$ healthy men. $ISI_{MATSUDA}$, Matsuda insulin sensitivity index.

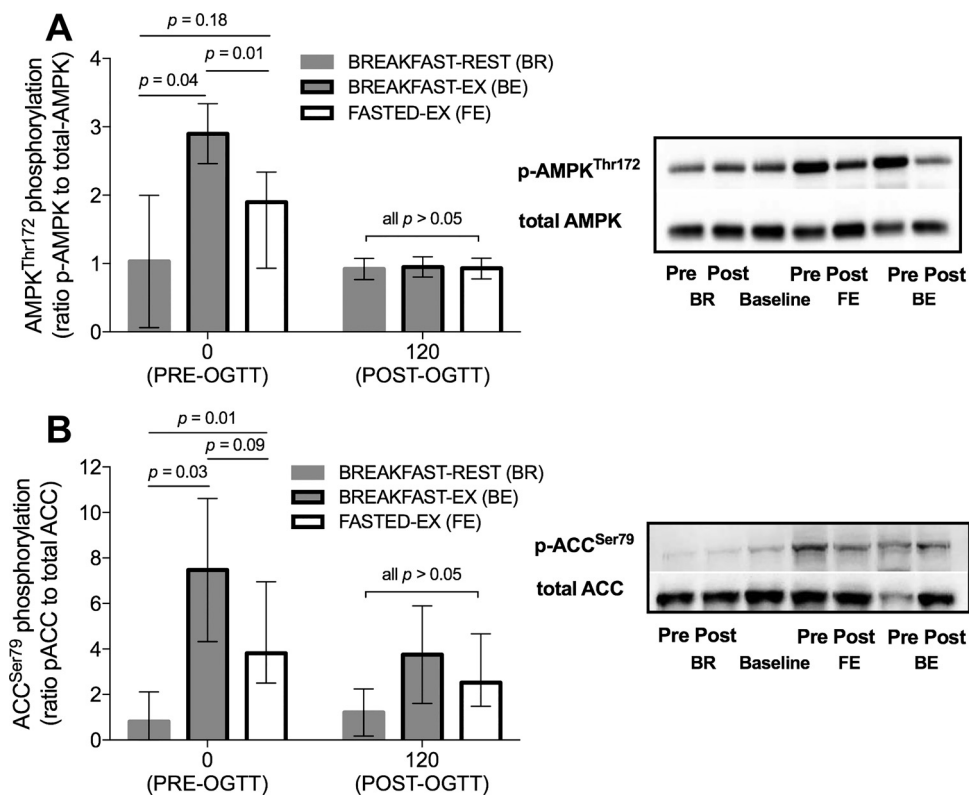
triglyceride concentrations (Fig. 3E, $P > 0.05$) but a time \times trial interaction effect was apparent ($F = 3.994$, $P < 0.01$). There was an effect of trial for the within-laboratory (300 min) and OGTT (120 min) triglyceride AUC, which tended to be lower in FE versus BE but with post hoc adjustment this difference between trials was less clear (Table 2; $P > 0.05$). Plasma lactate concentrations at baseline were not different across trials ($P > 0.05$) but were lower in BR versus BE and FE in the exercise period (rest in BR) and at OGTT 0 min but were then higher in BR versus BE during the OGTT (time \times trial; $F = 20.305$, $P < 0.01$). No effect of the trial was detected for the total within-laboratory (300 min) lactate AUC, but a main effect of the trial was detected for the lactate OGTT (120 min) AUC, which was higher in BR versus BE (Table 2; $P < 0.01$).

Plasma I-FABP concentrations. There was no difference between trials at baseline for plasma I-FABP concentrations (Fig. 3F; $P > 0.05$), but these were lower after breakfast (time \times trial interaction effect; $F = 6.844$, $P < 0.01$) in BR and BE versus FE (both $P < 0.05$). During and after exercise (or rest in BR), I-FABP concentrations were lower in BR and BE versus FE and remained lower in BR versus FE until OGTT 20 min (all $P < 0.05$). The within-laboratory (300 min) I-FABP AUC was lower in BR and BE versus FE (Table 2; $P = 0.01$ and $P = 0.05$, respectively).

Activation of exercise and insulin signaling pathways in skeletal muscle. Time \times trial interaction effects were apparent for AMPK^{Thr172} (ratio p-AMPK to total AMPK) and ACC^{Ser79} (ratio p-ACC to total ACC) phosphorylation, if normalized to the baseline muscle sample (Fig. 5A; $F = 5.154$, $P = 0.04$ and Fig. 5B, $F = 5.881$, $P = 0.02$, respectively). Compared with the BR trial, skeletal muscle AMPK^{Thr172} phosphorylation was higher after exercise (or after rest in BR) in the BE trial [by 1.9 fold (95% nCI: 0.9- to 2.8-fold); $P = 0.04$] and was also higher in BE versus the FE trial [by 1.0-fold (95% nCI: 0.2- to 2.0-fold); $P = 0.01$]. A similar pattern was apparent for ACC phosphorylation, which was higher after exercise (or after rest in BR) in BE versus BR [by 6.7-fold (95% nCI: 5.4- to 8.0-fold); $P = 0.03$] but did not differ between BE and FE ($P = 0.09$). By OGTT 120 min, ACC^{Ser79} and AMPK^{Thr172} phosphorylation had returned to baseline levels in all three trials (all $P > 0.05$). No time \times trial interaction ($F = 2.110$, $P = 0.16$) nor a main effect of trial ($F = 0.098$, $P = 0.83$) was detected for Akt^{Ser473} (ratio p-Akt^{Ser473} to total-Akt2) phosphorylation (Fig. 6A). A main effect of time ($F = 9.907$, $P = 0.01$) was observed, where Akt^{Ser473} phosphorylation was elevated at OGTT 120 min in all trials. Similarly, no time \times trial interaction ($F = 1.533$, $P = 0.25$) nor a main effect of trial ($F = 0.484$, $P = 0.56$) was detected for Akt^{Thr308} (ratio p-Akt^{Thr308} to total-Akt2) phosphorylation (Fig. 6B). A main effect of time ($F = 10.598$, $P = 0.01$) was also detected for this phosphorylation site, whereby Akt^{Thr308} phosphorylation was elevated at OGTT 120 min in all trials. For AS160^{Thr642} phosphorylation (ratio p-AS160^{Thr642} to total AS160), a time \times trial interaction was detected (Fig. 6C; $F = 4.430$, $P = 0.03$), whereby the AS160^{Thr642} phosphorylation was not different between BR and BE at any time, was higher pre-OGTT in BE compared with FE ($P = 0.04$) but was not different between BE and FE at 120 min post-OGTT ($P = 0.69$).

Substrate utilization. Across the duration of the trial, carbohydrate utilization was higher in BE versus BR [Fig. 7; by 514 kcal (95% nCI: 452 to 576 kcal) and higher in BE versus FE (by 124 kcal (95% nCI: 18 to 230 kcal), both $P < 0.01$]. This difference in carbohydrate utilization between BE and FE was derived from a higher utilization of plasma glucose and other carbohydrate sources (i.e., primarily muscle glycogen but also plasma lactate) in BE ($P = 0.02$ and $P = 0.04$, respectively). Within-laboratory fat utilization did not differ between BR and BE ($P = 0.25$) but was higher in FE versus BE [by 138 kcal (95% nCI: -6 to 224 kcal), $P = 0.03$]. Muscle glycogen utilization during exercise (g/kg body mass) was positively correlated ($R = 0.64$, $P < 0.01$) with skeletal muscle ACC^{Ser79} phosphorylation (ratio p-ACC to total ACC) after exercise conducted following BE or extended FE.

Fig. 5. The phosphorylation of 5' AMP-activated protein kinase (phospho AMPK^{Thr172}, ratio p-AMPK to total AMPK) (A) and the phosphorylation of acetyl-CoA carboxylase (phospho ACC^{Ser79}, ratio p-ACC to total ACC) (B) before (PRE-OGTT) and after (POST-OGTT) an oral glucose tolerance test (OGTT) that was conducted after breakfast followed by rest (BREAKFAST-REST), breakfast followed by exercise (BREAKFAST-EX), or extended overnight fasting followed by exercise (FASTED-EX). All samples were taken from the vastus lateralis. Samples were normalized to the baseline muscle sample for each participant (collected in the resting fasted state on the first main trial for each participant). Data are means \pm normalized 95% confidence intervals. $n = 9$ healthy men. BE, Breakfast-Exercise; BR, Breakfast-Rest; FE, Fasted-Exercise.



DISCUSSION

This is the first study, to our knowledge, to assess the effect of preexercise feeding on postprandial plasma glucose kinetics after exercise. Our data demonstrate that preexercise feeding increases plasma glucose disposal during meals consumed after exercise, despite lower insulinemia in this condition. Characterizing glucose flux at meals is important because this determines postprandial glycemia, a predictor of cardiovascular disease risk (47, 48). Previous work has only studied postprandial glucose flux after fasted-state exercise. As most people consume food and perform exercise while still in a postprandial period from a prior meal (13, 23, 55) our results describe the physiological responses to feeding that are more readily applicable to scenarios that are representative of normal daily living. Moreover, our novel data demonstrate that metabolic responses to exercise conducted in an overnight fasted state and to meals that are consumed after exercise cannot be easily extrapolated to conditions where breakfast has been consumed.

The disposal of plasma glucose (the Rd) into skeletal muscle is elevated after exercise, via insulin-dependent and -independent pathways (25). We observed a higher postprandial plasma glucose disposal rate with breakfast versus fasting before exercise. The higher postprandial plasma glucose rate of disposal with preexercise breakfast ingestion was apparent despite lower insulinemia in the breakfast-exercise trial. At rest, breakfast consumption improves glucose tolerance and insulin sensitivity at subsequent meals [known as the “second-meal effect” (5, 20, 26)]. Mechanisms likely relate to delayed gastric emptying (19), a potentiation of early phase insulinemia at the second meal (37) and enhanced glucose uptake into muscle because of increased GLUT4 trafficking (18). Our findings show that breakfast ingestion (vs. fasting) before exercise

enhances subsequent glucose disposal at postexercise meals in the presence of lower insulinemia, suggesting that the second-meal effect is maintained even if exercise is performed between meals. Although the effects of preexercise feeding on the metabolic responses during subsequent exercise is well characterized (15, 43), our data therefore provide new insights regarding postprandial glucose metabolism after exercise in the fed versus fasted state.

Molecular insulin-signaling pathways are instrumental mediators of glucose disposal in response to exercise and/or nutrition (11). We therefore determined the activation status of key proteins involved in glucose uptake in skeletal muscle [the primary site of postprandial glucose disposal (14)]. Akt activation (Thr³⁰⁸ and Ser⁴⁷³ phosphorylation) 2 h after OGTT began, was unaffected by prior exercise or prior breakfast ingestion. However, the timing of muscle sampling could be responsible for this result. It is possible that differences in Akt activation between trials may have been apparent earlier in the postprandial period, as peak Akt phosphorylation can be variable, occurring as early as 30 min after an OGTT (35). Thus, despite the lack of a measurable difference, we cannot rule out a role for insulin signaling in the glucose disposal responses we observed. Distal proteins within the insulin signaling pathway can be activated after exercise, without detectable differences in Akt phosphorylation (61). We therefore measured AS160^{Thr642} activation as this phosphorylation site has been previously shown to be activated by both insulin and exercise stimulation (58). However, our data show that AS160^{Thr642} phosphorylation was not different 2 h post-OGTT with breakfast versus fasting before exercise, providing further evidence that differences in early stages of the activation of the insulin signaling pathway were not responsible for the higher plasma

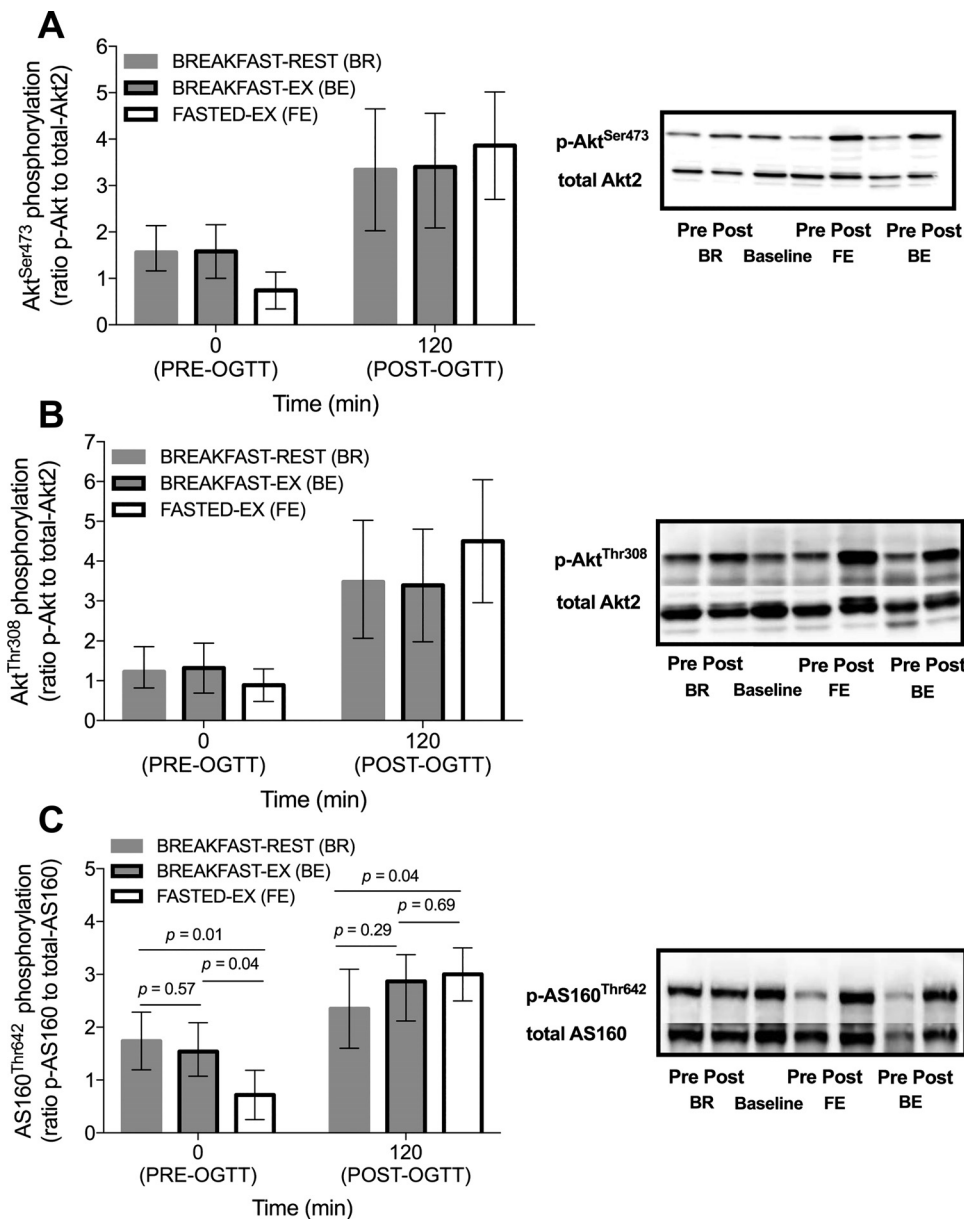


Fig. 6. The phosphorylation of Akt2 [(A) phospho Akt^{Ser473}, ratio p-Akt^{Ser473} to total Akt2 and phospho Akt^{Thr308} (B), ratio p-Akt^{Thr308} to total Akt2] and the phosphorylation of AS160 [(C) AS160^{Thr642}, ratio p-AS160^{Thr642} to total AS160] before (PRE-OGTT) and after (POST-OGTT) an oral glucose tolerance test (OGTT) that was conducted after breakfast followed by rest (BREAKFAST-REST), breakfast followed by exercise (BREAKFAST-EX), or extended overnight fasting followed by exercise (FASTED-EX). All samples were taken from the vastus lateralis. Samples were normalized to the baseline muscle sample for each participant (collected in the resting fasted state on the first main trial for each participant). Data are means \pm normalized 95% confidence intervals. $n = 9$ healthy men. AS160, Akt substrate of 160 kDa; BE, Breakfast-Exercise; BR, Breakfast-Rest; FE, Fasted-Exercise.

glucose disposal rate we observed when breakfast was ingested before exercise.

AMPK activity also plays a key role in muscle glucose uptake and stimulate GLUT4 translocation (27). The greater postexercise skeletal muscle AMPK activation with breakfast before exercise may have contributed to the higher glucose disposal rate in that trial. This AMPK response seems to be specific to skeletal muscle, as we have previously shown that postexercise adipose tissue AMPK content is unaffected by preexercise feeding (12). The increase in skeletal muscle AMPK activity with preexercise feeding that we report in the present study may seem surprising given that the ingestion of large amounts (>200 g) of carbohydrate before and during exercise can blunt AMPK signaling in muscle (3, 29). This blunting is partly because low muscle glycogen concentrations stimulate AMPK activity (42). The modest amount (65 g) of carbohydrate ingested by participants in our study may explain why we did not observe an elevated AMPK response in our

fasted-exercise trial. For example, when smaller carbohydrate doses are ingested before and/or during exercise (~ 120 g or less) the exercise induced increase in the phosphorylation of AMPK, and ACC is not always suppressed compared with when a placebo is ingested (2, 36, 58), although in one study this result was apparent despite a suppression of $\alpha 2$ -AMPK activity when carbohydrate was ingested (2).

The heightened AMPK response observed in the current study with breakfast before exercise may be explained by the type of carbohydrate ingested before exercise (high-glycemic index) in the BE trial, as this can stimulate muscle glycogen use during exercise, especially when no carbohydrate is ingested during the activity (62). Thus, the high glycemic index breakfast with a modest carbohydrate content in the present study, may have stimulated muscle glycogen utilization during exercise without supplying sufficient carbohydrate to replace additional glycogen utilization, resulting in lower postexercise muscle glycogen concentrations with breakfast versus fasting

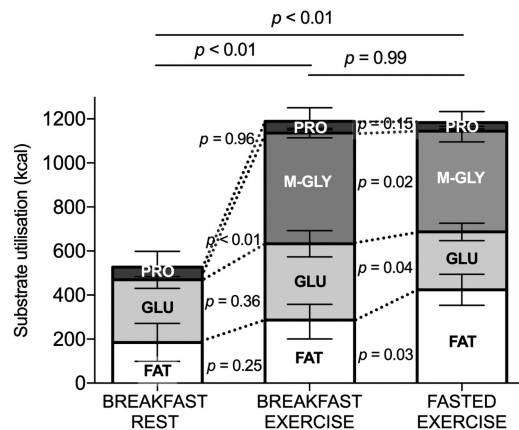


Fig. 7. Within-laboratory (300 min) substrate utilization after breakfast followed by rest (BREAKFAST REST), breakfast followed by exercise (BREAKFAST EXERCISE), or extended overnight fasting followed by exercise (FASTED EXERCISE). Within-laboratory substrate utilization was calculated assuming that the plasma glucose disappearance rate was equivalent to plasma glucose utilization. Glycogen utilization during exercise (on breakfast exercise and fasted exercise only) was calculated as total carbohydrate utilization (from indirect calorimetry) minus plasma glucose utilization during exercise. This estimate of muscle glycogen utilization will therefore include the utilization of other nonglucose (e.g., lactate) carbohydrates. CHO, carbohydrate; GLU, glucose; M-GLY, muscle glycogen; PRO, protein. Data are presented as means \pm 95% confidence intervals. $n = 12$ healthy men.

before exercise. This could explain the enhanced exercise-induced AMPK response if breakfast was consumed before exercise. However, we had insufficient tissue to measure muscle glycogen concentrations in all participants, and our data only provide rates of muscle glycogen utilization (which was higher with feeding versus fasting before exercise). Nevertheless, in the two participants for whom we had sufficient tissue to perform glycogen analyses, these data were in agreement with the tracer-derived calculations of muscle glycogen utilization (data not shown) and AMPK signaling assessed by Western blotting. Consistent with this, the present data demonstrated that postexercise ACC^{Ser79} phosphorylation—as a marker of activation of the AMPK pathway—positively correlated with muscle glycogen utilization during exercise. Thus, taken together our results suggest that the increased postexercise plasma glucose disposal rates with preexercise feeding versus fasting may have been mediated through enhanced AMPK signaling, which is itself a consequence of altered fuel use during exercise. AMPK signaling and resulting muscle glycogen concentrations following exercise after breakfast should now be explored.

The higher plasma glucose disposal rate (despite lower plasma insulin concentrations), which we observed in the BE trial, may be because of differences in GLUT4 trafficking downstream of the signaling proteins we measured. Insulin-stimulated GLUT4 translocation and insulin sensitivity of skeletal muscle are increased in vitro, if muscle is pretreated with insulin, without differences in Akt^{Ser473} or Akt^{Thr308} activation, or the transport activity of GLUT4 (18). This suggests priming of skeletal muscle by prior insulin exposure enhances subsequent insulin action. Pretreatment with insulin and exercise augments this response, possibly because insulin (33) and exercise (28) stimulate GLUT4 translocation from different intracellular stores (8, 49). As such, in the current work, prior breakfast and exercise (the multiple stimuli in BE)

may have enhanced skeletal muscle GLUT4 translocation during the OGTT. Although technically challenging, future work should now quantify GLUT4 trafficking with fed versus fasted state exercise to confirm this. It is also possible that hepatic glucose disposal accounts for some of the increase in glucose disposal following BE versus FE. Similarly to skeletal muscle, insulin-stimulated hepatic glucose uptake is enhanced by prior exposure to insulin in dogs (44, 45). Thus, assuming that this response persists in humans and after exercise, priming of the liver by prior breakfast could also contribute to greater glucose disposal during meals consumed post-exercise.

The increases in postexercise glucose disposal with prior breakfast consumption were, however, offset by alterations in plasma glucose appearance. As such, postprandial glucose concentrations did not differ between the two exercise trials. Postprandial plasma glucose appearance rates are determined by three main factors: 1) the appearance of glucose from the meal, 2) residual appearance of glucose from previous meals, and/or 3) liver glucose output (glycogenolysis or gluconeogenesis). We showed that alterations in the postprandial plasma glucose appearance rate (Ra_{TOTAL}) after exercise with prior breakfast was mostly driven by increased appearance of glucose from the postexercise OGTT. These alterations in the Ra_{TOTAL} suggest that differences in gut function (i.e., increased intestinal damage or absorptive capacity) and/or splanchnic blood flow altered the Ra_{TOTAL} . Therefore, breakfast before exercise may alter postprandial glycemia via factors related to intestinal absorption and splanchnic handling of glucose, rather than just glucose metabolism by skeletal muscle.

There are several potential mechanisms that may explain the differences in glucose appearance rates because of intestinal absorption and/or splanchnic handling of glucose. Plasma I-FABP concentrations are used as a marker of damage to intestinal epithelial cells (60). We noted lower plasma I-FABP concentrations in the BE versus FE trial, despite increased plasma glucose appearance rates of the orally ingested glucose after exercise. It is therefore unlikely that increased intestinal damage was responsible for the higher plasma glucose appearance rates we observed with feeding versus fasting before exercise. The better maintenance of splanchnic perfusion during exercise with prior feeding is a likely explanation for this I-FABP response (15). If splanchnic perfusion was better maintained during exercise with prior feeding, this may have also directly facilitated higher OGTT-derived glucose appearance rates in that trial versus the FE trial. It should be acknowledged, however, that this intestinal damage response may be specific to cycling and could differ with other exercise modalities (e.g., running), within the context of preexercise feeding. An alternative mechanism for higher appearance rate of orally ingested glucose with feeding before exercise could also be that apical glucose transporters were primed by the prior breakfast ingestion (40). Although further underlying mechanism(s) remain unclear, and should therefore be investigated with future work, we showed that a major determinant of postexercise glycemia (plasma glucose appearance rates) are altered by preexercise feeding and that this is unlikely to be explained by increases in intestinal damage.

Our results show that the metabolic and intramuscular signaling responses to exercise conducted in a fed state cannot be readily inferred from responses observed with exercise in a

fasted state. As well as a continual investigation of the mechanisms responsible for differences in postprandial glucose metabolism with altered preexercise feeding, future work should study whether the results we observed are apparent with different postexercise meals (including the coingestion of fat and protein). Moreover, if the acute alterations in postprandial metabolism translate into longer-term differences in insulin sensitivity with repeated bouts of exercise in the fed versus fasted state and in overweight and obese populations, should now be a focus for future work.

To conclude, eating breakfast (vs. fasting) before exercise increases postexercise plasma glucose disposal rates, but this is offset by increases in appearance rates of (mainly) orally ingested glucose, a result that does not appear to be explained by a greater intestinal damage response to the exercise. We showed that preexercise breakfast consumption lowers insulinemia at meals that are consumed after exercise, providing new evidence that the second meal effect is maintained even when exercise is performed between eating occasions.

ACKNOWLEDGMENTS

The authors thank the volunteers for their time and effort in participating in the study. We also thank Sewa Abdullah for assistance with mass spectrometry analysis. Supporting data are available as online supplemental material.

GRANTS

This work was supported by the European Society for Clinical Nutrition and Metabolism and The Rank Prize Funds (both to J. T. Gonzalez). F. Koumanov is funded by the Medical Research Council (Grant No. MR/P002927/1).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.A.B., D.T., G.A.W., D.L.H., E.J.S., K.D.T., and J.T.G. conceived and designed research; R.M.E., A.H., H.A.S., R.L.T., F.K., J.A.B., J.-P.W., G.A.W., and J.T.G. performed experiments; R.M.E., A.H., H.A.S., F.K., J.A.B., G.A.W., and J.T.G. analyzed data; R.M.E., F.K., G.A.W., and J.T.G. interpreted results of experiments; R.M.E. and J.T.G. prepared figures; R.M.E. and J.T.G. drafted manuscript; R.M.E., A.H., H.A.S., R.L.T., F.K., J.A.B., D.T., J.-P.W., G.A.W., D.L.H., E.J.S., K.D.T., and J.T.G. edited and revised manuscript; R.M.E., A.H., H.A.S., R.L.T., F.K., J.A.B., D.T., J.-P.W., G.A.W., D.L.H., E.J.S., K.D.T., and J.T.G. approved final version of manuscript.

REFERENCES

- Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J. Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. *J Clin Invest* 53: 1080–1090, 1974. doi:10.1172/JCI107645.
- Akerstrom TC, Birk JB, Klein DK, Erikstrup C, Plomgaard P, Pedersen BK, Wojtaszewski J. Oral glucose ingestion attenuates exercise-induced activation of 5'-AMP-activated protein kinase in human skeletal muscle. *Biochem Biophys Res Commun* 342: 949–955, 2006. doi:10.1016/j.bbrc.2006.02.057.
- Bartlett JD, Louhelainen J, Iqbal Z, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP. Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. *Am J Physiol Regul Integr Comp Physiol* 304: R450–R458, 2013. doi:10.1152/ajpregu.00498.2012.
- Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, Cobelli C. Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *Am J Physiol Endocrinol Metab* 284: E55–E69, 2003. doi:10.1152/ajpendo.00190.2001.
- Betts JA, Chowdhury EA, Gonzalez JT, Richardson JD, Tsintzas K, Thompson D. Is breakfast the most important meal of the day? *Proc Nutr Soc* 75: 464–474, 2016. doi:10.1017/S0029665116000318.
- Betts JA, Richardson JD, Chowdhury EA, Holman GD, Tsintzas K, Thompson D. The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults. *Am J Clin Nutr* 100: 539–547, 2014. doi:10.3945/ajcn.114.083402.
- Betts JA, Thompson D. Thinking outside the bag (not necessarily outside the lab). *Med Sci Sports Exerc* 44: 2040, 2012. doi:10.1249/MSS.0b013e318264526f.
- Bradley H, Shaw CS, Bendtsen C, Worthington PL, Wilson OJ, Strauss JA, Wallis GA, Turner AM, Wagenmakers AJ. Visualization and quantitation of GLUT4 translocation in human skeletal muscle following glucose ingestion and exercise. *Physiol Rep* 3: e12375, 2015. doi:10.14814/phy2.12375.
- Breen L, Philip A, Shaw CS, Jeukendrup AE, Baar K, Tipton KD. Beneficial effects of resistance exercise on glycemic control are not further improved by protein ingestion. *PLoS One* 6: e20613, 2011. doi:10.1371/journal.pone.0020613.
- Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, Wolever TM. Glycaemic index methodology. *Nutr Res Rev* 18: 145–171, 2005. doi:10.1079/NRR2005100.
- Cartee GD. Roles of TBC1D1 and TBC1D4 in insulin- and exercise-stimulated glucose transport of skeletal muscle. *Diabetologia* 58: 19–30, 2015. doi:10.1007/s00125-014-3395-5.
- Chen YC, Travers RL, Walhin JP, Gonzalez JT, Koumanov F, Betts JA, Thompson D. Feeding influences adipose tissue responses to exercise in overweight men. *Am J Physiol Endocrinol Metab* 313: E84–E93, 2017. doi:10.1152/ajpendo.00006.2017.
- de Castro JM. The time of day of food intake influences overall intake in humans. *J Nutr* 134: 104–111, 2004. doi:10.1093/jn/134.1.104.
- DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* 76: 149–155, 1985. doi:10.1172/JCI111938.
- Enevoldsen LH, Simonsen L, Macdonald IA, Bülow J. The combined effects of exercise and food intake on adipose tissue and splanchnic metabolism. *J Physiol* 561: 871–882, 2004. doi:10.1113/jphysiol.2004.076588.
- Febbraio MA, Keenan J, Angus DJ, Campbell SE, Garnham AP. Preexercise carbohydrate ingestion, glucose kinetics, and muscle glycogen use: effect of the glycemic index. *J Appl Physiol* (1985) 89: 1845–1851, 2000. doi:10.1152/jappl.2000.89.5.1845.
- Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr* 76: 5–56, 2002. doi:10.1093/ajcn/76.1.5.
- Geiger PC, Han DH, Wright DC, Holloszy JO. How muscle insulin sensitivity is regulated: testing of a hypothesis. *Am J Physiol Endocrinol Metab* 291: E1258–E1263, 2006. doi:10.1152/ajpendo.00273.2006.
- Gentilecore D, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, Wishart JM, Rayner CK, Horowitz M. Effects of fat on gastric emptying and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *J Clin Endocrinol Metab* 91: 2062–2067, 2006. doi:10.1210/jc.2005-2644.
- Gonzalez JT. Paradoxical second-meal phenomenon in the acute postexercise period. *Nutrition* 30: 961–967, 2014. doi:10.1016/j.nut.2013.12.001.
- Gonzalez JT, Fuchs CJ, Smith FE, Thelwall PE, Taylor R, Stevenson EJ, Trenell MI, Cermak NM, van Loon LJ. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Metab* 309: E1032–E1039, 2015. doi:10.1152/ajpendo.00376.2015.
- Gonzalez JT, Richardson JD, Chowdhury EA, Koumanov F, Holman GD, Cooper S, Thompson D, Tsintzas K, Betts JA. Molecular adaptations of adipose tissue to 6 weeks of morning fasting vs. daily breakfast consumption in lean and obese adults. *J Physiol* 596: 609–622, 2018. doi:10.1113/JP275113.
- Gonzalez JT, Stevenson EJ. Assessment of the post-exercise glycemic response to food: considering prior nutritional status. *Nutrition* 30: 122–123, 2013. doi:10.1016/j.nut.2013.05.015.
- Gonzalez JT, Veasey RC, Rumbold PL, Stevenson EJ. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *Br J Nutr* 110: 721–732, 2013. doi:10.1017/S0007114512005582.
- Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 49: 235–261, 1998. doi:10.1146/annurev.med.49.1.235.

26. Hamman L, Hirschman I. Studies on blood sugar. *Arch Intern Med (Chic)* XX: 761–808, 1917. doi:10.1001/archinte.1917.00090050122007.
27. Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 19: 121–135, 2018. doi:10.1038/nrm.2017.95.
28. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol (1985)* 99: 338–343, 2005. doi:10.1152/jappphysiol.00123.2005.
29. Impey SG, Hammond KM, Shepherd SO, Sharples AP, Stewart C, Limb M, Smith K, Philp A, Jeromson S, Hamilton DL, Close GL, Morton JP. Fuel for the work required: a practical approach to amalgamating train-low paradigms for endurance athletes. *Physiol Rep* 4: e12803, 2016. doi:10.14814/phy2.12803.
30. Jentjens RL, Underwood K, Achten J, Currell K, Mann CH, Jeukendrup AE. Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat. *J Appl Physiol (1985)* 100: 807–816, 2006. doi:10.1152/jappphysiol.00322.2005.
31. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* 26, Suppl 1: S28–S37, 2005. doi:10.1055/s-2004-830512.
32. Jeukendrup AE, Wagenmakers AJM, Stegen JH, Gijzen AP, Brouns F, Saris WHM. Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. *Am J Physiol* 276: E672–E683, 1999. doi:10.1152/ajpendo.1999.276.4.E672.
33. Kahn CR, White MF. The insulin receptor and the molecular mechanism of insulin action. *J Clin Invest* 82: 1151–1156, 1988. doi:10.1172/JCI113711.
34. Knudsen SH, Karstoft K, Pedersen BK, van Hall G, Solomon TP. The immediate effects of a single bout of aerobic exercise on oral glucose tolerance across the glucose tolerance continuum. *Physiol Rep* 2: e12114, 2014. doi:10.14814/phy2.12114.
35. Krusenstjerna-Hafström T, Madsen M, Vendelbo MH, Pedersen SB, Christiansen JS, Møller N, Jessen N, Jørgensen JO. Insulin and GH signaling in human skeletal muscle in vivo following exogenous GH exposure: impact of an oral glucose load. *PLoS One* 6: e19392, 2011. doi:10.1371/journal.pone.0019392.
36. Lee-Young RS, Palmer MJ, Linden KC, LePlastrier K, Canny BJ, Hargreaves M, Wadley GD, Kemp BE, McConnell GK. Carbohydrate ingestion does not alter skeletal muscle AMPK signaling during exercise in humans. *Am J Physiol Endocrinol Metab* 291: E566–E573, 2006. doi:10.1152/ajpendo.00023.2006.
37. Lee S-H, Tura A, Mari A, Ko S-H, Kwon H-S, Song K-H, Yoon K-H, Lee K-W, Ahn Y-B. Potentiation of the early-phase insulin response by a prior meal contributes to the second-meal phenomenon in type 2 diabetes. *Am J Physiol Endocrinol Metab* 301: E984–E990, 2011. doi:10.1152/ajpendo.00244.2011.
38. Liljeberg Elmståhl H, Björck I. Milk as a supplement to mixed meals may elevate postprandial insulinaemia. *Eur J Clin Nutr* 55: 994–999, 2001. doi:10.1038/sj.ejcn.1601259.
39. Loftus GR, Masson MEJ. Using confidence intervals in within-subject designs. *Psychon Bull Rev* 1: 476–490, 1994. doi:10.3758/BF03210951.
40. Mace OJ, Affleck J, Patel N, Kellett GL. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* 582: 379–392, 2007. doi:10.1113/jphysiol.2007.130906.
41. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22: 1462–1470, 1999. doi:10.2337/diacare.22.9.1462.
42. McBride A, Ghilagaber S, Nikolaev A, Hardie DG. The glycogen-binding domain on the AMPK β subunit allows the kinase to act as a glycogen sensor. *Cell Metab* 9: 23–34, 2009. doi:10.1016/j.cmet.2008.11.008.
43. Montain SJ, Hopper MK, Coggan AR, Coyle EF. Exercise metabolism at different time intervals after a meal. *J Appl Physiol (1985)* 70: 882–888, 1991. doi:10.1152/jappl.1991.70.2.882.
44. Moore MC, Smith MS, Farmer B, Coate KC, Kraft G, Shiota M, Williams PE, Cherrington AD. Morning hyperinsulinemia primes the liver for glucose uptake and glycogen storage later in the day. *Diabetes* 67: 1237–1245, 2018. doi:10.2337/db17-0979.
45. Moore MC, Smith MS, Farmer B, Kraft G, Shiota M, Williams PE, Cherrington AD. Priming effect of a morning meal on hepatic glucose disposition later in the day. *Diabetes* 66: 1136–1145, 2017. doi:10.2337/db16-1308.
46. Nilsson M, Elmståhl H, Björck I. Glucose and insulin responses to porridge and gruel meals intended for infants. *Eur J Clin Nutr* 59: 646–650, 2005. doi:10.1038/sj.ejcn.1602115.
47. Ning F, Zhang L, Dekker JM, Onat A, Stehouwer CD, Yudkin JS, Laatikainen T, Tuomilehto J, Pyörälä K, Qiao Q; DECODE Finnish and Swedish Study Investigators. Development of coronary heart disease and ischemic stroke in relation to fasting and 2-hour plasma glucose levels in the normal range. *Cardiovasc Diabetol* 11: 76–84, 2012. doi:10.1186/1475-2840-11-76.
48. Pekkanen J, Tuomilehto J, Qiao Q, Jousilahti P, Lindström J; DECODE Study Group. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. *Lancet* 354: 617–621, 1999. doi:10.1016/S0140-6736(98)12131-1.
49. Ploug T, van Deurs B, Ai H, Cushman SW, Ralston E. Analysis of GLUT4 distribution in whole skeletal muscle fibers: identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J Cell Biol* 142: 1429–1446, 1998. doi:10.1083/jcb.142.6.1429.
50. Radziuk J. An integral equation approach to measuring turnover in nonsteady compartmental and distributed systems. *Bull Math Biol* 38: 679–693, 1976. doi:10.1007/BF02458642.
51. Radziuk J, Norwich KH, Vranic M. Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol* 234: E84–E93, 1978. doi:10.1152/ajpendo.1978.234.1.E84.
52. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev* 93: 993–1017, 2013. doi:10.1152/physrev.00038.2012.
53. Rizza RA, Toffolo G, Cobelli C. Accurate measurement of postprandial glucose turnover: why is it difficult and how can it be done (relatively) simply? *Diabetes* 65: 1133–1145, 2016. doi:10.2337/db15-1166.
54. Rose AJ, Howlett K, King DS, Hargreaves M. Effect of prior exercise on glucose metabolism in trained men. *Am J Physiol Endocrinol Metab* 281: E766–E771, 2001. doi:10.1152/ajpendo.2001.281.4.E766.
55. Ruge T, Hodson L, Cheeseman J, Dennis AL, Fielding BA, Humphreys SM, Frayn KN, Karpe F. Fasted to fed trafficking of fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage. *J Clin Endocrinol Metab* 94: 1781–1788, 2009. doi:10.1210/jc.2008-2090.
56. Steele R, Bjerknes C, Rathgeb I, Altszuler N. Glucose uptake and production during the oral glucose tolerance test. *Diabetes* 17: 415–421, 1968. doi:10.2337/diab.17.7.415.
57. Tarnopolsky MA, Pearce E, Smith K, Lach B. Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. *Muscle Nerve* 43: 716–725, 2011. doi:10.1002/mus.21945.
58. Treebak JT, Pehmøller C, Kristensen JM, Kjøbsted R, Birk JB, Schjerling P, Richter EA, Goodyear LJ, Wojtaszewski JF. Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle. *J Physiol* 592: 351–375, 2014. doi:10.1113/jphysiol.2013.266338.
59. Van Thienen R, D'Hulst G, Deldicque L, Hespel P. Biochemical artifacts in experiments involving repeated biopsies in the same muscle. *Physiol Rep* 2: e00286, 2014. doi:10.14814/phy2.286.
60. van Wijck K, Lenaerts K, van Loon LJ, Peters WH, Buurman WA, Dejong CH. Exercise-induced splanchnic hypoperfusion results in gut dysfunction in healthy men. *PLoS One* 6: e22366, 2011. doi:10.1371/journal.pone.0022366.
61. Vendelbo MH, Møller AB, Treebak JT, Gormsen LC, Goodyear LJ, Wojtaszewski JF, Jørgensen JOL, Møller N, Jessen N. Sustained AS160 and TBC1D1 phosphorylations in human skeletal muscle 30 min after a single bout of exercise. *J Appl Physiol (1985)* 117: 289–296, 2014. doi:10.1152/jappphysiol.00044.2014.
62. Wee S-L, Williams C, Tsintzas K, Boobis L. Ingestion of a high-glycemic index meal increases muscle glycogen storage at rest but augments its utilization during subsequent exercise. *J Appl Physiol (1985)* 99: 707–714, 2005. doi:10.1152/jappphysiol.01261.2004.
63. Wolfe RR. *Radioactive and Stable Isotope Tracers In Biomedicine: Principles And Practice Of Kinetic Analysis*. New York: Wiley Liss, 1992.