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The metabolic interplay between dietary carbohydrate and exercise and its role in acute appetite regulation in males: a randomized controlled study

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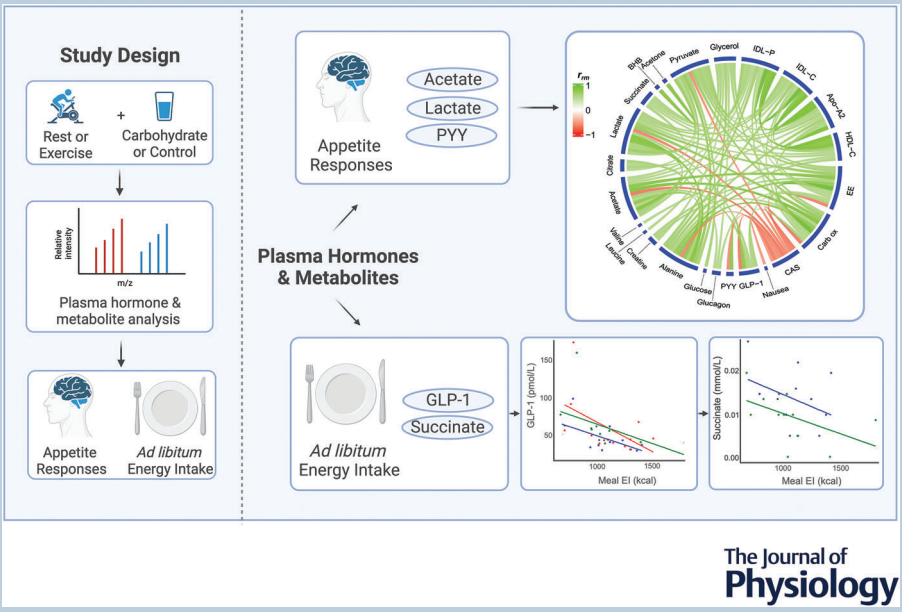

Abstract An understanding of the metabolic determinants of postexercise appetite regulation would facilitate development of adjunctive therapeutics to suppress compensatory eating behaviours and improve the efficacy of exercise as a weight-loss treatment. Metabolic responses to acute exercise are, however, dependent on pre-exercise nutritional practices, including carbohydrate intake. We

James Frampton Following his third-rate career as a non-league footballer for Weymouth FC, James Frampton decided to join the noble pursuit of scientific knowledge, which culminated in him obtaining his PhD in Clinical Medicine Research from Imperial College London in 2022. His research focused on the interaction between diet and exercise and its implications for appetite regulation, in addition to the role of the gut microbiota in skeletal muscle metabolism. He now works as a Research Associate for Thermo Fisher Scientific.

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therefore aimed to determine the interactive effects of dietary carbohydrate and exercise on plasma hormonal and metabolite responses and explore mediators of exercise-induced changes in appetite regulation across nutritional states. In this randomized crossover study, participants completed four 120 min visits: (i) control (water) followed by rest; (ii) control followed by exercise (30 min at ∼75% of maximal oxygen uptake); (iii) carbohydrate (75 g maltodextrin) followed by rest; and (iv) carbohydrate followed by exercise. An *ad libitum* meal was provided at the end of each 120 min visit, with blood sample collection and appetite assessment performed at predefined intervals. We found that dietary carbohydrate and exercise exerted independent effects on the hormones glucagon-like peptide 1 (carbohydrate, 16.8 pmol/L; exercise, 7.4 pmol/L), ghrelin (carbohydrate, −48.8 pmol/L; exercise: −22.7 pmol/L) and glucagon (carbohydrate, 9.8 ng/L; exercise, 8.2 ng/L) that were linked to the generation of distinct plasma ^{1}H nuclear magnetic resonance metabolic phenotypes. These metabolic responses were associated with changes in appetite and energy intake, and plasma acetate and succinate were subsequently identified as potential novel mediators of exercise-induced appetite and energy intake responses. In summary, dietary carbohydrate and exercise independently influence gastrointestinal hormones associated with appetite regulation. Future work is warranted to probe the mechanistic importance of plasma acetate and succinate in postexercise appetite regulation.

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Abstract figure legend Our work aimed to explore hormonal and metabolic mediators of exercise-induced changes in appetite and energy intake across nutritional states. Twelve male participants completed four study visits involving intake of water (control) or carbohydrate with a 30 min rest or high-intensity exercise session. Plasma samples were collected throughout the 120 min study periods to quantify gastrointestinal hormone release and ^{1}H nuclear magnetic resonance metabolite profiles. Visual analog scales were used to investigate appetite responses, and an *ad libitum* meal was provided at the end of each study visit to evaluate energy intake. Temporal changes in acetate, lactate and peptide YY were associated with supressed appetite responses in both exercise conditions. A consistent negative association between glucagon-like peptide 1 and succinate levels with meal energy intake was found in both exercise conditions.

Key points

- Carbohydrate and exercise independently influence key appetite-regulating hormones.
- Temporal changes in postexercise appetite are linked to acetate, lactate and peptide YY.
- Postexercise energy intake is associated with glucagon-like peptide 1 and succinate levels.

Introduction

Chronic exercise interventions typically produce only modest weight loss, especially in comparison to dietary energy restriction (Miller et al., 1997). This relative lack of efficacy is attributed primarily to compensatory eating behaviours, in which the energy expended through exercise is compensated for by an increase in energy intake (Martin et al., 2019). Nevertheless, subjective appetite responses and compensatory energy intake following acute exercise display marked inter-individual variability (Goltz et al., 2018; Hopkins et al., 2014). An understanding of the mechanisms that regulate exercise-induced changes in appetite regulation (appetite and energy intake responses) would facilitate the development of targeted therapeutics for obesity.

Exercise-induced appetite and energy intake responses have largely been attributed to changes in the systemic concentrations of gastrointestinal hormones implicated in appetite regulation, including glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and (active) ghrelin (Martins et al., 2007). However, acute exercise exerts changes beyond gastrointestinal hormone release, producing large-scale shifts in metabolism that might also drive exercise-induced changes in appetite and energy intake (Contrepois et al., 2020).

Evidence is growing that the metabolic effects of acute exercise are dependent on pre-exercise nutritional

practices, including carbohydrate intake (Edinburgh et al., 2022; Frampton et al., 2021; Hargreaves et al., 2004; Vieira et al., 2016). Acute carbohydrate ingestion also modulates GLP-1, PYY and ghrelin concentrations (Karhunen et al., 2008), in addition to producing extensive shifts in the plasma metabolite profile (Ho et al., 2013). We therefore hypothesized that the concurrent provision of carbohydrate and exercise would produce interactive hormonal and metabolic responses that would have important downstream implications for appetite regulation.

The aim of our work was to determine the interactive effects of carbohydrate and exercise on the plasma hormonal and metabolic responses and explore potential mediators of exercise-induced changes in appetite and energy intake across nutritional states.

Methods

Study approval

This study was granted ethical approval (South West – Frenchay Research Ethics Committee; 19/SW/007) and conducted in line with the *Declaration of Helsinki*. All participants provided written informed consent before enrolment. This study is registered at clinicaltrials.gov (NCT04019418).

Participants

This study recruited 12 healthy male participants aged 18–40 years old, with a body mass index of 18–30 kg/m^2 (inclusive) between February 2019 and February 2020. Females were excluded owing to the influence of the menstrual cycle on appetite control, gastrointestinal hormone release and exercise metabolism (Brennan et al., 2009; Dye & Blundell, 1997; Oosthuyse & Bosch, 2010). Full eligibility criteria are provided at ClinicalTrials.gov.

Participants had an age of 24 ± 5 years (mean ± SD), with a body mass index of 21.9 ± 2.1 kg/m^2 and a maximal oxygen uptake ($\dot{V}_{O_2\,max}$) of 40.2 ± 8.7 ml/kg/min. Detailed participant characteristics are provided via the online repository.

Study design

This study was a semi-double-blind, randomized, four-period crossover, placebo-controlled design.

Initially, all participants attended a screening visit to assess eligibility. Eligible participants returned to undertake a $\dot{V}_{O_2\,max}$ test to determine the absolute exercise intensity for study visits. Participants then completed four study visits in a randomized order: (i) control beverage and rest session; (ii) control beverage and exercise session; (iii) carbohydrate beverage and rest session; and (iv) carbohydrate beverage and exercise session. Study visits were separated by a minimum of 3 days.

The study design is described as semi-double-blind because both participants and researchers were blinded to the nature of the beverage, but not to the rest or exercise session.

All study visits and assessments were performed at the National Institute for Health and Care Research (NIHR) Imperial Clinical Research Facility at Hammersmith Hospital (London, UK).

Screening visit. Participants attended a screening visit after fasting for ≥4 h. A blood test (glucose and full blood count), height and weight measurements and an ECG were performed to determine participant eligibility. Participants were excluded if they had had an abnormal ECG, blood values outside the reference range, a body mass index of <18 or >30 kg/m^2, a history of metabolic disease, and/or had started a new medication within the last 3 months that was likely to interfere with energy metabolism, appetite regulation and hormonal balance.

Assessment of $\dot{V}_{O_2\,max}$. All assessments were performed on an ergometrics 900 bicycle ergometer (ergoline, Germany). Participants began the assessment by cycling at 50 W, after which the intensity was increased by 25 W every 3 min until volitional exhaustion. Pulmonary gas measurements (oxygen consumption and carbon dioxide production) were taken throughout the assessment via a Quark CPET metabolic cart (COSMED, Italy). If participants did not achieve a respiratory quotient ≥1.1 at the point of volitional exhaustion, the assessment was repeated at a separate study visit. After completion of the assessment, participants were presented with an *ad libitum* meal identical to that received during study visits to facilitate familiarization.

Study visits. Participants were asked to refrain from strenuous exercise, caffeine and alcohol intake, and to standardize their evening meal on the day before each study visit. Participants were also asked to fast overnight from 20.00 h the evening before each study visit (drinking water was permitted).

Participants arrived at the research facility at 09.00 ± 1 h, upon which an I.V. cannula was inserted into the antecubital vein to permit serial blood sampling. Participants were also asked to void their bladder, with all urine thereafter collected for the remainder of the study visit. After the collection of baseline measurements, participants consumed either a control beverage or a carbohydrate beverage. Participants were given 5 min to consume the beverage, after which they either rested or exercised for 30 min. After completion of the rest or

exercise session, participants rested for a further 90 min, at which point an *ad libitum* meal was provided.

Venous blood samples and 100 mm visual analog scale (VAS) scores were collected at baseline and at 15 min intervals after beverage ingestion (time $t = 15$, 30, 45, 60, 75, 90, 105 and 120). Pulmonary gas measurements were also performed at 15 min intervals throughout the visit (baseline, $t = 15-30$, 45−60 and 105−120).

Interventions

Beverages. The control beverage consisted of 300 ml of water only. The carbohydrate beverage constituted 300 ml of water with 75 g of maltodextrin [equating to 75 g of carbohydrate (300 kcal); MyProtein, UK]. This amount of carbohydrate has previously been shown to modulate gastrointestinal hormone release (Meek et al., 2021). Carbohydrate was provided in liquid form as non-sweet maltodextrin and served in opaque bottles at 4°C to facilitate blinding.

Rest and exercise sessions. Participants lay semi-recumbent on a bed for 30 min for the rest session. For the exercise session, participants exercised on an ergometrics 900 bicycle ergometer (ergoline, Germany) for 30 min at 75% $\dot{V}_{O_2\,max}$ at a cadence of ≥80 pedal revolutions per minute. This exercise intensity has previously been shown to modulate gastrointestinal hormone release (Ueda et al., 2009).

Beverage–exercise interval. Most studies investigating the effect of carbohydrate provision prior to exercise on energy intake have involved participants undertaking exercise several hours after carbohydrate ingestion (Bachman et al., 2016; Edinburgh et al., 2019; Gonzalez et al., 2013). The influence of carbohydrate ingestion on physiological responses such as subjective appetite, GLP-1 and PYY release is transient (Steinert et al., 2011) and thus largely attenuated by the time exercise is performed. As a result, any potential interaction between acute carbohydrate ingestion and exercise on these responses is lost. We therefore used a beverage–exercise interval of 5 min to ensure that changes in appetite-related hormones attributable to both carbohydrate ingestion and exercise coincided.

***Ad libitum* meal.** The *ad libitum* meal consisted of dried durum wheat semolina pasta (Tesco, UK), tomato and herb pasta sauce (Tesco, UK) and olive oil (Tesco, UK). This meal possessed an energy density of 5.3 kJ/g (1.3 kcal/g) and contained 21.2 g of carbohydrate, 3.0 g of fat and 3.3 g of protein per 100 g.

Participants were given 20 min to consume the meal and were asked to eat until 'comfortably full'. Participants were also asked to refrain from using mobile telephones, laptops or other distractions during this period.

Measurements

Primary outcome measures. Blood samples were analysed for GLP-1 and PYY using previously established in-house radioimmunoassays (Adrian et al., 1985; Kreymann et al., 1987) at all time points. Active ghrelin was measured using a commercial enzyme-linked immunosorbent assay (EZGRA-88K, Merck, Germany) at $t = 0$, 30, 60 and 120 only.

Secondary outcome measures. Blood samples were analysed for glucose using a colorimetric enzymatic activity assay (GL364, Randox, UK) at all time points. Insulin (HI-14K, Merck, Germany) and glucagon (GL-32K, Merck, Germany) were measured using commercial radioimmunoassays at $t = 0$, 15, 30, 45, 60, 90 and 120. Commercial assays were performed as specified by the manufacturer's instructions. Intra- and interassay coefficients of variability are available via the online repository.

Pulmonary gas measurements (oxygen consumption and carbon dioxide production) and the urea content of urine were used to calculate substrate oxidation (carbohydrate and fat oxidation) (Frayn, 1983) and total energy expenditure (Weir, 1949).

Ad libitum meal energy intake (EI) was calculated by measuring the difference in mass of the *ad libitum* meal before and after consumption. Total energy intake was calculated by adding the energy content of beverages to *ad libitum* meal energy intake. Acute energy balance was calculated by subtracting total energy expenditure from total energy intake.

Subjective appetite and nausea were measured by VAS scores. Subjective appetite was assessed using four questions relating to hunger, pleasantness, prospective consumption and fullness. These scales were then converted into a single composite appetite score (CAS) using the formula: CAS = [hunger + pleasantness + prospective consumption + (100 − fullness)]/4.

^{1}H Nuclear magnetic resonance metabolic profiling analysis of blood plasma. Water-suppressed ^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed at 310 K using a 600 MHz Bruker Avance III NMR spectrometer [*in vitro* diagnostics research (IVDr)] equipped with a 5 mm BBI Z-gradient probe, high-order shims, and automated tuning and matching (Bruker Biospin, Rheinstetten, Germany). Before the analysis, a quantitative calibration was performed using the standard high-throughput protocol (Dona et al., 2014). Bruker IVDr (B.I.) methods were used to extract lipoprotein data

(B.I. LISA) and to quantify small molecule metabolites (B.I. Quant-PS 2.0) (Jiménez et al., 2018). For each sample, three experiments were acquired in automation: two one-dimensional (1D) ^{1}H NMR spectra were acquired using standard 1D pulse sequences, the first one with saturation of the water resonance only (noesygppr1d, standard Bruker pulse program), and the second one with the same feature and with a block to filter out macromolecular/protein signals from the spectrum (Carr–Purcell–Meiboom–Gill, cpmgpr1d); ^{1}H−^{1}H two-dimensional *J*-resolved (*J*-Res) experiment with water suppression was also acquired (jresgpprqf). The parameter sets used for acquisition and processing were according to the literature (Dona et al., 2014).

Randomization and blinding

Randomization sequences were generated using an online randomization tool (randomizer.org). Sequences were then placed into opaque envelopes, sealed, and allocated to participants before their first study visit by a member of the study team. Participants were informed of the type of study visit (exercise or rest session) upon arrival for each study visit.

Beverages were created and labelled (A or B) by an external researcher before study visits. Beverage contents were revealed after VAS scoring, assays and statistical analysis had been performed.

Statistics

All statistical analyses included data from the 12 participants who competed all study visits.

Robust linear mixed model analysis. The time-averaged area under the curve (AUC) was calculated for all longitudinal outcomes and used for subsequent robust linear mixed effect model analysis. Unadjusted values were used for *ad libitum* meal energy intake, total energy intake, total energy expenditure and acute energy balance.

Robust linear mixed effects models were fitted using the R package 'robustlmm' (Koller, 2016) to evaluate the independent and interactive effects of carbohydrate and exercise on all outcomes. Fixed effects included in the model were carbohydrate and exercise, and an interaction between carbohydrate and exercise. Random effects included in the model were participant, an interaction between participant and carbohydrate, and an interaction between participant and exercise. Random effects with zero variance were removed from the model. The Satterthwaite's degrees of freedom method implemented in the R package 'lmerTest' (Kuznetsova et al., 2017) was used to derive *P*-values for fixed effects. If a significant interaction effect between carbohydrate and exercise was detected, multiple comparisons of estimated marginal means were performed using the R package 'emmeans' (Lenth, 2021), with the Tukey adjustment being applied. Independent effects were defined as a significant main effect of carbohydrate and/or exercise in the absence of a significant interaction effect. Interactive effects were defined as a significant interaction effect (Slinker, 1998). Estimated marginal means of carbohydrate and exercise were calculated only when no significant interaction effect was detected. Statistical significance was set at $P < 0.05$.

Nuclear magnetic resonance data processing and statistical analysis. The multivariate data analysis was performed on the 1D ^{1}H CPMG spectra. Each spectrum was automatically phased and baseline corrected using Topspin 3.5 pl 7 (Bruker BioSpin), then digitized over the range δ −0.5 to 10 where δ means ppm (chemical shift) and imported into MATLAB (2014a, MathWorks, Natick, MA, USA). The spectra were referenced to the doublet of the anomeric proton signal of a-glucose at δ 5.23. The spectral regions corresponding to the internal standard (δ −0.5 to 0.6), water (δ 4.3–5.1) and noise (δ 5.4–5.7, 5.8–6.7 and 8.5–10.00) were excluded to give full-resolution spectra (∼11.7 K spectral data points or variables per spectrum). Before multivariate data analysis, the spectra were normalized using the probabilistic quotient method (PQN) (Dieterle et al., 2006).

The data set was auto-scaled and modelled using partial least-squares discriminant analysis in a Monte Carlo cross-validation framework and repeated measures design. *P*-values were adjusted for multiple testing by calculating the False Discovery Rate (FDR) using Storey-Tibshirani method (*q*-value), where variables with $q \leq 0.05$ were considered as significant in the Monte Carlo cross-validation framework. The goodness of fit (R^2Y) was calculated using the training data, and the goodness of prediction (Q^2Y) from test data (Posma et al., 2018). For discriminant analysis, the number of the intervention was used as a dummy variable at each time point ($Y = 1, 2, 3$ and 4).

Relevant features from the NMR quantitative measurements (e.g. B.I. LISA and B.I. Quant-PS 2.0) were also subjected to linear mixed models, correlation networks and partial least-squares regression.

Identification of metabolites for ^{1}H NMR metabolic profiling analysis. Subset optimization by reference matching (STORM) was used for the identification of significant metabolites using the correlation structure of 1D ^{1}H NMR data (Posma et al., 2012). *J*-Res spectra were also used for identification purposes. Internal and external databases, such as the Human Metabolome Data Base (HMDB; http://hmdb.ca/) and/or the Biological Magnetic Resonance Data Bank (BMRB;

http://www.bmrb.wisc.edu), were used for confirmation of assignments.

Correlation network analysis. Correlation networks were created for outcomes measured at $t = 0$, 30 and 120 using the R package 'rmcorr' (Bakdash & Marusich, 2017). Only outcomes with a significant main effect of carbohydrate and/or exercise and/or a significant interaction effect were included. Correlations with a coefficient of $\geq$0.6 and P-value of $<$0.05 (after correction with false discovery rate (Q) = 5%; Benjamini et al., 2006) were displayed graphically using the R package 'circlize' (Gu et al., 2014). A cut-off of $\geq$0.6 was selected because this indicated a strong relationship between the two variables (Campbell & Swinscow, 1997) and was therefore more likely to be biologically meaningful.

Partial least-squares regression. Partial least-squares regression was performed using the R package 'mixOmics' (Rohart et al., 2017) using all outcomes measured in plasma/serum with a significant main effect of carbohydrate and/or exercise and/or a significant interaction effect. Leave-one-out cross-validation was used to select the number of latent variables for each model. Root mean square error of prediction and goodness of fit (R^2) were calculated via leave-one-out cross-validation using the selected number of latent variables. All variables were scaled and centred before analysis.

Determination of sample size. A formal sample size calculation was not performed because the interactive effect of carbohydrate and exercise has not been investigated before. Prior studies have, however, demonstrated that 12 participants are sufficient to show that carbohydrate ingestion (Steinert et al., 2011) and exercise (Ueda et al., 2009) influence gastrointestinal hormone release. Furthermore, 12 participants have been argued to be sufficient with respect to precision about the mean and variance when no prior information is available (Julious, 2005). The study was stopped once 12 participants had completed all study visits.

Results

Study design

In a randomized-crossover fashion, 12 male participants completed four study visits that involved the consumption of a control beverage (water) or carbohydrate beverage (75 g maltodextrin, 300 kcal), followed by a 30 min rest or exercise session (75% $\dot{V}_{O_2\,max}$ on a cycle ergometer). Maltodextrin was selected over other carbohydrate sources because of its low sweetness (BeMiller, 2019) and thus facilitated blinding. This created four study interventions: (i) control and rest (Con-Rest); (ii) control and exercise (Con-Ex); (iii) carbohydrate and rest (Carb-Rest); and (iv) carbohydrate and exercise (Carb-Ex). Serial blood samples, VAS assessments and pulmonary gas measurements were taken periodically during each 120 min visit, with an *ad libitum* meal presented at the end of the visit (Fig. 1*A*).

Independently and interactively, carbohydrate and exercise modulate the hormonal milieu

Initially, we performed robust linear mixed model analyses to identify the independent and interactive effects of carbohydrate and exercise on the hormonal milieu (Figs 1*B* and 2). Our results revealed that carbohydrate and exercise independently increase the anorexigenic hormone GLP-1 {Carb, 16.8 pmol/l [95% confidence interval (CI), 8.5–25.0 pmol/L]; Ex, 7.4 pmol [95% CI, 3.0–11.7 pmol/L]; Fig. 1*B*}, while acting independently to decrease circulating levels of the orexigenic hormone (active) ghrelin (Carb, −48.8 pmol/L [95% CI, −67.8 to −29.8 pmol/L]; Ex, −22.7 pmol/l [95% CI, −40.6 to −4.8 pmol/L]; Fig. 1*B*). Our results also showed that carbohydrate independently increased the anorexigenic hormone PYY (Carb, 3.0 pmol/L [95% CI, 0.5–5.4 pmol/L; Fig. 1*B*). In addition, we investigated the interactive effects of carbohydrate and exercise on insulin, glucagon and glucose concentrations owing to the posited role of glucose homeostasis in appetite regulation (Wyatt et al., 2021). Carbohydrate and exercise exhibited antagonistic and interactive effects on the insulin response, in which carbohydrate increased and exercise decreased circulating levels, and with exercise attenuating the rise in circulating insulin following carbohydrate ingestion (Fig. 2). Conversely, carbohydrate and exercise increased glucagon concentrations independently (Carb, 9.8 ng/L [95% CI, 2.1–17.6 ng/L]; Ex, 8.2 ng/L [95% CI, 3.1–13.4 ng/L]; Fig. 2). Glucose concentrations were unaffected by exercise, but, as expected, showed a marked increase after carbohydrate ingestion (Carb, 1.0 mmol/L [95% CI, 0.7–1.3 mmol/L]; Fig. 2).

Carbohydrate and exercise suppress appetite but exert opposite effects on acute energy balance

The independent and interactive effects of carbohydrate and exercise on appetite, acute energy balance and substrate oxidation are presented in Figs 1*B* and 2. Carbohydrate and exercise showed independent appetite-suppressive effects (Carb, −8 mm [95% CI, −14 to −2 mm]; Ex, −8 mm [95% CI, −14 to −2 mm]; Fig. 1*B*). However, there were no independent effects of carbohydrate or exercise changes on *ad libitum* meal energy intake (Fig. 1*B*). Given that *ad libitum* meal energy

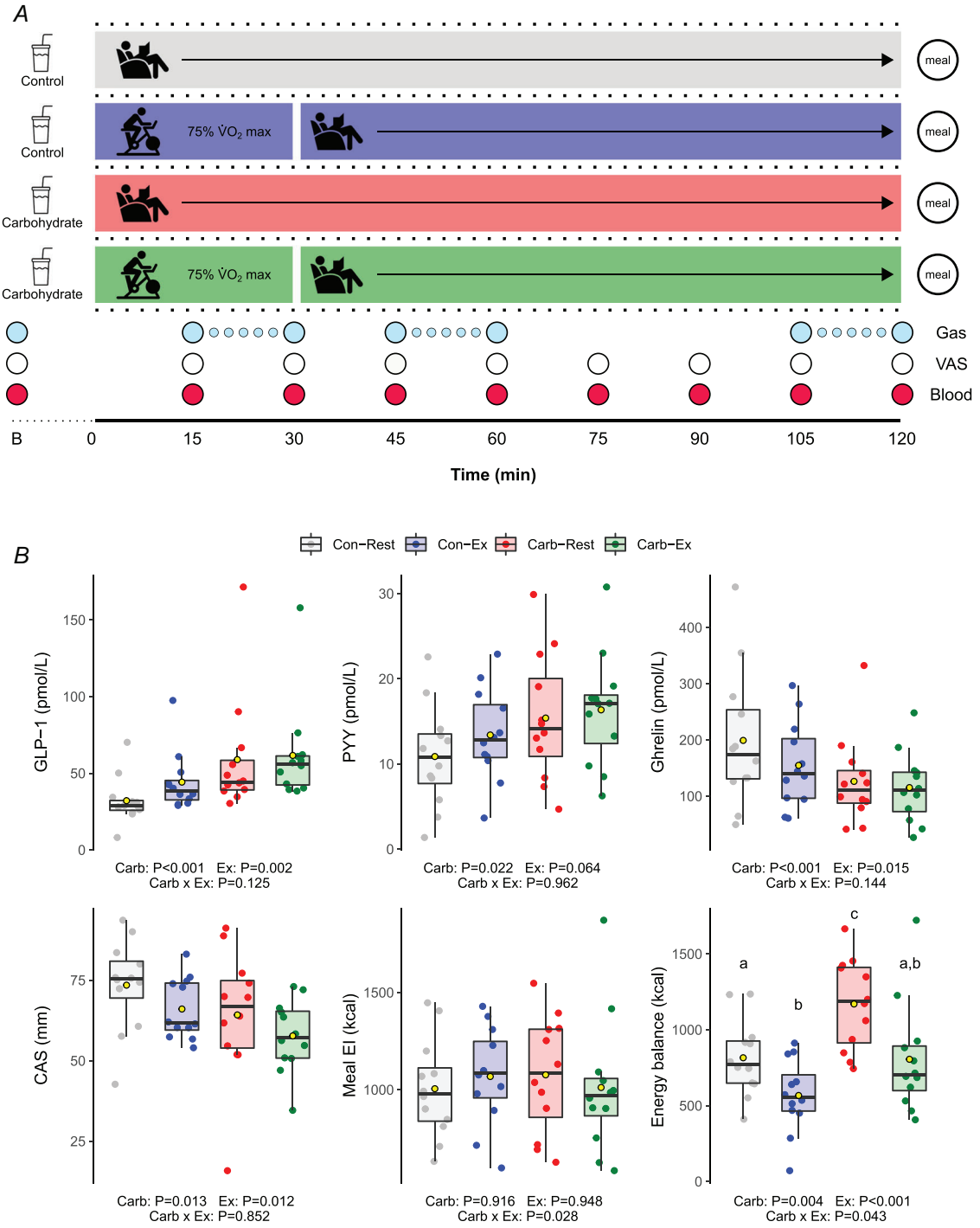

Figure 1. Study design to investigate the effect of dietary carbohydrate and exercise on gastrointestinal hormones, appetite, *ad libitum* energy intake and acute energy balance
A, overview of study interventions, including serial blood sampling scheme, visual analog scale assessments and pulmonary gas measurements. Abbreviation: B, baseline. *B*, the effect of dietary carbohydrate and exercise on glucagon-like peptide 1 (GLP-1), peptide PYY (PYY), (active) ghrelin, composite appetite score (CAS), *ad libitum* meal energy intake (EI) and acute energy balance. Data are the time-averaged area under the curve for all hormones and CAS. Dots represent values for individual participants, with yellow dots representing intervention means. The *P*-values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex) and for the interaction between carbohydrate and exercise (Carb × Ex) are presented. [Colour figure can be viewed at wileyonlinelibrary.com]

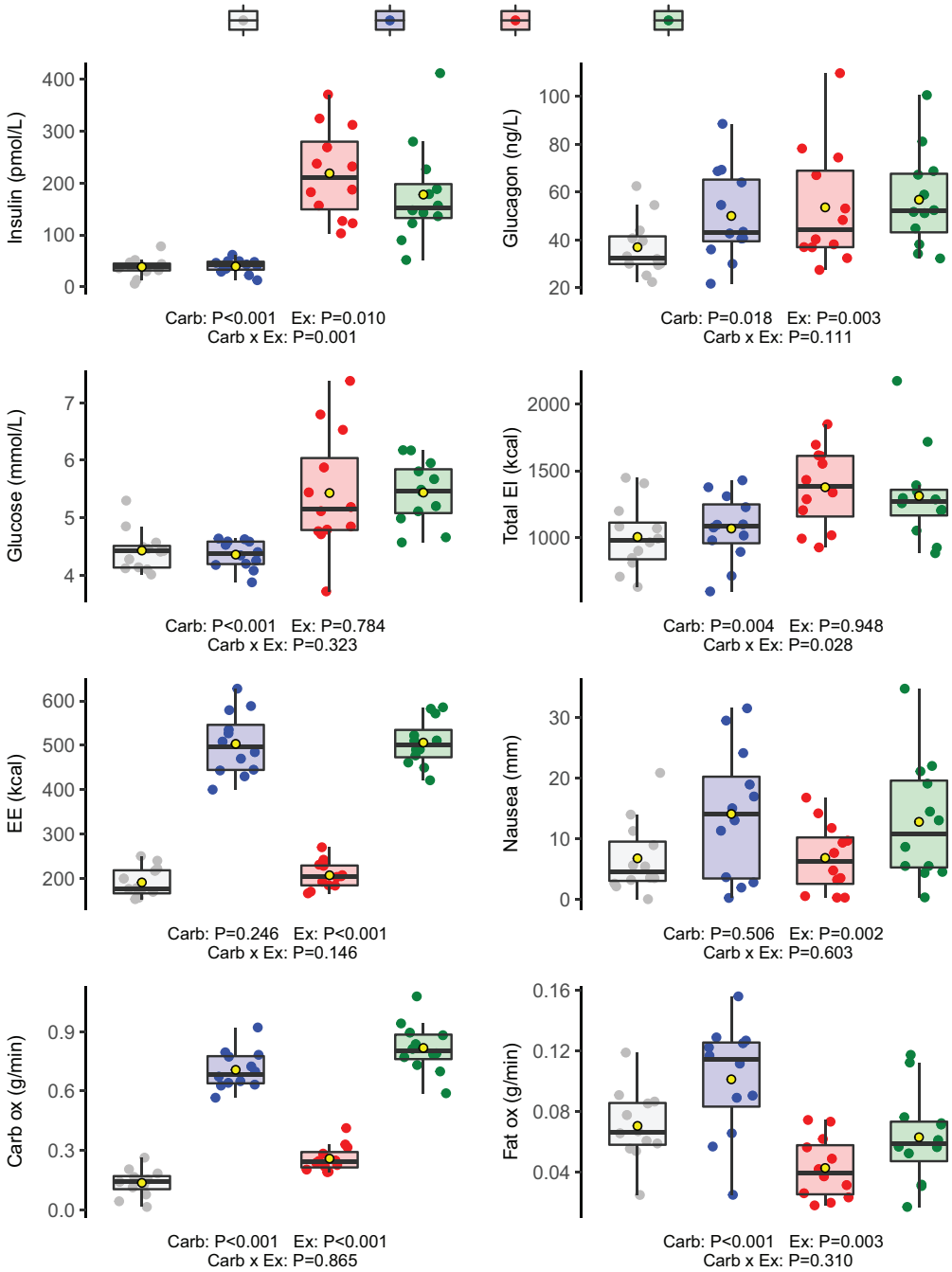

Figure 2. The effect of dietary carbohydrate and exercise on pancreatic hormones, components of acute energy balance and substrate oxidation
Data are the time-averaged area under the curve for insulin, glucagon, glucose, nausea, carbohydrate oxidation (Carb ox) and fat oxidation (Fat ox) only. Dots represent values for individual participants, with yellow dots representing intervention means. The *P*-values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex) and for the interaction between carbohydrate and exercise (Carb × Ex) are presented. Interventions with different letters are significantly different from each other ($P < 0.05$). Abbreviations: EE, energy expenditure; EI, energy intake. [Colour figure can be viewed at wileyonlinelibrary.com]

intake was the sole component of acute energy balance that was allowed to vary in our study design (pre-exercise energy intake and exercise energy expenditure were fixed), *ad libitum* meal energy intake had the potential to exert a large influence over acute energy balance. Carbohydrate independently increased whereas exercise independently decreased acute energy balance (total energy intake minus total energy expenditure in each study intervention), thus exercise without carbohydrate ingestion resulted in the lowest acute energy balance (Fig. 1*B*).

Carbohydrate and exercise generate distinct metabolic phenotypes

Data obtained from ^{1}H NMR metabolic profiling analysis were modelled using repeated measures partial least-squares discriminant analysis to distinguish metabolic phenotypes between study interventions at predefined time points (t = 0, 30 and 120). The metabolic phenotype was highly distinguishable between all study interventions immediately post-exercise (T = 30), characterised by high a degree of explained variance ($R^2Y \geq 0.99$) and capability of prediction ($Q^2Y > 0.7$ with the exception of Con-Ex vs Carb-Ex; Figure 3*A*) that showed a lower capability of prediction ($Q^2Y = 0.48$). Differences between study interventions were present until the end of the study visit (t = 120) but were less pronounced at later time points (Fig. 3*B*). No differences between study interventions were detected at baseline ($t = 0$).

^{1}H Nuclear magnetic resonance metabolic profiling analysis identified 23 unique small metabolites that showed significant differences over time between study interventions (Fig. 3*A* and *B*). Quantitative measurements were available for 14 of these metabolites (Jiménez et al., 2018), which were used for subsequent analysis (Fig. 4). Carbohydrate ingestion produced differential amino acid and amino acid derivative responses characterized by an increase in creatine concentrations (Carb, 0.005 mmol/L [95% CI, 0.002–0.009 mmol/L) and a simultaneous decrease in branched chain amino acid [isoleucine (−0.01 mmol/L), leucine (−0.02 mmol/L) and valine (−0.01 mmol/L)] concentrations (Fig. 4). Exercise increased concentrations of creatine (Ex, 0.008 mmol/L [95% CI, 0.003–0.013 mmol/L]) and the amino acids alanine (Ex, 0.10 mmol/L [95% CI, 0.07–0.13 mmol/L]) and glutamate (Ex, 0.03 mmol/L [95% CI, 0.00–0.06 mmol/L]; Fig. 4). Concentrations of the TCA cycle intermediates citrate (Ex, 0.04 mmol/L [95% CI, 0.02–0.06 mmol/L]) and succinate (Ex, 0.011 mmol/L [95% CI, 0.009–0.013 mmol/L]), in addition to acetate (Ex, 0.08 mmol/L [95% CI, 0.06–0.09 mmol/L]) and lactate (Ex, 2.9 mmol/L [95% CI, 2.1–3.8 mmol/L]), also increased in response to exercise, with only lactate increasing in response to carbohydrate ingestion (Carb, 0.5 mmol/L [95% CI, 0.2–0.7 mmol/L]; Fig. 4). Exercise resulted in increased concentrations of 3-hydroxybutyrate (BHB; Ex, 0.037 mmol/L [95% CI, 0.007–0.067 mmol/L]) and pyruvate (Ex, 0.015 mmol/L [95% CI, 0.008–0.022 mmol/L]; Fig. 4). However, ketogenesis was suppressed by carbohydrate ingestion, as demonstrated by decreased BHB (Carb, −0.068 mmol/L [95% CI, −0.010 to −0.036 mmol/L]) and acetone (Carb, −0.04 mmol/L [95% CI, −0.06 to −0.02 mmol/L]) concentrations (Fig. 4). Carbohydrate and exercise exerted antagonistic and interactive effects on glycerol concentrations, with carbohydrate ingestion suppressing concentrations and exercise elevating concentrations, and with exercise generating higher concentrations when carbohydrate was not ingested (Fig. 4).

^{1}H Nuclear magnetic resonance metabolic profiling analysis also revealed differences between interventions in ^{1}H NMR peaks assigned to high-density lipoprotein (HDL) and low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) (Fig. 3*A* and *B*). Consequently, quantitative lipoprotein measurements were analysed (Jiménez et al., 2018), with data presented for main lipoprotein fractions and parameters (Fig. 5; sub-fraction data are available via the online repository). Carbohydrate ingestion resulted in an increase in high-density lipoprotein cholesterol (HDL-C; Carb, 2.0 mg/dl [95% CI, 0.2–3.8 mg/dl]; Fig. 5), whereas exercise resulted in an increase in intermediate-density lipoprotein particle number (IDL-P; Ex, 9.7 nmol/L [95% CI, 2.9–16.5 nmol/L]) and cholesterol concentrations (IDL-C; Ex, 1.7 mg/dl [95% CI, 0.6–2.8 mg/dl]), in addition to an increase in apolipoprotein A2 (Apo-A2; Ex, 1.3 mg/dl [95% CI, 0.3–2.3 mg/dl]; Fig. 5). No other main effects of carbohydrate and exercise nor any interaction between carbohydrate and exercise was detected.

Changes in circulating acetate, lactate and PYY are associated with exercise-induced appetite suppression

To identify temporal relationships between outcomes, we used repeated measures correlation analyses to create within-intervention correlation networks (Fig. 6). These analyses highlighted distinct relationships between outcomes that were dependent on carbohydrate intake and exercise (Fig. 6*A*). Acetate, alanine, Apo-A2, carbohydrate oxidation, total energy expenditure, lactate and valine showed a high degree of connectivity (total number of connections, ≥15) across multiple study interventions (Fig. 6*B*). Irrespective of carbohydrate intake, higher concentrations of acetate, lactate and PYY were associated with decreased appetite during the exercise study interventions (Fig. 6*A*). Furthermore, of

these outcomes, only higher concentrations of acetate were associated with decreased appetite in the resting state (Fig. 6*A*). Both GLP-1 and PYY showed positive correlations across most study interventions (Fig. 6*A*). GLP-1 also exhibited strong positive correlations with insulin after carbohydrate ingestion (Fig. 6*A*), and with total energy expenditure and carbohydrate oxidation during exercise study interventions (Fig. 6*A*). The only identified hormone–metabolite relationships were positive correlations between GLP-1 and acetate and between GLP-1 and lactate during exercise without carbohydrate ingestion (Fig. 6*A*).

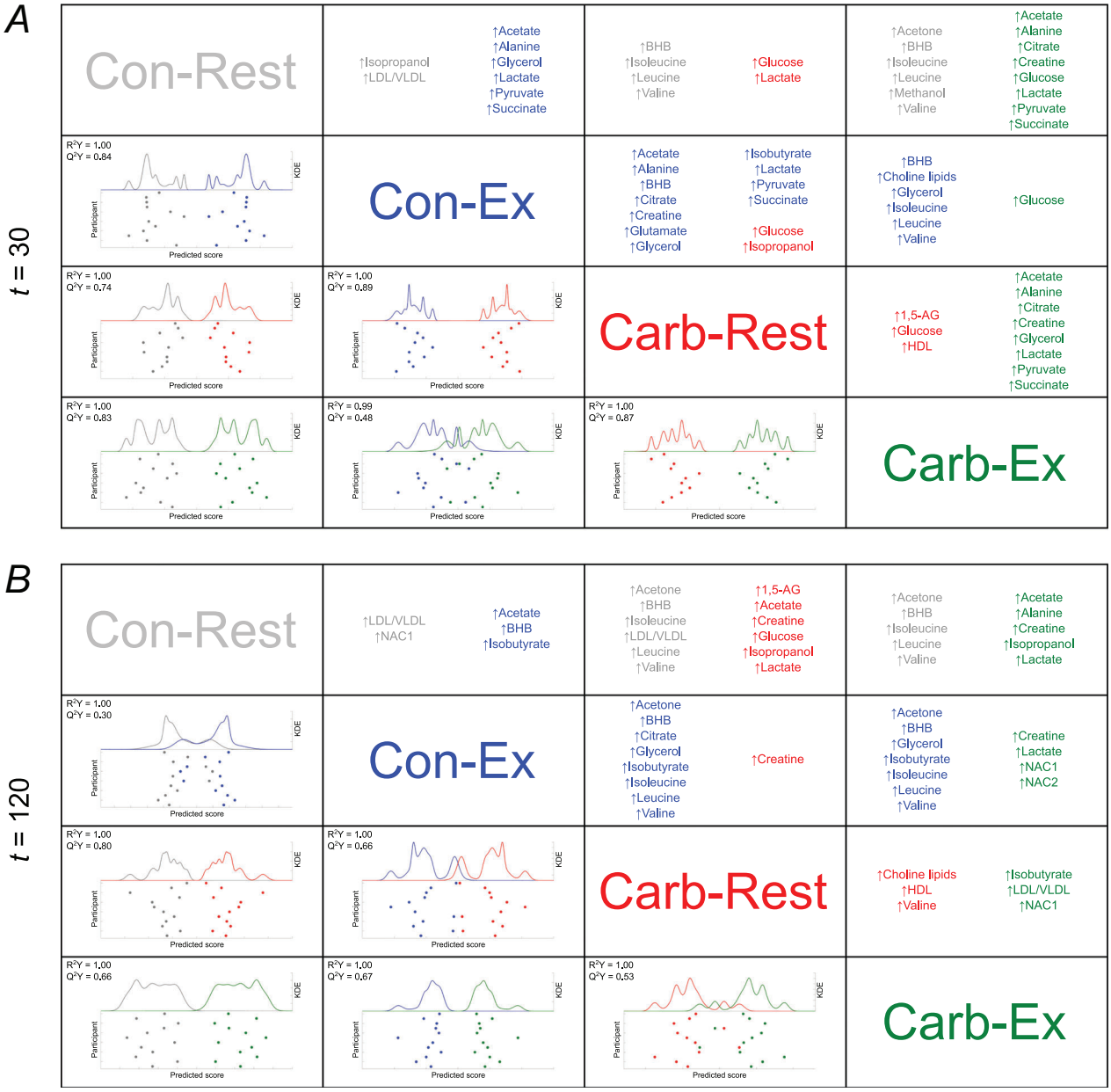

Figure 3. Partial least-squares discriminant analysis score plot and metabolite matrix
A, comparisons between study interventions at time t = 30. *B*, comparisons between study interventions at t = 120. In both *A* and *B*, the panels below the diagonal display score plots derived from partial least-squares discriminant analysis demonstrating differentiation in metabolic profiles between study interventions (Con-Rest, grey; Con-Ex, blue; Carb-Rest, Red; and Carb-Ex, green). Dots represent individual participant metabolic profiles. All models include kernel density estimates (KDE) of the predicted scores for both study interventions undergoing comparison. In both *A* and *B*, the panels above the diagonal display differences in metabolites between study interventions. Abbreviations: Q^2Y, capability of prediction; R^2Y, explained variance. The nuclear magnetic resonance peak assignments for significant metabolites are provided in the online repository. [Colour figure can be viewed at wileyonlinelibrary.com]

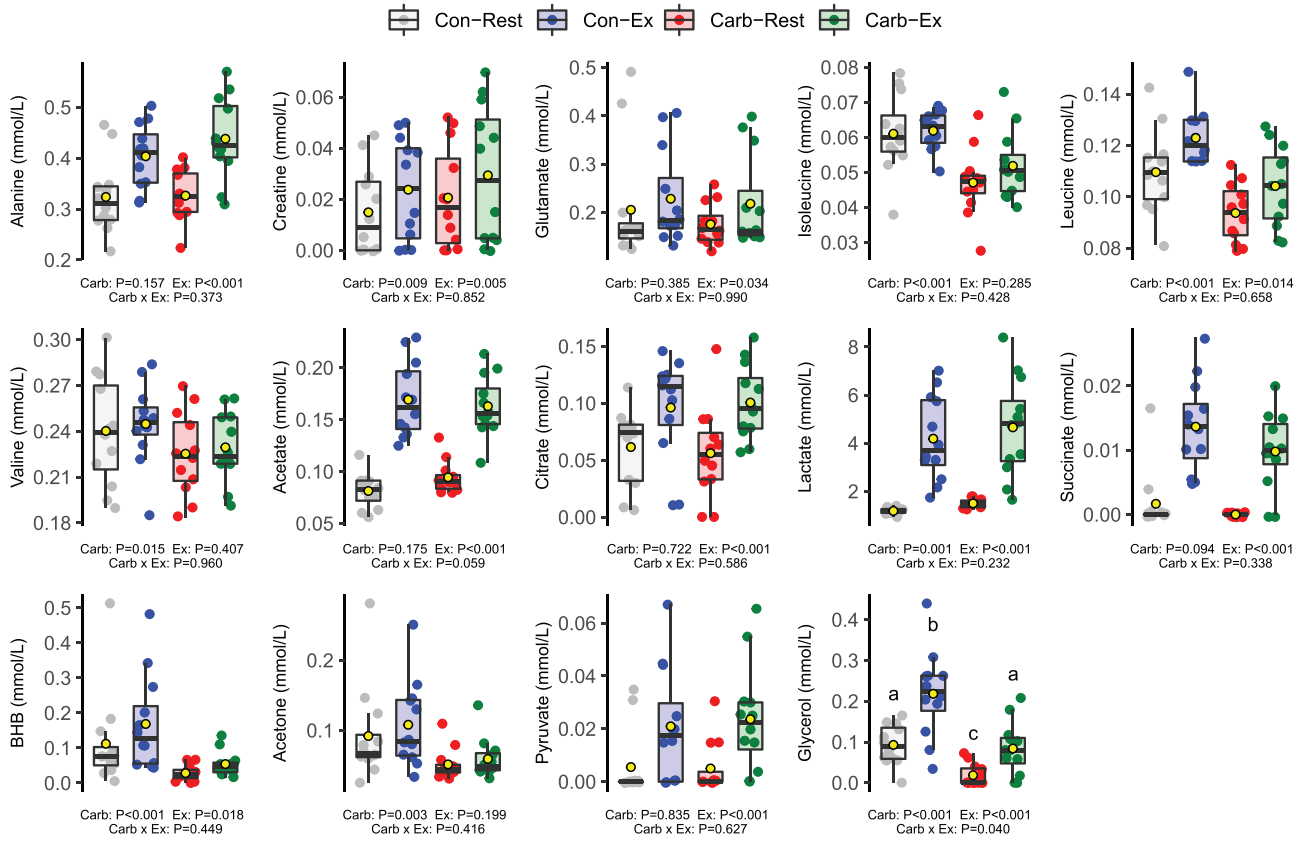

Figure 4. The effect of dietary carbohydrate and exercise on circulating small metabolites
Data are the time-averaged area under the curve for all outcomes. Dots represent values for individual participants, with yellow dots representing intervention means. The *P*-values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex) and for the interaction between carbohydrate and exercise (Carb × Ex) are presented. Interventions with different letters are significantly different from each other ($P < 0.05$). Abbreviation: BHB, 3-hydroxybutyrate. [Colour figure can be viewed at wileyonlinelibrary.com]

Identification of metabolic predictors of *ad libitum* energy intake

Congruent with prior research (Holt et al., 2017), subjective appetite was not correlated with subsequent *ad libitum* meal energy intake within study interventions (see online repository). Although subjective appetite might reflect eating latency (King et al., 2013), it does not appear to be predictive of energy intake, hence any observed relationship with subjective appetite might not necessarily be present for energy intake.

We therefore investigated the capacity of the preceding metabolic environment during the 120 min study visit period (represented as the time-averaged AUC) to predict subsequent *ad libitum* meal energy intake within each study intervention using partial least-squares regression (Fig. 7). For all study interventions, one latent variable was created, with variable importance in projection (VIP) scores of >1 (Kuhn & Johnson, 2013) used to identify outcomes important in the prediction of subsequent *ad libitum* energy intake (Fig. 7*A*). The VIP scores identified GLP-1 and creatine to be important predictors of subsequent energy intake, exhibiting VIP scores of >1 in three of the study interventions. Likewise, glucose, alanine, citrate, succinate, acetone, pyruvate, glycerol and Apo-A2 also appeared to be important predictors, possessing a VIP score of >1 in two of the study interventions. Of these metabolites, only succinate was an important predictor of meal energy intake in both exercise interventions irrespective of carbohydrate intake. Follow-up within-intervention simple univariate regression analyses were also performed to explore the direction of relationship between important predictors (VIP > 1) and meal energy intake (Fig. 7*B*) and highlight the consistent negative association between GLP-1 and succinate release with subsequent *ad libitum* meal energy intake in both exercise conditions.

Discussion

Dietary carbohydrate and exercise both exert profound effects on human metabolism that have important

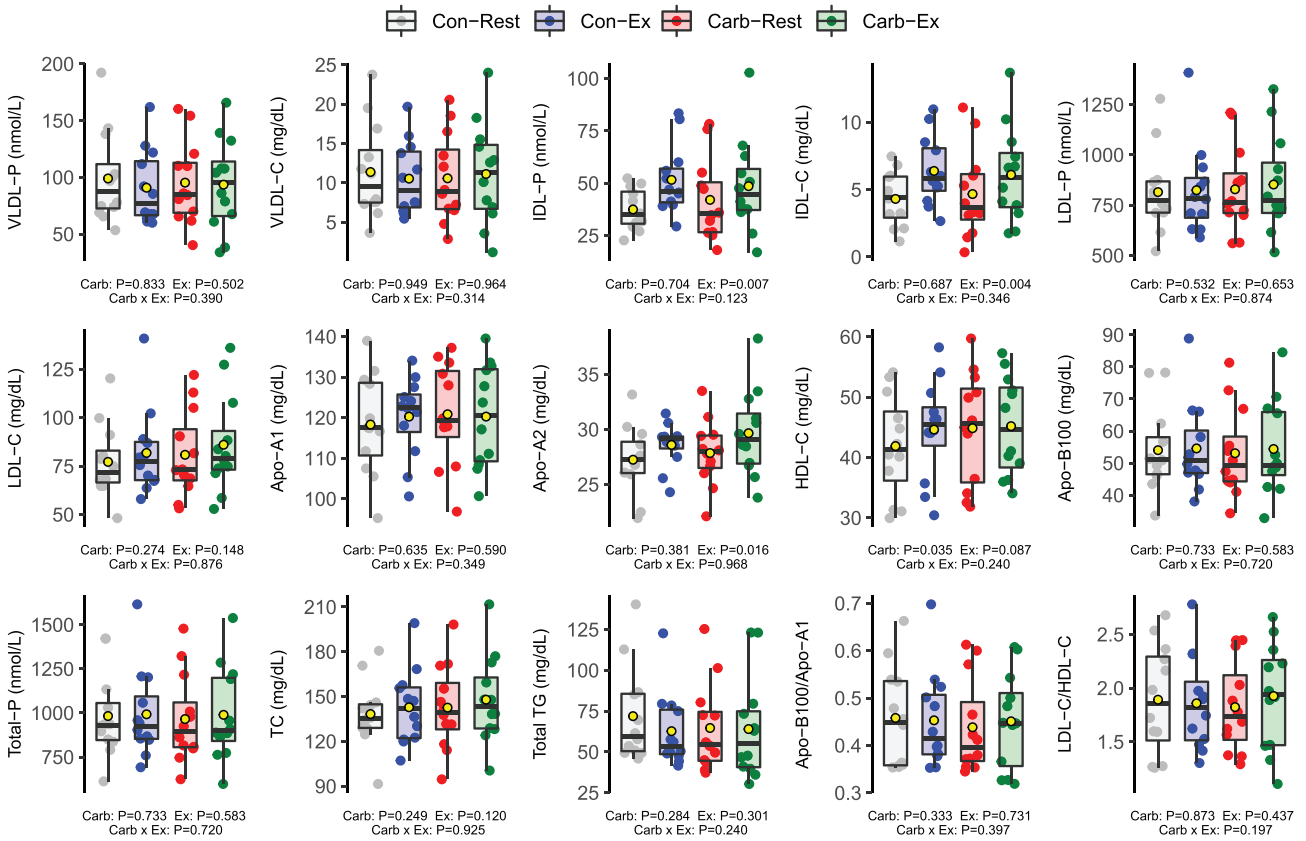

Figure 5. The effect of dietary carbohydrate and exercise on main lipoprotein fractions and parameters
Data are the time-averaged area under the curve for all outcomes. Dots represent values for individual participant, with yellow dots representing intervention means. The *P*-values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex) and for the interaction between carbohydrate and exercise (Carb × Ex) are presented. Abbreviations: Apo-A1, Apolipoprotein A1; Apo-A2, Apolipoprotein A2; Apo-B100, Apolipoprotein B100; Apo-B100/Apo-A1, Apolipoprotein B100/Apolipoprotein A1 ratio; HDL-C, High-density lipoprotein cholesterol; IDL-C, Intermediate-density lipoprotein cholesterol; IDL-P, Intermediate-density lipoprotein particle number; LDL-C, Low-density lipoprotein cholesterol; LDL-C/HDL-C, Low-density lipoprotein cholesterol/HDL-C, High-density lipoprotein cholesterol ratio; LDL-P, Low-density lipoprotein particle number; TC, Total cholesterol; Total TG, Total triglycerides; Total-P, Total particle number; VLDL-C, Very-low-density lipoprotein cholesterol; VLDL-P, Very-low-density lipoprotein particle number. [Colour figure can be viewed at wileyonlinelibrary.com]

implications for appetite regulation and energy intake. Here, we show that dietary carbohydrate and exercise generate independent or interactive effects on gastrointestinal and pancreatic hormones associated with appetite regulation. Using ^{1}H NMR metabolic profiling analysis, we also demonstrate that dietary carbohydrate and exercise generate distinct plasma metabolic phenotypes, leading to the identification of new putative mediators of exercise-induced effects on appetite and energy intake.

Glucagon-like peptide 1, PYY and ghrelin have well-recognized roles as key regulators of appetite and energy intake (Steinert et al., 2017), thus the primary outcome of the study was to establish the independent or interactive response of these hormones to carbohydrate intake and exercise. We show that dietary carbohydrate and exercise independently increase GLP-1 and decrease ghrelin concentrations (carbohydrate also increases PYY independently). Consequently, both carbohydrate and exercise create a hormonal milieu conducive to the suppression of appetite and energy intake. Despite the pattern of response for gastrointestinal hormones across study conditions being reflected in subjective appetite responses, this was not observed for meal energy intake, suggesting that other factors might contribute to the observed between-condition differences in energy intake.

In accord with previous research (Frampton et al., 2022), exercise without carbohydrate ingestion (which can also be regarded as 'fasted exercise') resulted in the lowest acute energy balance. Nevertheless, the long-term implications of this finding are unclear owing to the inherent trade-off between internal and ecological validity. For example, the time at which participants ate was fixed, and therefore any effect of eating latency

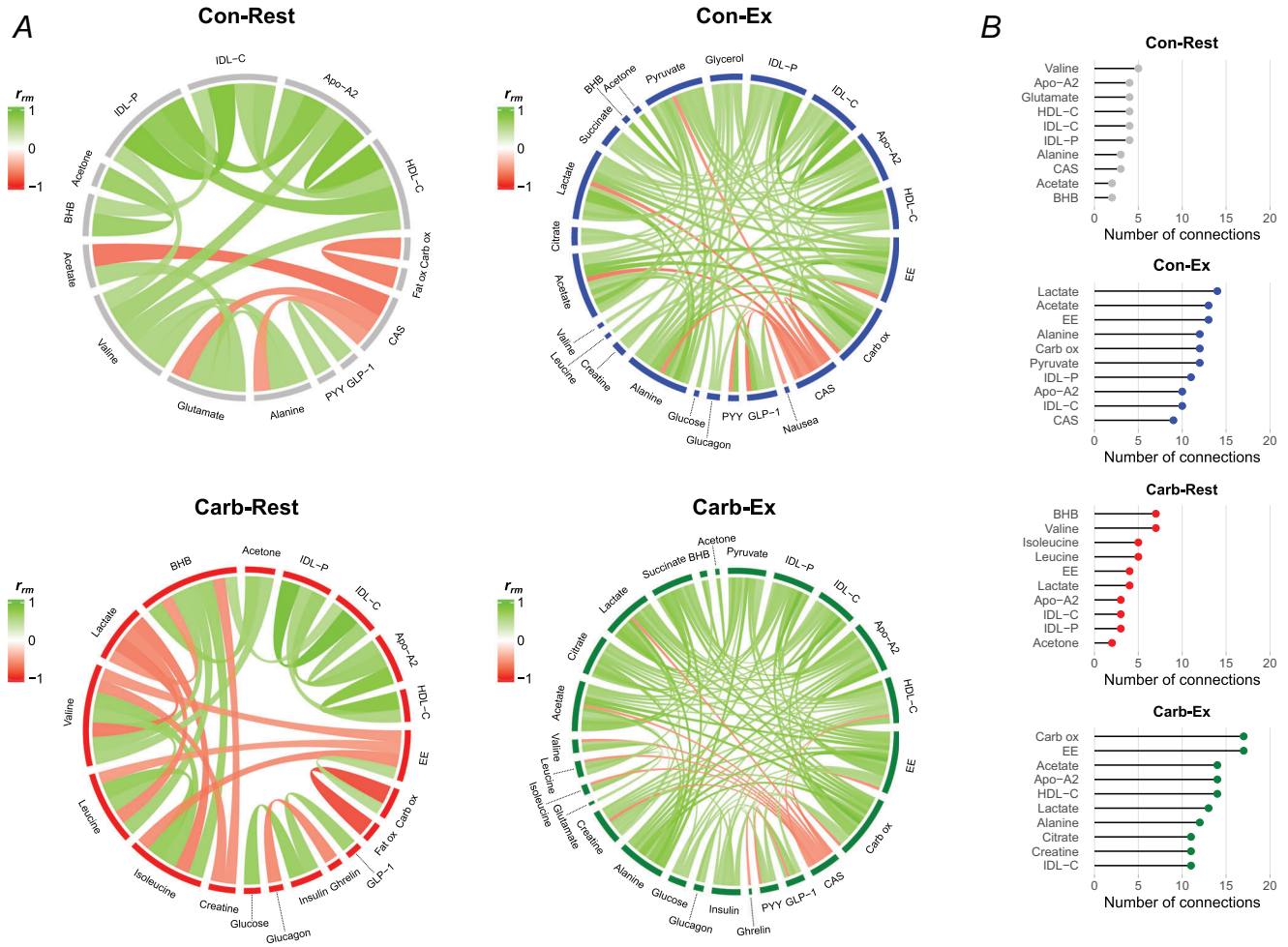

Figure 6. Correlation networks of temporal measurements across study interventions
Repeated measures correlation networks for Con-Rest, Con-Ex, Carb-Rest and Carb-Ex (*A*) and the top 10 outcomes with the highest number of connections for each study intervention (*B*). Outcomes linked by a green line showed a significant positive correlation, whereas outcomes linked by a red line showed a significant negative correlation. Only outcomes measured at t = 0, 30 and 120 with a significant main effect of carbohydrate and/or exercise and/or a significant interaction effect were included in the analyses. Correlations with a coefficient of ≥0.6 and adjusted P-value of <0.05 are displayed. Abbreviation: Apo-A2, Apolipoprotein A2; BHB, 3-hydroxybutyrate; Carb ox, Carbohydrate oxidation; CAS, Composite appetite score; EE, Energy expenditure; Fat ox, Fat oxidation; GLP-1, Glucagon-like peptide 1; HDL-C, High-density lipoprotein cholesterol; IDL-C, Intermediate-density lipoprotein cholesterol; IDL-P, Intermediate-density lipoprotein particle number; PYY, Peptide YY; r_{rm}, repeated measures correlation coefficient. [Colour figure can be viewed at wileyonlinelibrary.com]

(possibly as a result of gastrointestinal hormone and metabolite modulation) on subsequent energy intake was unknown. Further work is needed to investigate whether the lower acute energy balance when exercise is performed in the fasted state translates into greater weight loss with fasted exercise training.

We also provide, for the first time, extensive characterization of the acute lipoprotein response to exercise, showing a transient increase in IDL-P, IDL-C and Apo-A2 concentrations after acute exercise. Although chronic changes in fasting levels of these lipoprotein parameters are associated with modified cardiovascular disease risk (Birjmohun et al., 2007; Hodis et al., 1997), the increase observed with acute exercise is likely to be a physiological response to the increased energy demands imposed by exercise, and thus represents an increase in energy mobilization. Furthermore, characterization of the acute lipoprotein response to exercise might also facilitate the understanding of how chronic exercise modifies lipoprotein profiles and associated cardiovascular disease risk.

Exercise is often accompanied by a temporary suppression of appetite commonly referred to as exercise-induced anorexia (King et al., 1994). Correlation network analyses resulted in the identification of key relationships between the plasma metabolome

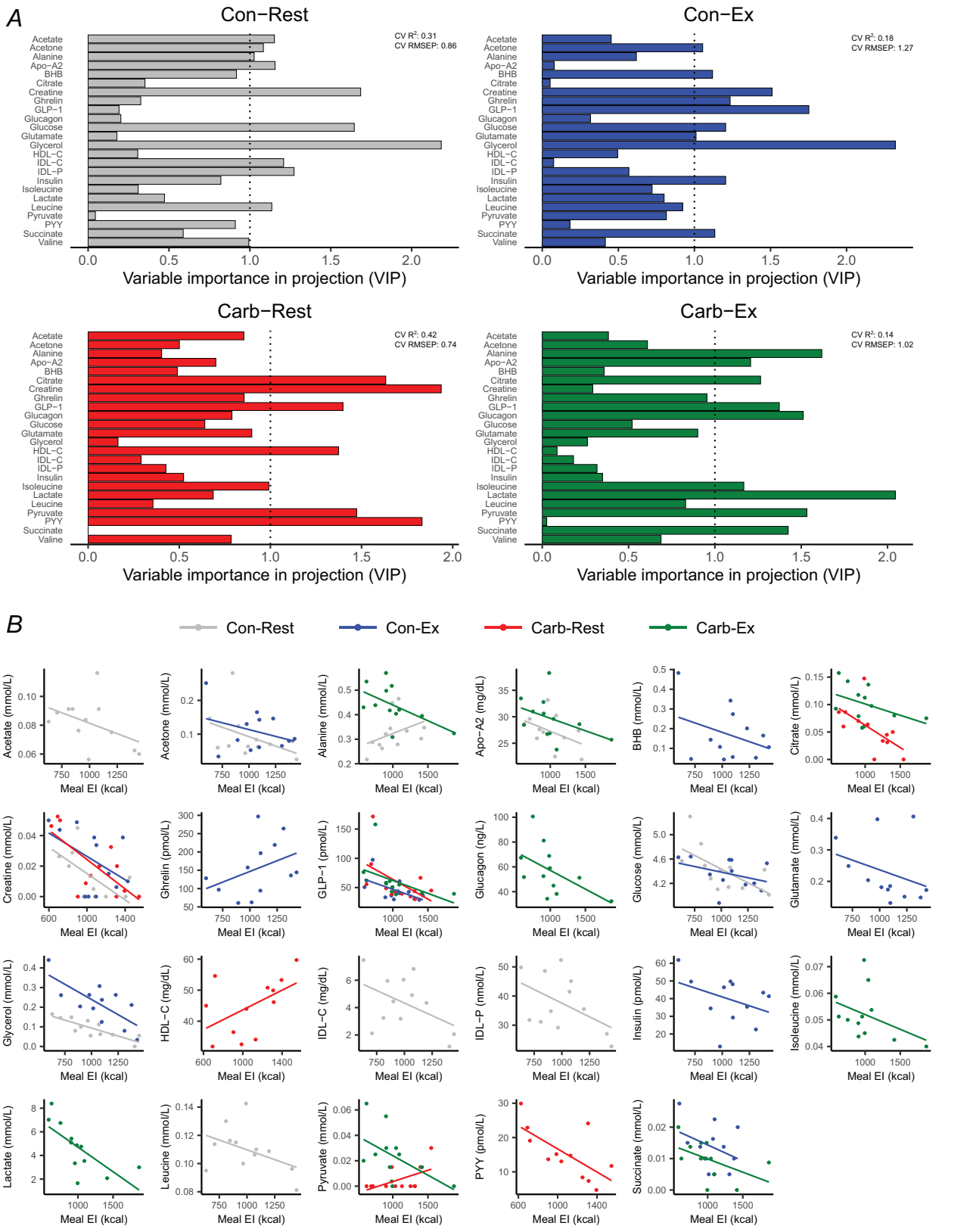

Figure 7. Prediction of *ad libitum* meal energy intake from preceding metabolic environment

A, variable importance in projection (VIP) scores from partial least-squares regression models for each study intervention. Only outcomes measured with a significant main effect of carbohydrate and/or exercise and/or a significant interaction effect were included in the analyses. *B*, simple univariate linear regression plots for outcomes with a VIP score of >1. The root mean square error of prediction (RMSEP) and explained variance (R^2) were calculated via leave-one-out cross-validation (CV). Abbreviation: Apo-A2, Apolipoprotein A2; BHB, 3-hydroxybutyrate; EI, energy intake; GLP-1, Glucagon-like peptide 1; HDL-C, High-density lipoprotein cholesterol; IDL-C, Intermediate-density lipoprotein cholesterol; IDL-P, Intermediate-density lipoprotein particle number; PYY, Peptide YY. [Colour figure can be viewed at wileyonlinelibrary.com]

and exercise-induced appetite suppression. Peptide YY, lactate and acetate all displayed strong negative correlations with appetite in both exercise interventions irrespective of carbohydrate intake. The relationships between PYY and lactate and exercise-induced appetite suppression are expected and have been reported previously (Martins et al., 2007; Vanderheyden et al., 2020). Indeed, a recent study highlighted that exercise-induced lactate is used as a precursor for the synthesis of *N*-lactoyl-phenylalanine, a metabolite shown to possess anorexic properties in rodents following pharmacological administration (Li et al., 2022). Although exercise was shown to raise circulating *N*-lactoyl-phenylalanine in humans, its association with the marked inter-individual exercise-induced changes in appetite and energy intake were not reported (Goltz et al., 2018; Hopkins et al., 2014).

In contrast, a relationship between appetite and acetate during exercise has not been identified previously. The intestine is the primary site of acetate production in the fasting and postprandial state (Kirschner et al., 2021), with skeletal muscle becoming a major contributor to acetate production during exercise (Van Hall et al., 2002). Despite limited human evidence, exogenous administration of acetate has been shown to suppress appetite in rodent models, acting via a central homeostatic mechanism (Frost et al., 2014). Indeed, increasing circulating acetate concentrations through I.V. infusions to levels similar to those achieved during exercise has been shown to increase GLP-1 concentrations (Freeland & Wolever, 2010), suggesting that acetate might regulate exercise-induced appetite suppression by direct and indirect mechanisms.

Appetite and energy intake are related but distinct constructs. Therefore, components of the plasma metabolome that were implicated in appetite suppression could not be assumed to be implicated in energy intake suppression. Consequently, we used partial least-squares regression analyses to identify potential metabolic predictors of energy intake. Glucagon-like peptide-1 and succinate were the only two components of the plasma metabolome to be identified as important predictors of energy intake in both exercise study interventions. Again, the identification of GLP-1 is unsurprising and highlights the importance of this hormone in the relationship between dietary carbohydrate, exercise, appetite and energy intake. Recent studies investigating GLP-1 receptor agonists and exercise training have reported greater weight loss when using both interventions concurrently *vs.* either intervention alone (Lundgren et al., 2021). This suggests that therapeutics based on mediators of exercise-induced changes in appetite and energy intake might be highly efficacious with respect to weight loss, particularly when used as a co-intervention alongside exercise training. A possible role for circulating succinate has, however, not been reported previously. Preclinical evidence indicates that succinate administration can decrease energy intake via an upregulation of intestinal gluconeogenesis (Wang et al., 2019) and might also play a key role in skeletal muscle remodelling after exercise (Reddy et al., 2020).

Like acetate, lactate and succinate are produced in substantial amounts by contracting skeletal muscle during exercise (Juel & Halestrap, 1999; Reddy et al., 2020), suggesting that muscle-derived metabolites might be key regulators of energy intake in the acute period after exercise. Although participants in the present study exercised at the same relative intensity, our data also highlight notable inter-individual differences in acetate, lactate and succinate responses across exercise interventions (Fig. 8*A*). Furthermore, acetate and lactate responses exhibited strong positive correlations between exercise interventions (Fig. 8*B*), suggesting that participants genuinely vary in their metabolic profile with exercise. It has been demonstrated recently that appetite and energy intake responses to exercise also show substantial inter-individual variation (Goltz et al., 2018; Hopkins et al., 2014). Differences in acetate, lactate and succinate concentrations might therefore contribute to the variation in postexercise appetite and energy intake responses and, consequently, be a key therapeutic target for interventions aiming to augment exercise-induced weight loss. However, acetate, lactate and succinate concentrations during exercise interventions were not related to body weight, fat-free mass or $\dot{V}_{O_2\,max}$ (Fig. 8*C*), suggesting that increasing skeletal muscle mass or aerobic fitness is unlikely to alter the concentration of these metabolites. In addition to being muscle derived, acetate, lactate and succinate are generated from gut microbial metabolic activity (Macfarlane & Macfarlane, 2003). Consequently, the observed variation in circulating levels of these metabolites could be explained, in part, by individual differences in gut bacterial composition and metabolism.

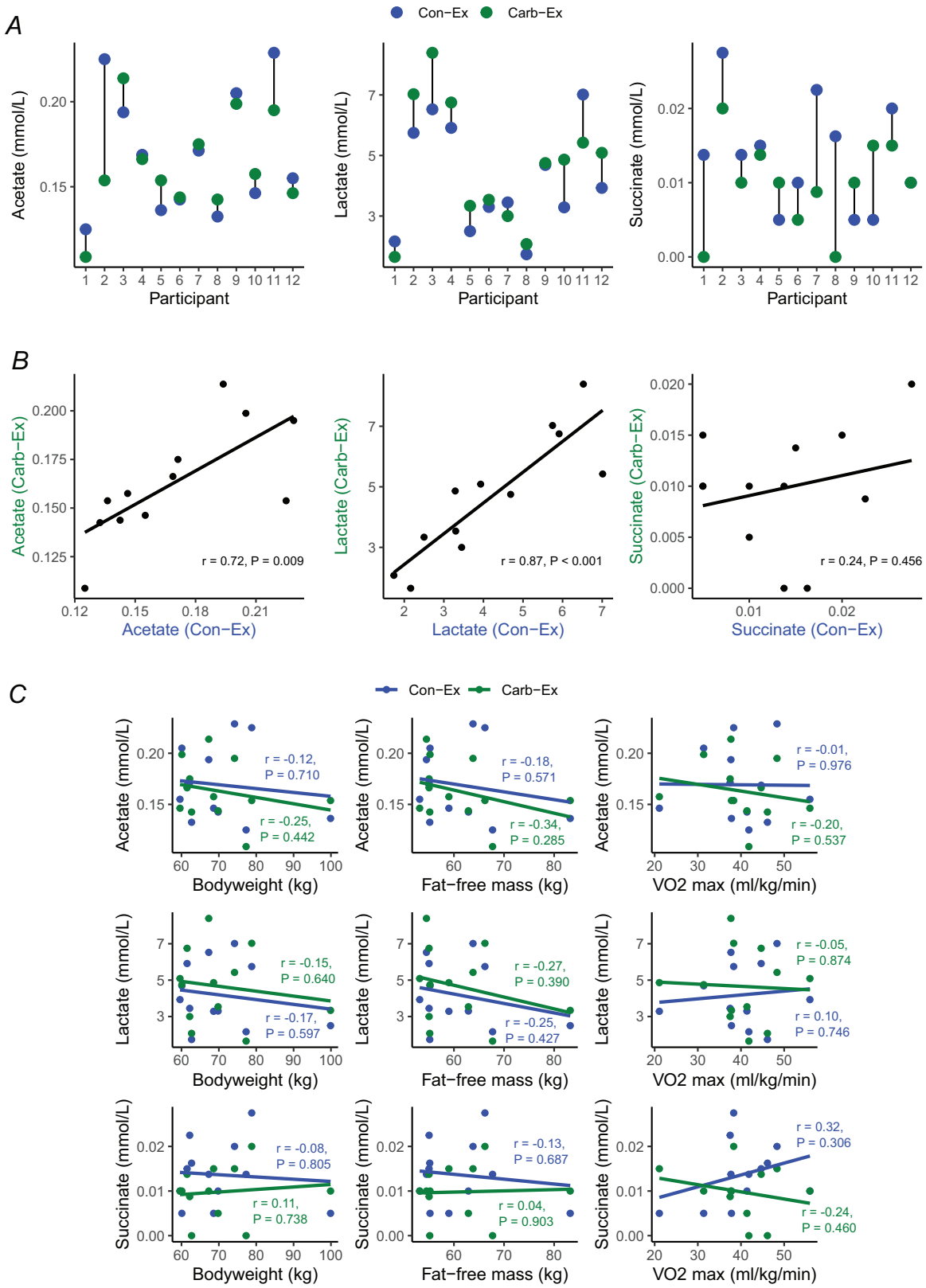

Figure 8. Inter-individual variation in acetate, lactate and succinate responses during exercise interventions and their relationship to anthropometric and physiological characteristics

A, time-averaged area under the curve data from each participant for both exercise interventions. The length of the black line represents the difference between exercise interventions. *B*, correlation between Con-Ex and Carb-Ex time-averaged area under the curve data. C, correlation between acetate, lactate, succinate and anthropometric (body weight, fat-free mass) and physiological characteristics (maximal oxygen uptake). Abbreviation: *r*, Pearson's correlation coefficient. [Colour figure can be viewed at wileyonlinelibrary.com]

The findings of our study must, however, be interpreted in the context of its limitations. In this study, we measured energy intake, hence energy balance, only in the immediate period after exercise completion. Therefore, any compensatory responses that might occur beyond this period would not be observed, hence our findings cannot necessarily be applied to exercise training studies. Quantitative measurements were available for only select metabolites, hence the responses of some metabolites (e.g. isobutyrate) that showed differential responses across study interventions in the ^{1}H NMR metabolic profiles were not entered into subsequent correlation and regression models. Our analysis might therefore have missed key metabolites involved in exercise-induced changes in appetite and energy intake. The cohort used in the present study included only young lean males; the translation of our findings to other populations (such as individuals with obesity or females) cannot be assumed. For example, we observed increases in glucagon concentrations after carbohydrate ingestion, a response that might be present only in this demographic, and not in individuals with obesity and associated metabolic diseases (Wagner et al., 2017). Furthermore, the identification of plasma metabolites involved in exercise-induced appetite and energy intake must be considered exploratory rather than confirmatory. Future investigations should attempt to manipulate succinate and acetate concentrations in the context of exercise, via the ingestion of oral agents that raise or lower their concentrations, to elucidate their role in energy intake around exercise. Lastly, the carbohydrate and exercise doses selected for this study were chosen in order to maximize the gastrointestinal hormone response and might therefore not reveal synergisms between carbohydrate and exercise that can be observed only at submaximal doses.

In conclusion, we show that dietary carbohydrate and exercise generate independent and interactive effects on various components of the plasma metabolome, resulting in the generation of distinct metabolic phenotypes. We also provide, for the first time, extensive characterization of the lipoprotein response to exercise and identify plasma acetate and succinate as new putative regulators of exercise-induced suppression of appetite and energy intake. Future investigations are needed to establish whether treatments to augment plasma acetate and succinate provide therapeutic opportunities to suppress postexercise appetite and energy intake responses.

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Additional information

Data availability statement

Data and R code used for analysis are available from the Open Science Framework at: https://osf.io/9t2bp/ (DOI: 10.17605/OSF.IO/9T2BP).

Competing interests

The authors declare no conflict of interest.

Author contributions

E.S.C. and K.G.M. conceived and designed the study. J.F., J.P. and A.S.Y.T. conducted the study. J.F., J.I.S.-C., I.G.P., G.F.B., J.P., A.S.Y.T., A.C.C.O., A.M. and E.S.C. performed sample analysis. J.F. and J.I.S.-C. performed statistical analysis. J.F. and E.S.C. wrote the first draft of the manuscript, which was critically revised by J.I.S.-C., I.G.P., G.F.B., J.P., A.S.Y.T., A.C.C.O., A.M., K.G.M. and G.F. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

appetite, carbohydrate, exercise, metabolism

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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