RESEARCH ARTICLE

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

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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 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 increases in 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 potently 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 potently 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 fastest 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.
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 rest, and then a 2-h oral glucose tolerance test (OGTT). In BE, the same breakfast was consumed before 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 ± SD] ambient temperature (23.7 ± 0.5°C on BR, 23.7 ± 0.6°C on BE, 23.6 ± 0.7°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 characteristics 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 (BCS43 Monitor, Tanita, Tokyo, Japan). A

| Characteristic   | Value  |
|-----------------|--------|
| Age (years)     | 23 (3) |
| Stature (cm)    | 179.8 (4.4) |
| Body mass (kg)  | 76.3 (7.9) |
| Body mass index (kg/m²) | 23.6 (2.90) |
| Fat mass (kg)†  | 10.6 (3.7) |
| Fat Mass Index (kg/m²)† | 3.26 (1.12) |
| Body fat (%)†   | 14 (4) |
| Fat free mass (kg)† | 65.5 (6.4) |
| VO2peak (l/min)* | 4.00 (0.72) |
| VO2peak (ml·kg⁻¹·min⁻¹)* | 53 (10) |
| Peak power output (W) | 317 (67) |
| HRMAX (beats/min) | 189 (10) |

Data are presented as means and (standard deviation). n = 12. HRMAX, max heart rate; VO2peak, 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.

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-¹³C] glucose ingestion and [6,6-²H₂] glucose infusion) and muscle biopsies were combined to assess postprandial plasma glucose kinetics and intramuscular signaling, respectively. Wmax, maximum power output.
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 3-1 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 (V̇O2peak) was calculated as the highest average V̇O2 over a rolling 30-s period.

Preliminary tests confirmed that 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, Cambridge, UK) for 24 h before all trials (pretrial 24-h physical activity energy expenditure [(mean ± SD) 988 ± 500 kcal on BR, 1,022 ± 521 kcal on BE, 992 ± 313 kcal on FE; all P > 0.05; n = 9]).

Participants arrived at the laboratory at 0800 ± 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 10-min OGTT. Whole blood was then dispensed into 0.5-ml aliquots and immediately frozen at -20°C before longer-term storage at -80°C.

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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 of (Quaker Oats) has a glycemic index of 69 (17)], as is commonly consumed in developed countries. Because of the coinigestion of milk, this breakfast would produce a high insulineemic 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 antebrachial vein (the contra lateral 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 μmol/kg; infusion: 0.35 μmol·kg⁻¹·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 (RaOGTT) 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-tracer 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 insulin 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 (Mercodia AB, Uppsala, Sweden; intra-assay CV 5.7%; interassay CV 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%; inter-assay 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 [6,6-2H2]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 heptfluorobutyric 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
Night at 4°C with primary antibodies against p-AMPKThr172, p-ACCser79, p-AktSer473, p-AktThr308, and p-AS160Thr642 (Cell Signal-
p-AS160Thr642, and total AS160). Gels were electro-blotted using a
concentrations (7). Expired O2 and CO2 concentrations were mea-

gene expression were recovered in 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 (99.9% N2, 0% O2 and CO2) and high
(balanced nitrogen mix, 16.0% O2, 5.0% 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 account-

tation-time curve (AUC or iAUC, respectively) for each plasma, metabolite or hormone was calculated using the trapezoidal 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):

\[
\text{ISI}_{\text{MATSUDA}}(au) = \frac{10,000}{\sqrt{\text{FFPI} \times \text{MPl} \times \text{MPI}}}
\]

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 Epilson Group, Charlottesville, VA). This model reduces errors in estimates of glucose kinetics that are apparent when using Steele’s (56) one-compartment model (53). The RaTOTAL
and glucose disappearance (Rd) were calculated as follows:

\[
\text{Ra}_{\text{TOTAL}}(t) = F \frac{E_1(t)}{E_1} \times V_1 \times G(t) - k_{21} \times sQ_2(t)
\]

\[
\text{Rd}(t) = \text{Ra}_{\text{TOTAL}} - V_1 \times G(t) - k_{21} \times V_1 \times G(t) + k_{12} \times sQ_2(t)
\]

where F is the [6,6-\text{H}_2] infusion, V_1 is the glucose volume of distribution [4% of body mass (kg)], E_1(t) is the [\text{^2H}_2] plasma glucose
enrichment (mole percent excess) at time t, E_1(t) is the change in E_1 over time (derivative of E_1), G(t) is the plasma glucose concentrations at time t, G(t) is the change in G over time (derivative of G), k_{12} and k_{21} are fixed rate constants between the peripheral and the accessible compartments, and sQ_2(t) is the amount of the tracer [\text{^2H}_2] and tracee in the peripheral compartment, respectively, evaluated by integrating the two-compartment model.

The [U-\text{^{13}}\text{C}] enrichment of the orally ingested glucose and the RaTOTAL (from Eqn. 1) were used to calculate the plasma rate of appearance of glucose from the OGTT (RaOGTT). In these equations, r_1 is the ratio of the infusion [\text{^2H}_2] and oral [U-\text{^{13}}\text{C}] glucose tracer concentrations in plasma, r_1(t) is the change in r_1 over time (derivative of r_1), g is the [U-\text{^{13}}\text{C}] glucose tracer in plasma, q_0 is the amount
of the [U-13C] tracer in the peripheral compartment (by integrating
the two-compartment model), and \( E_{\text{OGTT}} \) is the [U-13C] enrichment
of the OGTT.

\[
ra^{\text{OGTT}}(t) = ra^{\text{TOTAL}}(t) \frac{F}{r_1(t)} - \frac{V_1 \times g(t)}{r_1(t)} \times r_1(t) + \frac{k_{12} q_1^0(t)}{r_1(t)} - q_2^0(t) \quad (3)
\]

\[
Ra^{\text{OGTT}}(t) = ra^{\text{OGTT}}(t) \left( \frac{1}{E_{\text{OGTT}}} \right) \quad (4)
\]

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

\[
\text{Metabolic Clearance Rate} = \frac{Rd(t)}{G_1} \quad (5)
\]

Rates of whole-body fat and carbohydrate utilization were calcu-
lated 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 utili-
zation during exercise. Because of these methods, this estimate of
muscle glycogen utilization will include the utilization of other nonglycose 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 carbo-
hydrate oxidation during exercise. However, during short-duration,
moderate-intensity exercise this effect is negligible (31). Within-
laboratory energy expenditure was determined assuming that lip-
ids, glucose, and glycogen give 40.81, 15.64, and 17.36 kJ/g
respectively (31).

One-way, repeated measures ANOVAs were used to assess differ-
ences 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 \( \varepsilon < 0.75 \), with Huynh-Feldt corrections used for
less severe asphericity. If time \( \times \) trial interaction effects were iden-
tified, 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 (\( Ra^{\text{TOTAL}} \)) (\( C \)),
the rate of appearance of glucose in plasma
from the oral glucose tolerance test (\( Ra^{\text{OGTT}} \))
(\( D \)), and the plasma enrichments of [\( ^2\text{H}_2 \)]-
glucose (\( E \)) and [\( ^{13}\text{C} \)]-glucose (\( F \)) before and
during an oral glucose tolerance test (OGTT)
that was conducted after breakfast followed by
rest (BREAKFAST-REST), breakfast fol-
lowed by exercise (BREAKFAST-EX), or ex-
tended overnight fasting followed by exercise
(FASTED-EX). Data are means \( \pm \) normalized
95% confidence intervals, \( n = 12 \) healthy men.
\( \text{a} \) difference between breakfast rest vs. break-
fast exercise, \( \text{b} \) breakfast rest vs. fasted exer-
cise, and \( \text{c} \) breakfast exercise vs. fasted exercise
with \( P < 0.05 \). MPE, mole percent excess.
are means ± 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 × 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 R_{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 (Ra_{OGTT}) and a trial × time interaction was detected (Fig. 2D; F = 3.134, P = 0.04). A main effect of trial was detected for Ra_{TOTAL} during the OGTT [(g/120 min); F = 3.123, P = 0.05]].
detected for the total RaOGTT (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 [2H2]- and [13C]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 × 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 (Fig. 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 × 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 × 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 × 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

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**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.**

| Metabolite                  | Breakfast-Rest | Breakfast-Exercise | Fasted-Exercise |
|----------------------------|----------------|-------------------|----------------|
| Peak glucose conc., mmol/l | 10.62 (9.98, 11.25) | 9.65 (9.27, 10.03) | 9.82 (9.44, 10.45) |
| Time to peak glucose, min  | 36 (24, 47)    | 49 (36, 62)       | 49 (36, 61)    |
| Glucose AUCTOTAL, mmol/l   | 6.41 (6.21, 6.60) | 6.05 (5.90, 6.15) | 6.07 (5.93, 6.27) |
| Peak insulin conc., pmol/l | 286 (231, 341) | 209 (148, 269)    | 282 (222, 337) |
| Time to peak insulin, min  | 36 (26, 49)    | 36 (24, 64)       | 43 (33, 54)    |
| Insulin AUCTOTAL, pmol/l   | 88 (79, 97)    | 62 (54, 71)       | 76 (67, 85)    |
| NEFA AUCTOTAL, mmol/l      | 0.16 (0.12, 0.19) | 0.25 (0.24, 0.33) | 0.37 (0.31, 0.42) |
| Triglyceride AUCTOTAL, mmol/l | 0.085 (0.07, 0.09) | 0.24 (0.20, 0.29) | 0.26 (0.22, 0.31) |
| β-hydroxybutyrate AUCTOTAL, mmol/l | 0.03 (0.00, 0.06) | 0.08 (0.06, 0.13) | 0.14 (0.09, 0.19) |
| I-FABP AUCTOTAL, pg/ml    | 279 (242, 317) | 304 (267, 366)    | 415 (353, 476) |

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; AUCTOTAL, time-averaged area underneath the concentration-time curve for the total within-laboratory period (300 min); AUCOGTT, 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. *P < 0.05, difference between breakfast rest and breakfast exercise; **P < 0.05, difference between breakfast rest and fasted exercise; *P < 0.01, difference between breakfast rest and breakfast exercise; **P < 0.01, difference between breakfast rest and fasted exercise; ***P < 0.01, difference between breakfast exercise and fasted exercise.
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 × 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 × trial interaction effects were apparent for AMPKThr172 (ratio p-AMPK to total AMPK) and ACCSer79 (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 AMPKThr172 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, ACCSer79 and AMPKThr172 phosphorylation had returned to baseline levels in all three trials (all P > 0.05). No time × trial interaction (F = 2.110, P = 0.16) nor a main effect of trial (F = 0.908, P = 0.83) was detected for AktSer473 (ratio p-AktSer473 to total-Akt2) phosphorylation (Fig. 6A). A main effect of time (F = 9.907, P = 0.01) was observed, where AktSer473 phosphorylation was elevated at OGTT 120 min in all trials. Similarly, no time × trial interaction (F = 1.533, P = 0.25) nor a main effect of trial (F = 0.484, P = 0.56) was detected for AS160Thr642 (ratio p-AS160Thr642 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 AS160Thr642 phosphorylation was elevated at OGTT 120 min in all trials. For AS160Thr642 phosphorylation (ratio p-AS160Thr642 to total AS160), a time × trial interaction was detected (Fig. 6C; F = 4.430, P = 0.03), whereby the AS160Thr642 phosphorylation was not different between BR and BE at any time, but was 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 BR [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 ACCSer79 phosphorylation (ratio p-ACC to total ACC) after exercise conducted following BE or extended FE.

triglyceride concentrations (Fig. 3E, P > 0.05) but a time × 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 × 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 FE (Table 2; P < 0.01).
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 AS160Thr642 activation as this phosphorylation site has been previously shown to be activated by both insulin and exercise stimulation (58). However, our data show that AS160Thr642 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. 5. The phosphorylation of 5’ AMP-activated protein kinase (phospho AMPKThr172, ratio p-AMPK to total AMPK) (A) and the phosphorylation of acetyl-CoA carboxylase (phospho ACCSer79, 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. Data are means ± normalized 95% confidence intervals. n = 9 healthy men. BE, Breakfast-Exercise; BR, Breakfast-Rest; FE, Fasted-Exercise. ](https://doi.org/10.1152/ajpendo.00163.2018)
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 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.
work, prior breakfast and exercise (the multiple stimuli in BE) different intracellular stores (8, 49). As such, in the current (33) and exercise (28) stimulate GLUT4 translocation from and exercise augments this response, possibly because insulin enhances subsequent insulin action. Pretreatment with insulin suggests priming of skeletal muscle by prior insulin exposure, or the transport activity of GLUT4 (18). This demonstrated that postexercise ACCSer79 phosphorylation—as a Western blotting. Consistent with this, the present data dem-
ticated 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 non-glucose (e.g., lactate) carbohydrates. CHO, carbohydrate; GLU, glucose; M-GLY, muscle glycogen; PRO, protein. Data are presented as means ± 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 ACCSer79 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 muscle mass are increased in vitro, if muscle is pretreated with insulin, without differences in AktSer473 or AktThr308 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 (RaTOTAL) after exercise with prior breakfast was mostly driven by increased appearance of glucose from the postexercise OGTT. These alterations in the RaTOTAL suggest that differences in gut function (i.e., increased intestinal damage or absorptive capacity) and/or splanchnic blood flow altered the RaTOTAL. 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 sig-
naling 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 coinestion 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 insulinaemia 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.

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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.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.

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