Effect of High-Fat, High-Carbohydrate, and High-Protein Meals on Metabolism and Performance During Endurance Cycling

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The effect of pre-exercise meal composition on metabolism and performance in cycling were investigated in a crossover study. Twelve competitive cyclists ingested high-fat, high-carbohydrate, or high-protein meals 90 min before a weekly exercise test. The test consisted of a 1-hour pre-load at 55% peak power, five 10-min incremental loads from 55 to 82% peak power (to measure the peak fat-oxidation rate), and a 50-km time trial that included three 1-km and 4-km sprints. A carbohydrate supplement was ingested throughout the exercise. Relative to the high-protein and high-fat meals, the high-carbohydrate meal halved the peak fat-oxidation rate and reduced the fat oxidation across all workloads by a factor of 0.20 to 0.58 ($p = .002–.0001$). Reduced fat availability may have accounted for this reduction, as indicated by lower plasma fatty acid, lower glycerol, and higher pre-exercise insulin concentrations relative to the other meals ($p = .04–.0001$). In contrast, fat oxidation following the high-protein meal was similar to that following the high-fat meal. This similarity was linked to evidence suggesting greater lipolysis and plasma fat availability following high-protein relative to high-carbohydrate meals. Despite these substantial effects on metabolism, meal composition had no clear effect on sprint or 50-km performance.

Key Words: high-intensity cycling, insulin, glucagon, substrate oxidation

Introduction

Fatigue during prolonged endurance exercise is related to the depletion of glycogen stores or a reduction in plasma glucose concentration (reviewed in 19). Consequently, there has been considerable interest in nutritional interventions that offset fatigue either by increasing carbohydrate availability or by reducing carbohydrate utilization during exercise (19).
High-carbohydrate meals (>50 g carbohydrate), taken 1 to 4 hours before exercise, increase the amount of carbohydrate stored in the digestive tract, liver, and muscle prior to exercise (6, 29, 32) and can enhance moderate-intensity endurance performance in subjects who have fasted overnight (29, 32). Performance during prolonged exercise can also be enhanced with carbohydrate supplementation, which offsets the effects of declining plasma-glucose concentration resulting from inadequate hepatic glucose production (reviewed in 11, 19).

An intervention that may reduce carbohydrate utilization is the elevation of plasma fat availability with a high-fat pre-exercise meal (5, 35). A high-fat meal increases plasma chylomicron-triglyceride and free fatty-acid concentrations (12, 13, 39), which can be energy substrates for the exercising muscle (4). In addition, a high-fat meal with a low-carbohydrate content (<20 g) attenuates the post-prandial increase in plasma insulin, which in turn further increases fat oxidation (6, 7, 12, 39).

In trained cyclists the effect of high-carbohydrate versus high-fat meals on endurance performance is unclear. Whitley et al. (39) found a non-significant 5% enhancement in mean power in a 10-km time trial preceded by 2 hours at 70% of maximal oxygen uptake (% VO₂max) following a high-fat meal with 74% energy (%E) from fat relative to a high-carbohydrate meal (86%E). Pitsiladis et al. (27) reported an 8.2% enhancement in time to exhaustion (p = .001) during cycling at 75% VO₂max following a high-fat (90%E) meal, relative to a high-carbohydrate (70%E) meal; in this study the high-fat meal was followed by infusion of heparin to increase fat utilization. The use of heparin, and the fact that subjects did not consume a carbohydrate supplement in either study, make these findings difficult to apply to competitive athletes. Consequently, the first aim of the current study was to investigate the effect of a high-fat relative to a high-carbohydrate meal on cycling performance with carbohydrate supplementation.

Compared with research on high-carbohydrate pre-exercise meals, there has been little research on the effect of protein-rich pre-exercise meals on metabolism and performance in endurance exercise. Protein-rich meals (>50 g) are of interest to exercise scientists for several reasons. First, an increase in plasma branched-chain amino-acid concentration following a protein-rich meal could enhance performance via a mechanism involving attenuation of central fatigue (24), although it is reasonably clear that any such enhancement is at best small (8). Second, the glucoregulatory hormonal response (insulin and glucagon) following a high-protein meal is considerably different to that following a high-carbohydrate low-protein meal (38); this metabolic difference may impact performance because of the effects of these hormones on liver, adipose, and muscle fuel metabolism (3, 25, 36, 37). Finally, there is little research directly investigating the metabolic and performance effects of a meal containing 30%E protein, 40%E carbohydrate, and 30%E fat, which is popular with some endurance athletes (31). Consequently, the second aim of the current study was to investigate the effect of a high-protein meal on metabolism and performance relative to high-fat and high-carbohydrate meals.

A 50-km cycling time trial with 1-km and 4-km sprints was used to measure performance. The time trial was preceded by 110 min of pre-load exercise, which included five 10-min incremental loads to examine the effect of meal composition on peak fat oxidation rate and also on fuel utilization at higher intensities. The cyclists ingested a carbohydrate-electrolyte solution to simulate dietary practice in competitions and to offset the effects of declining plasma-glucose availability and dehydration (11).
Methods

Subjects

Twelve male cyclists and triathletes participated in the study. Subject characteristics (mean \(\pm SD\)) were: maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)), 4.8 \(\pm\) 0.5 L \(\cdot\) min\(^{-1}\) (64 \(\pm\) 6 ml \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\)); peak power, 367 \(\pm\) 33 W; mass, 76 \(\pm\) 7 kg; height, 178 \(\pm\) 5 cm; and age, 27 \(\pm\) 8 years. The study received ethical approval from the Otago Ethics Committee of the Southern Regional Health Authority.

Research Design

The design was a double-blind randomized crossover. Exercise tests were performed at weekly intervals over a 5-week period. There were two exercise procedures. The first was a short incremental test performed 7 days before the main experiment to measure peak-power output and \(\dot{V}O_{2\text{max}}\). The second procedure was used to measure the effect of meal composition on metabolism and performance. This test was first performed in the fasting condition 1 week before the first treatment test as a familiarization trial to improve reliability of performance outcomes in the subsequent tests. In addition, the familiarization test provided a fasting baseline for comparison of the effects of the pre-exercise meals on metabolism.

Each subject completed a 4-day dietary and 1-week training diary during the week before the familiarization test. The cyclists then repeated their normal weekly diet and training regimens throughout the 4 weeks of the study.

Peak Power and Maximal Oxygen Uptake

The test of peak power output and \(\dot{V}O_{2\text{max}}\) was performed on an electromagnetically-braked cycle ergometer (Rodby, Södertälje, Finland) with drop handlebars and fitted with the subject’s own pedals, cycling shoes, and seat. Peak power was determined using a procedure similar that of Kuipers et al. (22). Briefly, after a warm up of 10 min at 100 W, the work rate was increased by 50 W every 150 s until a heart rate of 160 beats \(\cdot\) min\(^{-1}\) was surpassed; thereafter, work rate increased by 25 W every 150 s until the test was terminated at the point of voluntary exhaustion, or when pedal cadence fell by greater than 20 revolutions per minute. Peak power was defined as the last completed work rate plus the fraction of time spent in the final non-completed workload multiplied by 25. Peak power was used to set workloads in appropriate stages of the metabolic and performance exercise procedure.

Expired respiratory gas was collected continuously throughout the test using an online gas analysis system for determination of oxygen consumption (\(\dot{V}O_{2}\)). Cyclists wore a mouthpiece and nose clip (Hans Rudolph, MO). Expired air passed down a 2-m flexible plastic tube into a 6-L mixing chamber. Mixed-expired air samples were drawn from the mixing chamber by vacuum through a sampling line for measurement of \(O_2\) (oxygen sensor N-22 and oxygen analyzer S-34, Applied Electrochemistry Inc., Sunnyvale, CA) and \(CO_2\) (Datex Normocap CD102, Finland) fractions. Inspired minute volume was measured using a pneumotachograph and flow transducer (Hewlett Packard, Waltham, MA). Instrument outputs were passed through an analogue-to-digital converter to a microcomputer where calculations were made of respiratory variables. Immediately prior to sampling, gas analyzers were calibrated with a two-point calibration using room air and analytical
grade concentrations of $O_2$ (~16%) and $CO_2$ (~4%) verified against a beta standard (BOC Gases, NZ). The volume meter was calibrated using a 3-L syringe. Calibration checks of the $O_2$ and $CO_2$ analyzers and volume meter were performed after each sampling block (e.g., duration of the incremental test, see below) and corrections made of gas fractions if necessary. $V_{O_2\max}$ was the $VO_2$ measured during the final fully-completed 20-s gas-sampling period, which corresponds to the oxygen utilization during the final work rate.

**Experimental Procedure**

The testing procedure is shown in Figure 1.

**Preparation.** To accommodate study or work commitments, the cyclists were given the option of performing all of the tests in either the morning or the evening. For the morning tests, the cyclists reported to the lab between 0600 and 0800 after an overnight fast. For an evening test, subjects arrived at the lab at 1700, with the final (non-test) meal taken between 1200 and 1300. After arrival at the lab, an indwelling cannula with a 30-cm extension line was placed into the forearm of the subject for subsequent sampling of venous blood. After the cannula was fitted, the subject remained seated for 20 min; during the final 6 min, respiratory gas was sampled online for calculation of fat and carbohydrate oxidation at rest in the fasting condition. Fasting gas exchange was sampled on only one occasion because subjects were assumed to be burning the same mixture of fuels before the ingestion of each pre-exercise meal.

![Figure 1](image_url) — The testing procedure consisted of a pre-exercise meal, a 1-hour pre-load (55% peak power), an incremental test (five 10-min workloads: 55, 65, 70, 75, and 82% of peak power), and a 50-km time trial with 1-km (thin bars) and 4-km (thick bars) sprints.
Following gas collection, cyclists ingested one of the three meals and water 90 min before the start of exercise. The cyclists finished the meal within 10 min. After the meal the subject rested seated in a chair until the beginning of exercise. Resting VO$_2$ and VCO$_2$ were measured shortly before exercise.

**Pre-exercise Meals.** The composition of the three pre-exercise meals is presented in Table 1. The meals where chocolate-coconut flavored custard mixtures with indistinguishable taste and texture. The meals were made in bulk, then frozen for later use in the experiments. On the day of the test, meals were thawed overnight, then beaten with an electric mixer until a smooth thick consistency developed suitable for serving. The meals were isoenergetic and provided at 63 kJ · kg$^{-1}$ (15 kcal · kg$^{-1}$). Water was provided with the meal to standardize the total pre-exercise water ingestion to 9 ml · kg$^{-1}$.

Soy-protein isolate (Supro®, Columbit, Auckland, NZ) was chosen as the protein source for the custard mixtures because, relative to casein-based protein isolates, it contains a ratio of amino acids shown to cause a stronger glucagon and a weaker insulin secretary response (18) and may therefore promote greater lipolysis and gluconeogenesis. The fat mixture used in the meals contained dairy cream, canola oil, coconut cream, and egg yolk at 7, 47, 42, and 4 g of food per 100-g mixture, respectively. Total fat content per 100 g of mixture was 19.9 g (61%) saturated, 9.4 g (29%) monounsaturated, and 3.7 g (10%) polyunsaturated. The composition contained a mixture of medium chain-length triglycerides for rapid digestion and early provision of fat fuels, and long-chain triglycerides to aid in the digestive response and for the longer-term provision of fat fuel as chylomicron.

**Table 1 Composition of the Test Meals**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mass (g)</th>
<th>Energy % of total</th>
<th>kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat meal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>28</td>
<td>10</td>
<td>475</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>15</td>
<td>5</td>
<td>237</td>
</tr>
<tr>
<td>Fat</td>
<td>102</td>
<td>85</td>
<td>4034</td>
</tr>
<tr>
<td>High-protein meal</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>83</td>
<td>30</td>
<td>1432</td>
</tr>
<tr>
<td>Carbohydrate</td>
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<td>40</td>
<td>1909</td>
</tr>
<tr>
<td>Fat</td>
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<td>30</td>
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<tr>
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<td>4034</td>
</tr>
<tr>
<td>Fat</td>
<td>6</td>
<td>5</td>
<td>237</td>
</tr>
</tbody>
</table>

*Note.* Macronutrient allocations are the mean for the study group. Individual quantities were adjusted accordingly to body mass.
triglycerides. For the carbohydrate source, a mixture consisting of sago starch and sucrose was used in a 50:50 ratio by mass.

**Pre-load and Incremental Test.** The 1-hour pre-load and the incremental loads were performed on the Rodby ergometer. Respiratory gas was collected for 5 min from the beginning of the 25th and 55th minutes of the 1-hour pre-load. The average of the final 3 min of the 5-min gas sample was used in calculations of $\overline{V}\text{O}_2$ and $\overline{V}\text{CO}_2$. Expired gas was collected throughout the incremental test, although the mouthpiece was removed for 2 min at the beginning of the second and fourth stages to allow for the ingestion of the supplement.

The oxidation rate ($\text{g} \cdot \text{min}^{-1}$) for carbohydrate and fat were calculated during the 1-hour pre-load and the incremental test using the biochemical and physical data of Peronnet and Massicotte (26):

\[
C = 4.5850 \cdot \overline{V}\text{CO}_2 - 3.2255 \cdot \overline{V}\text{O}_2 \\
F = -1.7012 \cdot \overline{V}\text{O}_2 + 1.6946 \cdot \overline{V}\text{CO}_2
\]

where $C$ and $F$ are the rates of oxidation carbohydrate and fat, respectively.

A correction was made to $\overline{V}\text{CO}_2$ during the latter incremental loads to account for the evolution of CO$_2$ from declining body bicarbonate stores (buffer CO$_2$). Buffer CO$_2$ production was calculated from the decline in blood standard HCO$_3^-$ concentration (SBC) that occurred during the latter stages of the incremental test. Venous blood samples were drawn into capillary tubes from the forearm cannulae at 1–2-min intervals over the duration of the incremental test. The capillary tubes were sealed using rubber caps and stored on ice for 80–90 min before being assayed for SBC (the HCO$_3^-$ concentration of the sample at PCO$_2$ of 40 mmHg and 37 ºC) using a blood-gas analyzer (ABL50, Radiometer, Copenhagen, Denmark). The SBC provided a direct index for the effect of electrochemical changes in body fluid during exercise relative to the resting condition and circumvented complications involved with anaerobic sampling of arterial blood for analysis of the actual HCO$_3^-$ concentration.

The rate of decline in SBC was determined by fitting a first-order monoexponential equation to data from the 1st to 10th minute of each workload: $y = a + be^{-ct}$, where $y$ is the SBC (mmol · L$^{-1}$) at time $t$, $a$ is the asymptotic concentration, $e$ is the standard exponential term, and $b$ and $c$ are constants. The SBC at the beginning and end of the respiratory-gas sampling period (the 7th and 10th minutes of each stage) were determined from the equation.

The fractional change in SBC was assumed to equal the fractional change in the labile whole body CO$_2$ store over the 3-min gas-sampling period. The assumption was based on the observations that exercise-induced changes in strong ion concentrations (La$^+$, Na$^+$, K$^+$, Cl$^-$) and CO$_2$ are distributed rapidly and relatively evenly across the extracellular fluid compartment and most intracellular fluid compartments, with the exception of the active muscle which behaves differently (20). The volume of the labile whole body CO$_2$ store was taken as the whole body water volume minus the intracellular fluid volume of the active muscle (10.6% total body mass), because the CO$_2$ content of the active tissue compartment changes little during sustained heavy exercise (20). Assuming no substantial change of compartmental fluid volume between rest and exercise, a change in the molar SBC, therefore, represents a calculable mass of CO$_2$ expired at the lung:
Buffer CO₂ production (ml · min⁻¹) = \( \frac{(SBC_B - SBC_E)}{SBC_R} \cdot CO_{2R} \cdot \frac{kg \cdot (0.894 \cdot SBC_R / 24.5)}{22.26/3} \)

where CO₂ is a literature estimate of the labile body CO₂ store at rest (9.2 mmol · kg⁻¹; 10), \( SBC_R \) is the SBC at rest, \( SBC_B \) and \( SBC_E \) are the SBC at the beginning and end of the expired gas sampling period for each workload, respectively, 0.894 is the correction for the active muscle compartment, 24.5 is the standard resting SBC, and 22.26 is the gas constant for CO₂. No correction was made to the respiratory data for protein oxidation.

50-km Time Trial. After completion of the incremental loads, the cyclists were transferred to a cycle ergometer (Kingcycle, High Wycombe, UK) for the 50-km time trial. The time trial was modeled on the 100-km time trial in the reliability study of Schabort et al. (30). The CV for the 100-km time trial was 1.7% for 100-km time and 1.9 to 2.0% for sprint time, derived from the mean of the four sprints. The sprints were included to provide additional measures of performance and to simulate the variable-intensity nature of bicycle road races (30).

The Kingcycle was calibrated at 250 W immediately before the time trial. Cyclists were instructed to ride as fast as possible for the sprint distance. The sprints were three 1-km sprints beginning immediately as the rider passed 7, 22, and 37 km, and three 4-km sprints at 11, 26, and 41 km (Figure 1). Sprint time was record by hand with stopwatches. Subjects were provided with a view of the course profile on the wall of the laboratory and the elapsed distance and heart rates from the Kingcycle output during each ride. The best performance times were posted on the laboratory wall to encourage competition. Subjects were informed of their performance times upon the completion of each test.

Carbohydrate-Electrolyte Supplement. At 30 min into the 1-hour pre-load, cyclists began ingesting a 6% carbohydrate solution (PR*Nutrition, San Diego, CA). The solution contained a mixture of 50 g fructose and 50 g maltodextrin per 100 g carbohydrate, 110 mg · L⁻¹ sodium, 155 mg · L⁻¹ potassium, and traces of other molecules. The rate of ingestion during the 1-hour pre-load and the incremental test was 6 ml · kg⁻¹ · 30 min⁻¹ (~55 g carbohydrate · h⁻¹). During the time trial, it was 8 ml · kg⁻¹ · 25 km⁻¹ (~58 g carbohydrate · h⁻¹).

Laboratory Conditions

Environmental conditions in the laboratory were 19–21 °C and 45–55% humidity. Cyclists were provided with a sound system on which they could play music of their choice. The cyclists played the same style of music for all tests. A fan was positioned 80 cm in front of the head of the subject with the airflow velocity set to maximum (~2.0 m · s⁻¹).

Blood Sampling and Analysis

Blood was drawn from the forearm cannula into 7-ml EDTA vacutainers 5 min before and ~30, and ~75 min after meal ingestion. During exercise, blood was drawn as the cyclists passed the 30th and the 60th minutes of the 1-hour pre-load, upon completion of the incremental loads, and at the end of the 50-km time trial. Between sampling, the line and cannula were flushed with sterile heparinized saline (5 IU ·
ml⁻¹) to prevent clotting. Saline and residual blood was removed from the dead space in the line (~750 ml) before samples were drawn. After collection, blood was spun at 3000 g and 2 °C for 12 min. Separated plasma was then aliquoted into Eppendorf tubes and frozen (~80 °C) for later analysis. Tubes designated for glucagon assay contained protease inhibitor (Trasylsol, Bayer, Pymble, NSW) and were silicon coated to prevent binding of the peptide hormone to the polyurethane.

Plasma glucagon and insulin concentrations were determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). The assay coefficient of variation (CV) determined from control sera for insulin and glucagon concentrations were 3.6 and 17.0%, respectively. Enzymatic colorimetric assay was used to determine plasma free fatty-acid (Boehringer, Mannheim, Germany), glycerol (Sigma Diagnostics, St. Louis, MO), and glucose (Roche, Auckland, NZ) concentrations. The assay coefficient of variation (CV) for free fatty acids, glycerol, and glucose was 3.5, 6.1, and 3.0%, respectively. The Department of Human Nutrition at the University of Otago performed the glucagon, insulin, free fatty acid, glucose, and glycerol assays.

**Statistical Analysis**

The effects of the meals on metabolic and performance variables were estimated using a repeated-measures analysis provided by Proc Mixed in the Statistical Analysis System (SAS Institute, Cary, NC). The familiarization trial was omitted from the analysis of performance.

Measures of centrality and spread for subject descriptive and dietary variables are means and standard deviations. Most outcome variables were analyzed after log transformation, to reduce or eliminate effects of non-uniformity of error (15). Spread for these variables were therefore best represented by percentage standard deviations or factor (3/4) standard deviations generated from the repeated-measures analyses. For example, for a hormone concentration of 40 mmol · L⁻¹ with a between-subject standard deviation of 20%, the typical variation is 40 ± 1.20 to 40 ± 1.20, or 33 to 48 mmol · L⁻¹. Performance and metabolic data in graphs and text are shown as least-squares means to eliminate the artefactual variation that would otherwise be apparent with raw means when there are missing values for some levels of a repeated measure. Precision of the estimates is shown as 95% confidence limits with corresponding *p* values. Reliability of sprint and 50-km time is expressed as the within-subject error (coefficient of variation, CV) derived from the repeated-measures analyses.

**Results**

**Performance**

The durations of the 1-km sprints, 4-km sprints, and 50-km time trial averaged over all meal treatments were 81 ± 8 s, 352 ± 26 s, and 77 ± 6 min, respectively (least-squares means ± composite between-subject SD). Although mean performance times were shortest following the high-fat meal, there was no clear effect: relative to the high-carbohydrate and high-protein meals, the high-fat meal reduced times by 0.8 to 2.8% with an uncertainty (95% confidence limits) of ±3.2% to ±4.2%. The within-subject error derived from the repeated-measures analysis for 50-km time was 3.7%; errors for the mean of the three 1-km and the three 4-km sprints were 4.4 and 3.3%, respectively.
Substrate oxidation rates during the exercise procedure are shown in Figure 2. There was little change in fat-oxidation rate between the middle and end of the 1-hour pre-load for the three meal conditions (Figure 2). At the end, the rate was lowest in the high-carbohydrate condition by a factor of 0.63 relative to high-fat (95% confidence limits: 0.48 to 0.84, \( p = .003 \)), 0.59 relative to high-protein (0.45 to 0.77, \( p = .0005 \)), and 0.63 relative to fasting (0.47 to 0.85, \( p = .004 \)). Rates for carbohydrate oxidation were correspondingly higher.

**Figure 2 — Fat and carbohydrate oxidation rates the exercise procedure. Bars are between-subject standard deviations.**

**Fat and Carbohydrate Oxidation**

Substrate oxidation rates during the exercise procedure are shown in Figure 2.

**1-hour Pre-load**

There was little change in fat-oxidation rate between the middle and end of the 1-hour pre-load for the three meal conditions (Figure 2). At the end, the rate was lowest in the high-carbohydrate condition by a factor of 0.63 relative to high-fat (95% confidence limits: 0.48 to 0.84, \( p = .003 \)), 0.59 relative to high-protein (0.45 to 0.77, \( p = .0005 \)), and 0.63 relative to fasting (0.47 to 0.85, \( p = .004 \)). Rates for carbohydrate oxidation were correspondingly higher.
**Incremental Test**

Respiratory data for the five incremental loads are shown in Table 2. The high-carbohydrate meal condition raised the RER during all five workloads relative to the high-fat meal, high-protein meal, and fasting condition (all comparisons $p < .05$). There was no clear difference in the RER between the high-fat, high-protein, and fasting conditions.

Data for the buffer-CO$_2$ correction are not shown, but these accounted for a small increase in fat oxidation at 75% peak power ($0.10 \pm 0.12 \text{ g \cdot min}^{-1}$) but a larger increase at 82% peak power ($0.26 \pm 0.16 \text{ g \cdot min}^{-1}$). The high-carbohydrate meal reduced fat-oxidation rate across all workloads by a factor of 0.20 to 0.58 (0.09 to 0.89, $p = .0001$–.008) relative to the other conditions. When moving from moderate to heavy exercise (75% to 82% peak power), the fat oxidation rate in the high-carbohydrate condition was lowered by a factor of 2.0 (1.6 to 2.5, $p = .0001$) relative to the change in the high-fat condition, 1.83 (1.41 to 2.26, $p = .0002$) in high-protein, and 1.69 (1.23 to 2.14, $p = .003$) in the fasting condition. The mean peak fat-oxidation rates (with the corresponding energy contribution from fat as percentage of total energy) were 0.64 g \cdot min$^{-1}$ (34%E) in the fasting condition, 0.63 g \cdot min$^{-1}$ (33%E) in the high-protein condition, 0.53 g \cdot min$^{-1}$ (28%E) in the high-fat condition, and 0.32 g \cdot min$^{-1}$ (20%E) in the high-carbohydrate condition (between-subject SD for peak fat-oxidation rate: $3/4 \pm 1.43$; for percentage energy, $\pm 7$). The respective mean intensity at which these rates occurred was 77, 79, 78, and 73% $\dot{V}O_2^{\text{max}}$ (between-subject SD: $\pm 9$%). The peak fat-oxidation rate was lower in the high-carbohydrate condition by a factor of 0.49 to 0.59 (0.36 to 0.81, $p = .002$–.0002) relative to the other conditions; the corresponding reduction in the percentage contribution from fat was 8.5 to 14%E (1.7 to 21%E, $p = .02$–.0002).

| Table 2 Mean Oxygen Uptake and Respiratory Exchange Ratio During the Incremental Test |
|-------------------------------------|-----|-----|-----|-----|-----|
| Variable                           | 1   | 2   | 3   | 4   | 5   |
| Intensity                          |     |     |     |     |     |
| $\dot{V}O_2$ (L \cdot min$^{-1}$)  | 2.8 | 3.2 | 3.5 | 3.8 | 4.2 |
| % $\dot{V}O_2^{\text{max}}$       | 58  | 67  | 73  | 79  | 86  |
| RER                                |     |     |     |     |     |
| Fasting                            | 0.90| 0.89| 0.90| 0.91| 0.93|
| High carbohydrate                  | 0.95| 0.94| 0.95| 0.95| 0.96|
| High fat                           | 0.91| 0.91| 0.92| 0.92| 0.92|
| High protein                       | 0.90| 0.90| 0.91| 0.91| 0.91|
| Standard deviations (%)            |     |     |     |     |     |
| $\dot{V}O_2$                       | 11  | 12  | 12  | 10  | 10  |
| $\dot{V}O_2^{\text{max}}$         | 5   | 6   | 5   | 5   | 5   |
| RER                                | 3   | 4   | 3   | 3   | 3   |
Plasma Fuels, Metabolites, and Hormones

Plasma glucose, free fatty-acid, and glycerol concentrations during the testing procedure are shown in Figure 3.

Glucose. There was no significant effect of meal composition on plasma-glucose concentration before and during exercise; although, the mean concentrations during exercise were raised by 0.5–1.0 mmol·L⁻¹ following the high-protein meal relative to the high-fat and high-carbohydrate meals (p = .15 to .22).

![Figure 3 — Plasma glucose (A), free fatty-acid (B), and glycerol (C) concentrations before and during exercise. Bars are between-subject standard deviations. Dashed lines in A and B indicate extrapolation from the fasting sample.](image-url)
Free Fatty Acids. The only clear effect of meal composition on plasma free fatty-acid concentration at rest before exercise was a reduction following the high-carbohydrate meal, by a factor of 0.63 (0.40 to 0.99, \( p = .05 \)) relative to the high-protein meal. During exercise there were few consistent differences in free fatty acids between meal conditions except: (a) at the end of the incremental test, where fatty acids in the fasting, high-fat, and high-protein conditions were 1.6 to 1.4 (1.0 to 2.4, \( p = .04–.01 \)) times higher than in the high-carbohydrate condition; and (b) at the end of the 50-km time trial, where fatty acids were lower in the high-fat condition by a factor of 0.62 to 0.79 (0.24 to 1.05, \( p = .03–.10 \)) relative to other conditions. The high-carbohydrate meal tended to lower the overall free fatty-acid concentration relative to the high-fat and the high-protein meals by a factor of 0.88 to 0.83, but there was no clear overall effect (0.69 to 1.12, \( p = .13–.3 \)).

Glycerol. During exercise, the overall glycerol concentration following the high-carbohydrate meal was lower by factors of 0.66 (0.58 to 0.78, \( p = .0001 \)) and 0.59 (0.53 to 0.69, \( p = .0001 \)) relative to that following the high-fat and high-protein meals respectively (Figure 3, section C). Although the high-protein condition elevated the overall glycerol concentration by a factor of 1.17 relative to the high-fat condition, the effect was not clear cut (95% confidence limits: 0.95 to 1.39, \( p = .13 \)).

Insulin. By 40 min after ingestion, insulin had increased relative to fasting by factors of 8.4 (5.5 to 12.9, \( p = .0001 \)) following the high-carbohydrate meal, 4.2 (2.7 to 6.6, \( p = .0001 \)) following the high-protein meal, and 3.3 (2.2 to 5.0, \( p = .0001 \)) following the high-fat meal (Figure 4, section A). Insulin following the high-carbohydrate meal was 2.6 (1.7 to 4.0, \( p = .0001 \)) times that after the high-fat meal. These differences remained similar at 80 min after meal ingestion (comparisons not shown).

Exercise decreased insulin concentration. Halfway into the 1-hour pre-load, the difference relative to fasting had decreased by a factor of 2.2 (1.5 to 3.2, \( p = .0002 \)) following high-protein, 1.9 (1.2 to 2.8, \( p = .001 \)) following high-carbohydrate, and 1.5 (1.1 to 2.2, \( p = .025 \)) following high-fat condition; there was no clear difference between meal conditions other than a 1.4-fold (1.0 to 2.1, \( p = .06 \)) elevation in the high-protein compared with the high-fat condition. The introduction of the supplement increased insulin at the end of the pre-load by 2–4 mU · L\(^{-1} \); the differences between treatments, however, remained similar to those at the halfway point.

At the completion of the 1-hour pre-load and the incremental test, insulin in the high-protein condition was 1.4 to 1.8 (1.0 to 2.6, \( p = .01–.008 \)) times higher than after the fasting and high-fat conditions. The remaining comparisons were of smaller magnitude and not statistically significant.

Glucagon. Relative to fasting, the high-protein and high-fat meals elevated resting glucagon by factors of 3.3 to 3.4 (2.0 to 5.6, \( p = .0001 \)), while the high-carbohydrate meal elevated glucagon by 2.0 (1.2 to 3.2, \( p = .008 \)); glucagon was 1.6 to 1.7 (1.0 to 2.9, \( p = .04–.05 \)) times that after the high-carbohydrate meal (Figure 4, section B).

Over the three exercise measurements, the glucagon concentration was higher by a factor of 2.1 to 3.2 (1.6 to 6.0, \( p = .001–.0001 \)) in the high-protein condition, 1.5 to 2.9 (1.0 to 5.4, \( p = .05–.002 \)) in the high-fat condition, and 1.3 to 1.4 (0.74 to 2.7,
Figure 4 — Plasma insulin (A) and glucagon (B) concentrations before and during exercise. Bars are between-subject standard deviations. Dashed lines indicate extrapolation from the fasting sample.

$p = .005–.29$) in the high-carbohydrate condition, relative to fasting. In the high-protein condition, glucagon was elevated by a factor of 1.3 to 2.2 (1.1 to 4.2, $p = .02$) relative to the high-carbohydrate condition. Although there was no clear difference at the end of the 1-hour pre-load, the high-protein condition elevated glucagon relative to the high-fat condition by a factor of 1.3 (1.1 to 1.6, $p = .02$) at the end of the incremental test, and by 1.5 (0.9 to 2.3, $p = .08$) at the end of the 50-km time trial.
Discussion

Performance

The aim of the current study was to compare the effect of high-fat, high-protein, and high-carbohydrate pre-exercise meals with carbohydrate supplementation on metabolism and performance during cycling. Despite substantial effects on plasma hormone concentrations and fuel utilization, the meals had no clear effect on performance. Burke et al. (1) also found no clear effect on ~2.5-hour cycling performance in response to two high-carbohydrate pre-exercise meals, one with a high- and the other with a low-glycemic index. The pre-exercise meals modified plasma insulin and substrate concentrations, but did not substantially modify fuel utilization during exercise. The authors concluded that the ingestion of the carbohydrate supplement during exercise minimized any potential differences in the metabolic and performance responses to the glycemic index of the pre-exercise meal. Even though we observed a clear elevation in fat oxidation following the high-fat and high-protein meals, it is also possible that any potential differences in performance relative to the high-carbohydrate meal were minimized by the maintenance of blood-glucose concentration and high rates of carbohydrate oxidation associated with the ingestion of a carbohydrate supplement (11).

Poor reliability can be a factor contributing to the lack of a clear outcome in an intervention. In our study, the within-subject error for the 50-km time trial was 3.7%, which is approximately twice that of 100-km time in the reliability study of Schabort et al. (30). However, our time trial was preceded by a pre-load of approximately the same amount of work as the time trial itself. Such a pre-load could increase the apparent error in the time trial by a factor of about two (17). The reliability of 50-km time in our study was therefore similar to that for 100-km time, so there was probably no reduction in the power to detect a performance effect. The sprint times, however, were independent of total test duration and unlikely to be substantially influenced by the pre-load. A likely explanation for the lower reliability of the sprint times, therefore, is substantial individual responses to the treatments.

Metabolism

Relative to the high-fat and the high-protein meals, the high-carbohydrate meal raised RER by 0.03–0.05, caused a two-fold reduction in the peak fat-oxidation rate and a four-fold reduction fat-oxidation rate during heavy exercise (82% peak power). The two-fold greater pre-exercise plasma-insulin concentration after the high-carbohydrate meal was probably the main reason for the relative reduction in fat oxidation and enhanced carbohydrate oxidation. Insulin is a potent inhibitor of lipolysis of adipose triglyceride (2). Inhibition of lipolysis results in decreased circulating lipoprotein-triglyceride and free fatty-acid concentrations, and consequently lowers plasma fatty-acid availability to the muscle for oxidation during exercise (6, 7, 21). Although insulin returned to near baseline during exercise, lower plasma glycerol and free fatty-acid concentrations during exercise after the high-carbohydrate meal are consistent with a persistent effect of insulin on adipose tissue acting to decrease plasma fat availability (6, 7, 23).

Even though we made no direct measurement of muscle metabolism, the higher insulin in the high-carbohydrate condition was likely to have directly influenced fuel utilization in the muscle. Insulin has been shown to suppress muscle
lipoprotein-lipase activity resulting in reduced free fatty-acid uptake and availability of fatty-acid substrate to the mitochondria (21). Insulin has also been found to increase muscle-glucose uptake and oxidation rates (7, 21, 23). The resulting increase in glycolytic flux may have played a role in decreased fat oxidation in the muscle through inhibition of carnitine acyl-transferase by malonyl-CoA (7); as yet, however, there is no clear evidence to support this suggested mechanism during exercise in humans (reviewed in 33). Finally, it is important to note that any suppressive effect of insulin on the adipose and muscle tissues in response to meal ingestion was probably not confined to the high-carbohydrate meal condition, since preexercise insulin was also elevated after both the high-fat and high-protein meals, although to a lesser extent.

An interesting finding was the similarity in fuel utilization during the incremental test between the high-fat and high-protein meals, even though the high-protein meal contained eight times more carbohydrate, three times more protein, and three times less fat than the high-fat meal. The high-protein meal produced both higher plasma insulin and glycerol concentrations than the high-fat meal, which is somewhat of a paradox, given the normal response to insulin is suppression of lipolysis (2). Although the present venous-blood hormone measurements provide only limited insight, the apparent higher rate of lipolysis (higher plasma glycerol) following the high-protein meal could be linked to greater glucagon-stimulated lipolysis (3, 25), particularly later in the exercise, once the effect of the elevated preexercise insulin on the adipose tissue had subsided somewhat (23). If such stimulation of adipose tissue lipolysis by glucagon occurs, it is probably mediated via the action of cAMP cascades on hormone-sensitive lipase (3, 25) and may therefore accentuate the normal adipose response to elevated plasma epinephrine during exercise (36). Stimulation of adipose lipolysis by glucagon is an attractive idea, but the higher plasma-glycerol concentration and fat-oxidation rate in the high-protein condition may simply have resulted from the lipolysis of elevated circulating chylomicron-triglycerides derived from the meal (12, 13). However, evidence for lower exercising plasma glycerol and glucagon concentrations in the high-fat meal condition, despite the higher fat content relative to the high-protein meal, would appear to contradict the more simple explanation. The present data do not allow determination of the source of the oxidized fatty acids.

The purpose of the incremental loads in the present exercise procedure was to investigate the effect of pre-exercise meal composition on the maximal rate of fat oxidation and the pattern of fuel utilization during heavy-intensity exercise. Interestingly, the peak fat-oxidation rate following the high-fat meal was ~55% of that following 2 weeks of high-fat dietary adaptation; in contrast, the peak fat-oxidation rate following the high-carbohydrate meal in the present study was similar to that following a 2-week high-carbohydrate diet (unpublished observations). The suppressive effect of the high-carbohydrate meal on the peak fat-oxidation rate is probably due to reduced fat availability and impaired muscle fat metabolism relating to the greater insulin response, whereas the lack of an elevation in the peak fat-oxidation rate following the high-fat meal relative to fasting is probably due to intrinsic rate-limiting factors associated with fat transport and fat oxidation (21, 34, 39).

During the final heaviest workload of the incremental test (86% $\dot{V}O_{2\text{max}}$), the fat-oxidation rate relative to the previous more moderate-intensity workload (79% $\dot{V}O_{2\text{max}}$) was substantially reduced by the high-carbohydrate meal but affected comparatively little by the high-protein and high-fat meals (Figure 2). Romijn et al. (28)
found that relative to moderate-intensity cycling (65% \( \overline{\text{VO}_2} \)), heavy-intensity cycling (85% \( \overline{\text{VO}_2} \)) in the fasting condition reduced the rates of plasma free fatty-acid appearance, intramuscular-triglyceride lipolysis, and fat oxidation. The authors suggested the reduction in plasma fatty-acid appearance during high intensity may be due to entrapment of free fatty acids within adipose tissue, owing to reduced blood flow to this tissue during heavy exercise (15). Suppression of adipose lipolysis by elevated insulin, as discussed above, could have accentuated the entrapment phenomenon, whereas the sustained fat-oxidation rate could be due to elevated circulating fat availability (12, 13) in the high-fat and high-protein meal conditions. For example, Hawley et al. (14) found that relative to a pre-exercise high-carbohydrate meal, the fat-oxidation rate during exercise for 1 hour at 80% \( \overline{\text{VO}_2} \) was twice that following a high-fat meal with heparin infusion. Because high-fat feeding (>80 g fat) alone can raise the oxidation rate of ingested fats in the muscle during exercise (13), the addition of heparin may not be necessary to obtain a carbohydrate-sparing effect. Indeed, Dyck et al. (9) found that raising plasma fatty-acid concentration with lipid infusion increased fat oxidation during cycling at 85% \( \overline{\text{VO}_2} \) relative to fasting. It would be interesting to investigate whether substantial pre-exercise fat feeding could enhance performance during a long-duration cycling test (>4 hours) with repeated high-intensity work components, where substantial depletion of muscle glycogen is likely. In such a test, a carbohydrate-rich supplement should be ingested to simulate dietary practice in competitions.

In conclusion, the high-carbohydrate meal given 90 min before exercise elevated pre-exercise plasma-insulin concentration and suppressed fat oxidation during exercise, relative to the high-fat and high-protein meals. In contrast, fat oxidation following the high-protein and the high-fat meals was similar to that of fasting, probably because of more fat in circulation relative to that following the high-carbohydrate meal. In the high-protein meal condition, the elevated fat availability could be mediated by a metabolic effect associated with the higher-protein content of the meal. Despite the effects of these meals on metabolism, there was no clear effect on performance.

References


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