Effects of High-Fat and High-Carbohydrate Diets on Metabolism and Performance in Cycling

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We compared the effects of high-fat and high-carbohydrate dietary conditions on metabolism and short- and ultra-endurance cycling performance. Seven cyclists ($\dot{V}O_2$max 72 ± 7 mL · kg^{-1} · min^{-1}) underwent a 2-week adaptation to each of the following 3 diets: 14-day high carbohydrate with 70 ± 9 percent energy (%E) carbohydrate, 16 ± 5%E fat, and 14 ± 2%E protein; 14-day high fat with 66 ± 10%E fat, 20 ± 3%E protein, and 15 ± 4%E carbohydrate; and 11.5-day high-fat diet followed by 2.5-day carbohydrate-loading. The conditions included a pre-exercise meal of the same composition as the preceding diet. Each diet condition was preceded by a 2-week standardizing normal diet. The exercise test lasted approximately 5 hours and comprised a 15-minute trial, an incremental test to measure the peak fat-oxidation rate, and a 100-km trial. Sports bars and a 5% carbohydrate solution were ingested during the tests. The diets had no statistically significant effect on 15-minute performance, although the high-fat condition tended to reduce distance covered by -2.4% (95% confidence interval: -5.4% to +0.6%, *P* = .11) relative to the fat with carbo-loading condition. In the 100-km time trial, the high-fat and the fat with carbohydrate-loading conditions attenuated the decline in power output observed in the high-carbohydrate condition (*P* = .03 to .07), although the corresponding improvement in performance time of 3% to 4% (-2% to 10%) was not statistically significant (*P* = .16 to .22). Power output during the final 5 km of the time trial in the fat with carbo-loading condition was 1.3-fold (1.0 to 1.6, *P* = .04) greater than in the high-carbohydrate condition. Overall, for every 10%E increase in dietary fat, 100-km mean power increased by 2% (-0.0% to 4%, *P* = .06). Relative to the high-carbohydrate condition, the high-fat conditions resulted in the following metabolic changes consistent with greater lipolysis and fuel availability: lower plasma insulin concentration before exercise (*P* < .0001), and during exercise a 10% to 20% higher plasma-glucose concentration (*P* < .01), higher plasma glycerol (*P* < .05), and a 2.5-fold to 2.9-fold increase in the peak fat-oxidation rate (*P* < .0001). In conclusion, high-fat dietary conditioning increased fat oxidation, and although the main effects were not statistically significant, there was some evidence for enhanced ultra-endurance cycling performance relative to high-carbohydrate.

FATIGUE DURING ENDURANCE exercise is often related to the depletion of muscle-glycogen or a reduction in blood glucose supply.[1] Fatigue can be attenuated by elevating pre-exercise liver- and muscle-glycogen content with a high-carbohydrate diet for 1 to 3 days prior to exercise[2-4] and by ingesting carbohydrates during exercise.[1,5] Consequently, most investigators advise endurance athletes to eat a diet of at least 60 to 70 percent energy (%E) carbohydrate to maximize pre-exercise glycogen stores to enhance competition and training performance.[1,6] Despite evidence that short-term (1 to 3 days) high-carbohydrate diets improve endurance relative to high-fat diets, few studies have found this effect applies to longer-term (> 5 days) chronic high-carbohydrate diets compared with moderate-carbohydrate or high-fat diets in well-trained cyclists (for reviews, see Hawley et al[7] and Helge[8]).

Chronic high-fat (> 50%E) diets are associated with metabolic adaptations that enhance muscle-triglyceride storage and the activities of enzymes involved in fat oxidation and fat transport across plasma and mitochondrial membranes.[9-11] Such adaptation appears to compensate for lower carbohydrate availability and probably account for the enhanced performance capacity observed after chronic high-fat diet exposure relative to short-term high-fat diets.[7] Evidence for muscle-enzyme adaptation to a high-fat diet has been observed after as little as 5 to 10 days in cyclists,[9] and therefore, a 1- to 2-week high-fat diet may be a practical intervention for endurance athletes.

In well-trained cyclists, 2- to 4-week high-fat diets improved[12] or had no clear effect[9,13] on 1- to 3-hour endurance performance relative to carbohydrate-rich diets. In studies in which well-trained cyclists ingested a high-carbohydrate diet for 1 to 3 days following a 5- to 10-day high-fat diet, elevated fat oxidation and apparent muscle-glycogen sparing[14,15] was related to enhanced performance in one study[14] and to nonsignificant trends for enhancement in one using a 2.5-hour endurance model[16] and in another using a 5-hour ultra-endurance model.[15] Despite some evidence for enhanced endurance performance after high-fat dietary adaptation, the results remain equivocal and largely untested in ultra-endurance (> 4 hours) models.

It has been suggested that an increased maximal capacity to generate power from fat oxidation following fat adaptation may be of particular benefit to performance in longer duration endurance events (ultra-endurance, > 4 hours).[17] In such events, the preformed muscle-glycogen store can be depleted before completion necessitating an increased reliance on fats and plasma glucose for energy production.[18] With an elevated fat-oxidation rate following fat adaptation, less carbohydrate is required to maintain a given power output, which may enhance prolonged endurance performance by increasing overall energy availability.[9,17]

On the other hand, carbohydrate provides most of the energy

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for high-intensity endurance exercise (85% to 100% $\dot{V}O_2$max). Consequently a high-carbohydrate diet could be more appropriate for athletes competing in endurance events because of greater pre-exercise muscle-glycogen availability in the heavily recruited muscle fibers and enhanced glycolytic metabolism relative to a high-fat diet.[17] Furthermore, the burning of carbohydrate is marginally more oxygen efficient than the burning of fat,[19] which may provide benefits at intensities close to $\dot{V}O_2$max.

An important issue in diet studies is the inclusion of energy-rich meals and supplements in testing procedures because of their effects on substrate availability and fuel utilization. High-fat pre-exercise meals elevate circulating fat availability[20,21] and should therefore complement the enhanced fat oxidation following high-fat diet adaptation. High-carbohydrate meals, on the other hand, increase carbohydrate availability, but suppress lipolysis and fat oxidation.[22-24] Carbohydrate ingested during exercise can help sustain plasma-glucose oxidation rate,[25] while supplemental protein and fat may also contribute to energy metabolism.[26,27] Furthermore, pre-exercise meals and supplements are commonly used by endurance athletes during training and competition.[24]

Accordingly, the aim of this study was to compare the effects on cycling metabolism and performance of a 2-week high-fat diet/pre-exercise meal condition, with and without carbohydrate loading, against a high-carbohydrate diet/pre-exercise meal condition, as the control. The cyclists ingested a standard caloric supplement during exercise. The effect of the diet conditions on the peak capacity for fat oxidation was determined, and performance was measured using a 15-minute test (short-endurance), and a 100-km time trial following 2.5 hours of other riding (ultra-endurance).

MATERIALS AND METHODS

Design

Each subject performed metabolic and performance tests before and after each of the three 2-week dietary treatment conditions (Fig 1). The order of treatments was randomized. Each diet condition was preceded by a 2-week standard normal diet (50%E carbohydrate, 35%E fat, 15%E protein) to act as a controlled lead in. Performance outcomes in response to the treatment conditions were therefore compared relative to a normal mixed diet. There were 2 exercise procedures in each 2-week treatment block: a short incremental test on day 11 to measure peak power and $\dot{V}O_2$max, and the 5-hour testing procedure on day 14 to measure the effects of the diets on metabolism and performance (Fig 1).

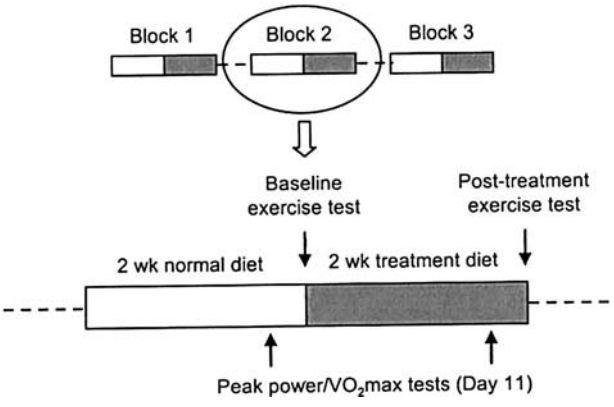

Fig 1. Study design. Dashed lines before and after the dietary conditioning periods represent time away from the study if required by the subjects.

Subjects

Seven nationally competitive male cyclists and triathletes participated in the study during their competitive off-season. Subject characteristics were: age, 27 ± 5 years; height, 178 ± 7 cm; mass, 74 ± 9 kg; sum of 7 skin folds, 57 ± 18 mm; body fat, 8 ± 3 percentage body mass (%BM). Prestudy maximal oxygen uptake ($\dot{V}O_2$max) and peak power were 5.2 ± 0.5 L · min^{-1} (72 ± 7 mL · kg^{-1} · min^{-1}) and 378 ± 41 W (5.2 ± 0.8 W · kg^{-1}), respectively. Training volumes in the week before the study were 430 ± 100 minutes of cycling and 410 ± 190 minutes of other training, which comprised swimming, running, or gym training. Subjects had been training in their sport for 7 ± 4 years. The research received ethical approval from the Otago Ethics Committee of the Southern Regional Health Authority.

Diets

The diets were prescribed as: (1) high-fat, low-carbohydrate (high-fat: 70%E fat, 15%E protein, 15%E carbohydrate); (2) high-carbohydrate, low-fat (high-carbohydrate: 70%E carbohydrate, 15%E protein, 15%E fat); and (3) 11.5-day high-fat, followed by 2.5-day on high-carbohydrate (fat with carbo-loading). During the fat with carbo-loading condition, subjects changed from the high-fat diet to the high-carbohydrate diet from 3:00 PM on the 11th day of the 2-week conditioning period.

The high-fat diet consisted of ad libitum consumption of high-fat meats, eggs and dairy products, nuts and seeds, low-starch vegetable products, and oils. The high-carbohydrate and carbo-loading diets consisted of ad libitum consumption of standard high-carbohydrate foods (cereals, pasta, bread, rice, and potatoes), vegetables with a high-starch content, low-fat confectionery, lean meats, and fish.

Blinding the subjects and the investigators to the dietary treatment conditions was not possible. To reduce bias, subjects were not informed of their performances until the completion of the study. To assist in adherence to the prescribed diets, subjects were provided with food composition tables, measuring containers, and a large range of menus specific for the dietary condition. Appropriate food items were purchased for the subjects during the dietary treatments, and regular contact between the investigators and the subjects was maintained to encourage compliance.

Dietary Analysis

Dietary compliance was monitored with 4-day dietary diaries completed during the first 5 days of each 2-week diet block. These diaries were processed rapidly using computer software based on the nutrient composition of New Zealand foods (Diet Cruncher, NutriComp, Dunedin, New Zealand). Subjects were notified if any modifications to the diet were required and asked to complete a second 4-day diary during the second week, which was subsequently processed and taken as representative of the dietary intake during the conditioning period.

Training

Subjects designed their own training programs based on a 14-day cycle. Light training or rest was scheduled on the day before the 5-hour test. Daily training volume and intensity were recorded in training diaries that included 10-point Likert scales for psychological state (mood, energy, dissatisfaction, limb pain).

Supplement and Fluid Replacement

Subjects consumed a 5.0% carbohydrate/electrolyte solution (PR Nutrition, San Diego, CA) and sports bars (PR Nutrition) throughout the 5-hour procedure at defined rates. The solution contained a 50:50 mix of fructose and maltodextrin (5 g · 100 mL^{-1}). The carbohydrate solution was ingested at approximately 12 mL · kg^{-1} · h^{-1} and the bar at 1 · h^{-1}. Each sports bar contained 14 g protein, 19 g carbohydrate, and 6 g fat. The average rate of carbohydrate ingestion over the 5-hour exercise period was 58 g · h^{-1}.

Peak Power and Maximal Oxygen Uptake

The test of peak power output and $\dot{V}O_2$max was performed on an electronically 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 to that of Kuipers et al.[28] After a warm-up of 10 minutes at 100 W, the work rate was increased by 50 W every 150 seconds until a heart rate of 160 min^{-1} was surpassed, thereafter work rate increased by 25 W every 150 seconds until the test was terminated at the point of voluntary exhaustion, or when pedal cadence fell by greater than 20 revolutions/minute. Peak power was defined as the last completed work rate plus the fraction of time spent in the final noncompleted workload multiplied by 25. Peak power was used to set workloads in appropriate stages of the 5-hour procedure.

Expired respiratory gas was collected continuously throughout the test using an on-line gas analysis system for determination of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Cyclists wore a mouthpiece and nose clip (Hans Rudolph, Kansas City, 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 (Applied Electrochemistry; oxygen sensor N-22 and oxygen analyzer S-34, Sunnyvale, CA) and CO_2 (Datex Normocap CD102, Helsinki, 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 2-point calibration using room air and analytical grade concentrations of O_2 ($\approx$ 16%) and CO_2 ($\approx$ 4%) verified against a beta standard (BOC Gases, Auckland, New Zealand). 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 (eg, duration of the incremental test, see below) and corrections made of gas fractions if necessary. Smith[29] verified the validity of the gas analysis system. $\dot{V}O_2$max was the $\dot{V}O_2$ measured during the final fully completed 20-second gas-sampling period, which corresponds to the oxygen utilization during the final work rate.

Blood samples were collected for measurement of blood-lactate concentration during the final 30 seconds of each workload (YSI 1500-L; Yellow Springs Instruments, Yellow Springs, OH). Blood lactate versus power output was modeled using a logarithmic function (TableCurve; SPSS Science, Chicago, IL) for determination of the effect of diet on the power output at blood-lactate concentrations of 2 and 4 mmol · L^{-1}.

Five-Hour Exercise Procedure

The testing procedure is shown in Fig 2. The warm-up, the 15-minute test, and the 100-km time trial were performed on a Kingcycle ergometer (Kingcycle, High Wycombe, UK). The 45-minute ride and the incremental test were performed on the Rodby ergometer. Anthropometry and a pre-exercise meal preceded the exercise test.

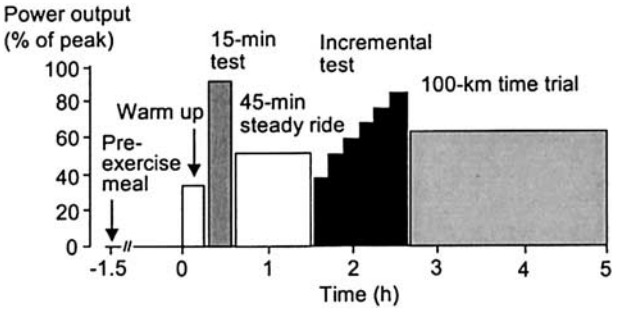

Fig 2. Testing procedure. Shown are the timings of the pre-exercise meal and the components of the 5-hour exercise test.

Preparation and anthropometry. Cyclists reported to the laboratory between 7:30 AM and 9:00 AM with their bicycle and riding outfit after an overnight fast. Report time was approximately 110 minutes before the beginning of cycling. Skin-fold thickness was measured at 7 sites and converted to the percentage body fat (%BM) using standard equations.

Pre-exercise meals. The cyclists ingested 1 of 3 pre-exercise meals between 80 and 90 minutes before beginning exercise. Each meal was designed to provide 42 kJ · kg^{-1} and 8 mL · kg^{-1} of water. The percentage macronutrient compositions of the meals were the same as the prescribed preceding diets. The meal for the normal condition consisted of a muffin, a meal replacement drink (PR Nutrition), and a carbohydrate drink (Horleys, Auckland, NZ). The meals for the high-fat and fat with carbo-loading conditions consisted of a dairy-based custard mixture: standard cream cheese, evaporated milk, fresh cream, whey protein powder, artificially sweetened custard mix, and water. The high-carbohydrate meal consisted of a sports bar (Sun Real Foods, Moorabbin, Australia), banana, carbohydrate-electrolyte drink, and canola oil. After eating, the cyclists rested in a chair until the beginning of exercise.

Fifteen-minute test. The cyclists warmed up for 15 minutes at 35% peak power. After a 2-minute rest, cyclists were asked to ride as far as possible in 15 minutes.

Forty-five–minute steady ride. The 45-minute steady-state ride at 50% of peak power was used as a part of the preload to the 100-km time trial and for recovery from the 15-minute test.

Incremental test. The purpose of the incremental test was to measure the effect of diet on fuel utilization at different exercise intensities, in particular, peak fat oxidation. The test consisted of 10-minute stages at 37.5%, 48.75%, 60%, 67.5%, 75%, and 82.5% of peak power (Fig 2). During each stage, expired gas was sampled continuously for determination of $\dot{V}O_2$ and $\dot{V}CO_2$. Gas from the final 3 minutes of each 10-minute stage was used to calculate fuel utilization.

One hundred–kilometer time trial. The cyclists were asked to ride the time trial as fast as possible. Expired respiratory gas was collected for 5 to 7 minutes for measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ as the rider passed the 10-, 30-, 70-, and 90-km marks. Mean power was calculated for each 5-km split from the Kingcycle data stream.

Environmental conditions. Temperature and humidity in the laboratory during the course of the experiments were 19°C to 21°C and 45% to 55%, respectively. A fan was positioned approximately 80 cm in front of the head of the cyclist with the airflow set at approximately 2 m · s^{-1}.

Blood Sampling and Urine Collection

A cannula with a 30-cm extension line was placed in a forearm antecubital vein for blood sampling before and during the exercise procedure. Blood was drawn into 10-mL syringes for analysis of

plasma insulin, glucagon, glucose, urea, free fatty acid, and glycerol concentration. The sample points were: 5 minutes before exercise, at the end of the 45-minute steady ride, at the end of the 1-hour incremental test, and as the riders passed the 30-, 70-, and 100-km points during the 100-km time trial. Blood was drawn into heparinized capillary tubes for measurement of blood lactate concentration (1) immediately before and as the rider passed the 5th, 10th, and 15th minute of the 15-minute test, (2) during the final minute in each stage of the incremental test, and (3) as the riders passed the 10-, 30-, 70-, 90-, and 100-km marks of 100-km time trial. Between sampling, the line and cannula were flushed with sterile heparinized saline (5 IU $\cdot$ mL^{-1}) to prevent clotting. Urine was collected for analysis of renal urea clearance immediately before exercise, before and after the incremental test, and at the end of the 100-km time trial.

Treatment of Blood Samples and Analysis

Blood samples were transferred from 10-mL syringe into 7-mL EDTA vaccutainers and spun immediately at 3,000 $\times$ *g*, 2°C for 12 minutes. Separated plasma was then aliquoted into Eppendorf tubes and frozen (-80°C) for later analysis. Eppendorf tubes designated for glucagon assay contained protease inhibitor (Trasylol, Bayer, Pymble, Australia), and were silicon coated to prevent binding of the peptide hormone to the polyurethane tubes. Plasma glucagon and insulin concentrations were determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). The within-assay error for the insulin and glucagon assays was 7.0% and 9.3%, respectively. Enzymatic colorimetric assay was used to determine plasma free fatty acid (Boehringer, Mannheim, Germany), glycerol (Sigma Diagnostics, St Louis, MO), and glucose (Roche Diagnostics, Auckland, New Zealand) concentrations. Plasma and urinary urea concentrations were determined using the urease/GLDH method (Roche Diagnostics, Auckland, New Zealand). The errors for free fatty acid, glycerol, glucose, and urea assays were 3.5%, 6.1%, 3.0%, and 6.7%, respectively. Owing to budgetary limitation, the effect of the 3 treatment diets on metabolic variables (plasma glucose, glycerol, free fatty acid, urea, insulin, and glucagon concentrations, and fuel utilization) was analyzed against the third normal diet trial only.

Metabolism

Protein oxidation. The total mass (g) of protein oxidized (Ptot) during the incremental test and during the 100-km time trial was 6.26 times nitrogen appearance.[30] Nitrogen appearance was the product of the increase in whole body urea store calculated from the change in plasma urea concentration, the urinary urea excretion rate, and an estimate of sweat urea loss.

The whole body urea store was calculated as the plasma urea concentration divided by 0.93 (to account for the water fraction of plasma) multiplied by the urea distribution volume (whole body water). Sweat nitrogen losses were not measured during the study. Literature estimates for sweat-urea loss during moderate exercise under normal dietary conditions range from 80% to 120% of the urinary excretion rate.[31,32] Taking a middle value of 100%, urinary urea excretion was therefore multiplied by a factor of 2 to account for the urea lost in sweat.

The total volumes of O_2 utilized and CO_2 produced in protein metabolism for the duration of the incremental test and for the duration of the 100-km time trial were calculated from the equations of Livesey and Elia[30]: $\dot{V}O_2$protein = Ptot $\cdot$ 1.01, $\dot{V}CO_2$protein = Ptot $\cdot$ 0.843. The gas equivalents were then used to compute the protein fraction of the measured $\dot{V}O_2$ and $\dot{V}CO_2$. The protein fraction was subtracted from the measured $\dot{V}O_2$ and $\dot{V}CO_2$ to generate a protein-corrected $\dot{V}O_2$ and $\dot{V}CO_2$ for each gas-sampling point during the incremental test and 100-km time trial. The $\dot{V}CO_2$ during the latter 3 stages of the incremental test was corrected for buffer CO_2 production (see below) before the protein correction.

Carbohydrate, fat, and protein oxidation rate. Carbohydrate and fat oxidation rates during exercise were calculated from protein-corrected $\dot{V}O_2$ and $\dot{V}CO_2$ using the equations of Peronnet and Massicotte.[33] The equations for the calculation of the oxidation rate and the oxygen equivalent for glucose (G) and fat (F) were: G ($g \cdot min^{-1}$) = (4.5850 $\cdot$ $\dot{V}CO_2$) - (3.2255 $\cdot$ $\dot{V}O_2$), F ($g \cdot min^{-1}$) = (-1.7012 $\cdot$ $\dot{V}O_2$) + (1.6946 $\cdot$ $\dot{V}CO_2$); $\dot{V}O_2$G ($L \cdot min^{-1}$) = G $\cdot$ 0.7455, $\dot{V}O_2$F ($L \cdot min^{-1}$) = F $\cdot$ 2.0092.[33] The protein oxygen equivalent and protein oxidation (P) for each sample point were calculated by difference: $\dot{V}O_2$protein ($L \cdot min^{-1}$) = $\dot{V}O_2$total - ($\dot{V}O_2$G + $\dot{V}O_2$F); P ($g \cdot min^{-1}$) = $\dot{V}O_2$protein/0.996. Energy equivalents ($kJ \cdot g^{-1}$) were 17.14, 16.18, and 40.76 for protein,[19] carbohydrate,[33] and fat,[33] respectively.

Correction for nonoxidative CO_2 production during the incremental test. Estimates of fuel utilization based on indirect calorimetry during heavy exercise (greater than 70% $\dot{V}O_2$max) may overestimate the contribution of carbohydrate due to the evolution of CO_2 from declining body HCO_3^- stores (buffer CO_2).[19,34] To correct for this problem, buffer CO_2 was subtracted from the $\dot{V}CO_2$ to derive the $\dot{V}CO_2$ attributable in the oxidation of fuel substrates. Buffer CO_2 production was calculated from the decline in blood standard HCO_3^- concentration (SBC, the HCO_3^- concentration of the sample at a standard PCO_2 of 40 mm Hg) during the latter stages of the incremental test. Venous blood samples were drawn into capillary tubes from the forearm cannula at 1- to 2-minute intervals over the duration of the incremental test. The capillary tubes were sealed using rubber caps and stored on ice for 80 to 90 minutes before being assayed for SBC using a blood-gas analyzer (ABL50; Radiometer, Copenhagen, Denmark). The SBC provided a direct index for the effect of electrochemical changes in the body fluid during exercise relative to the resting condition, and without the complication of changes in PCO_2 involved with the anaerobic sampling 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 ($mmol \cdot L^{-1}$) 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 minute of each stage) were determined from the equation. The prediction error for standard HCO_3^- using this method was calculated from standard error of the estimate for each curve: the range was 0.15 to 0.9 $mmol \cdot L^{-1}$.

The fractional change in SBC was assumed to equal the fractional change in the labile whole body CO_2 store over the 3-minute 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.[34] The labile whole body CO_2 store was taken as the whole body water volume minus the intracellular fluid volume of the active muscle ($\approx$ 10.6% total body mass), because the HCO_3^- concentration in the active tissue compartment changes little during sustained heavy exercise.[34] Assuming no substantial change of the fluid volumes of the body fluid compartments between rest and exercise, a change in the molar SBC, therefore, represents a calculable mass of CO_2 expired at the lung: buffer CO_2 production ($mL \cdot min^{-1}$) = ([SBC_b $-$ SBC_e]/SBC_r) $\cdot$ CO_{2r} $\cdot$ kg $\cdot$ (0.894 $\cdot$ SBC_r/24.5) $\cdot$ 22.26/3, where CO_{2r} is a literature estimate of the labile body CO_2 store at rest ($9.2 mmol \cdot kg^{-1}$),[35] 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 CO_2.

Statistical Analysis

The effects of the diets were estimated using a repeated-measures analysis provided by Proc Mixed in the Statistical Analysis System (SAS Institute, Cary, NC). For measures of performance, the dependent variable in the analyses was performance on the treatment diet minus performance on the standard normal diet. A term was included in the model to account for any practice effect between consecutive trials. For measures of metabolism, the dependent variable was the metabolic measure in the 3 treatment diets and the third normal diet.

Measures of centrality and spread for subject descriptive and dietary variables are means and standard deviations. Most variables were analyzed after log transformation to reduce or eliminate effects of nonuniformity of error.[36] Spread for these variables was therefore represented by a factor (×/÷) standard deviation 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 (CL) with corresponding *P* values. Reliability of performance is expressed as the within-subject error (coefficient of variation, CV) derived from the repeated-measures analyses.

Analysis of power output during the 100-km time trial. The trends in power output during the 100-km time trial were compared using within-subject modeling. For each subject and each dietary condition, a polynomial with linear, quadratic, and cubic components was fitted to the natural log of power for each 5-km split using Proc Reg in SAS; power output for the 3 normal diets was averaged and analyzed as one dietary condition. The coefficients of the linear-slope component of the polynomial for the power response were compared using an appropriate repeated-measures analysis in Proc Mixed.[37]

Analysis of percentage energy contribution of fat and carbohydrate. Proc Mixed was used to fit a repeated-measures quadratic polynomial model to the percentage energy from fat (dependent variable) versus exercise intensity for the incremental test, and versus distance for the 100-km time trial.

Analysis of blood lactate. Polynomial expressions containing linear and quadratic components were fitted to blood-lactate concentration for the 15-minute and incremental tests. The mean y-axis position and the linear and quadratic coefficients were compared between treatments.

Mechanisms analysis. Percent dietary fat and peak fat-oxidation rate were included as covariates in separate repeated-measures analyses with the treatment effect. Reduction of the treatment effect by the covariate indicates the extent to which changes in performance in the 100-km time trial were attributable to changes in diet or peak fat-oxidation rate.

RESULTS

Diet Composition

Percent macronutrient composition of the reported diets was similar to that of the prescribed diets (Table 1). Fat intake decreased by 20%E (95% CL: 12 to 27%E, $P < .0001$) on the high-carbohydrate diet and increased by 31%E (24 to 39%E, $P < .0001$) relative to the preceding normal diets.

Exercise Performance

Fifteen-minute test. The effect of diet on 15-minute performance is shown in Table 2. Although there was a reducing effect in the high-fat condition, there was no statistically significant effect of diet on 15-minute performance: change to the high-fat condition decreased mean distance covered by -2.4% (95% CL: -5.4% to +0.6%, $P = .11$) relative to the fat with carbo-loading condition and by -0.9% (-3.8% to +2.0%, $P = .51$) relative to the high-carbohydrate condition; corresponding reductions in mean power were -4.5% (-11.2% to +1.9%, $P = .16$) and -2.9% (-8.8% to +3.4%, $P = .34$), respectively. The within-subject errors were 1.9% and 4.0% for distance and power, respectively.

One hundred–kilometer time trial. The effect of diet on power output during the 100-km time trial is shown in Fig 3. There was a statistically significant decline in power output in the high-carbohydrate condition (change in linear slope as mean ± SD: -5.2% ± 2.6% · 10 km^{-1}, $P = .003$) and in the normal condition (-2.2% ± 1.8% · 10 km^{-1}, $P = .03$). The decline was not significant in the high-fat condition (-1.3% ± 2.6% · 10 km^{-1}, $P = .44$) or in the fat with carbo-loading condition (-0.8% ± 2.6% · 10 km^{-1}, $P = .58$). The slope was greater by -4.4% · 10 km^{-1} (95% CL: -8.3% to -0.05% · 10 km^{-1}, $P = .03$) and -3.8% · 10 km^{-1} (95% CL: -8.2% to +0.4% · 10 km^{-1}, $P = .07$) in the high-carbohydrate condition compared with the fat with carbo-loading and the high-fat conditions, respectively.

The effect of diet on 100-km performance is shown in Table 2. Although performance was improved in the high-fat conditions and impaired in the high-carbohydrate condition, the differences were not statistically significant. The enhancement following change to the high-fat condition was 4.0% (-1.9% to +9.6%, $P = .16$) for time and 11.4% (-4.2% to +29.6%, $P = .15$) for mean power relative to the high-carbohydrate condition. Similarly, the fat with carbo-loading condition reduced time by 3.3% (-2.2% to +8.5%, $P = .22$) and increased mean power by 8.4% (-5.8% to +24.6%, $P = .24$) relative to the

Table 1. Composition of Diets

	Diet				
				FATCHO	
	NORMAL	CHO	FAT	FAT	CHO
Protein (%E)	16 ± 5	14 ± 2	20 ± 3	20 ± 3	13 ± 4
Carbohydrate (%E)	49 ± 13	70 ± 9	15 ± 4	15 ± 3	63 ± 22
Fat (%E)	36 ± 17	16 ± 5	66 ± 10	65 ± 9	24 ± 10
Total energy (MJ · d^{-1})	16.1 ± 4.1	15.5 ± 2.4	19.9 ± 5.7	18.8 ± 2.4	13.6 ± 3.0

NOTE. Data are macronutrient composition of the experimental diets calculated from the 4-day dietary diaries as raw mean ± SD.

Abbreviations: NORMAL, mean of all 3 normal diets; CHO, high-carbohydrate; FAT, high-fat; FATCHO, fat with carbo-loading (day 1 to 11.5 FAT, day 11.5 to 13 CHO).

Table 2. Performance on the Three Diets

	CHO	FAT	FATCHO
15-min test			
Distance			
Baseline (km)	11.0 ± 0.5	11.0 ± 0.4	11.0 ± 0.5
Treatment effect (%)	−0.9 ± 2.0	−1.8 ± 2.0	0.5 ± 2.1
Power			
Baseline (W)	305 ± 40	310 ± 30	308 ± 38
Treatment effect (%)	−2.1 ± 4.3	−5.0 ± 4.1	−0.7 ± 4.3
100-km time trial			
Time			
Baseline (min)	156 ± 12	156 ± 13	155 ± 9
Treatment effect (%)	1.6 ± 4.1	−2.5 ± 4.3	−1.8 ± 3.7
Power			
Baseline (W)	217 ± 44	212 ± 40	223 ± 27
Treatment effect (%)	−4.5 ± 9.0	6.3 ± 12.9	3.4 ± 10.8

NOTE. Baseline data are raw mean ± SD, whereas treatment effect data are least-squares mean ± 95% CI. The treatment effect is the change in performance after the treatment diet relative to the normal baseline diet.

Abbreviations: CHO, high-carbohydrate; FAT, high-fat; FATCHO, fat with carbo-loading.

high-carbohydrate condition. There was no difference in time (+0.8%, -5.1% to +7.0%, $P = .77$) or mean power (-2.7%, -16.4% to +13.1%, $P = .70$) in the fat with carbo-loading condition relative to the high-fat condition.

Power output during the final 5 km of the time trial in the fat with carbo-loading condition was enhanced by a factor of 1.3 (1.1 to 1.6, $P = .04$) and 1.2 (1.0 to 1.5, $P = .02$) relative to that in the high-carbohydrate and the combined-normal conditions, respectively; there was also a trend for an enhancement of final 5-km power by a factor of 1.1 (-1.1 to 1.5) relative to the high-fat condition, but the effect was not significant ($P = .29$). There was no clear difference in final 5 km power between the remaining treatment comparisons. The within-subject errors for 100-km time and mean power were 3.5% and 9.1%, respectively.

The analysis of mechanisms revealed a positive relationship between fat in the diet and 100-km mean power: for every 10%E increase in dietary fat, mean power increased by 2% (-0.0% to 4%, $P = .06$). With percentage dietary fat as the covariate in the full repeated-measures analysis, the increase in mean power during the high-fat and the fat with carbo-loading conditions was made unclear at 3% (95% CL: -27% to 46%) and 0% (-30% to 42%), respectively, relative to the high-carbohydrate condition. Therefore, percentage energy from fat in the diet had a strong influence on the mean 100-km power response.

General Responses to Diet

In general, the subjects reported the high-fat diet to be of acceptable palatability, although 4 subjects experienced episodes of minor gastric discomfort (constipation, diarrhea), and 2 had bad breath. There were few palatability and no gastric problems with the high-carbohydrate diet, but most subjects reported reduced meal satiety and frequent hunger sensations during training and daily activities compared with the high-fat and normal diets.

Psychological Response During Training

In Likert units, the fat with carbo-loading diet decreased energy rating by 0.77 (0.04 to 1.50, $P = .03$), while on the high-fat diet energy rating tended to decrease by 0.54 (-0.19 to 1.27, $P = .14$) relative to the high-carbohydrate condition, respectively. There was no clear effect of diet condition on ratings of leg pain, dissatisfaction, or mood (data not shown).

Body Mass and Body Fat

Body mass after fat with carbo-loading was 2.5% (0.0% to 5.0%, $P = .05$) and 2.4% (0.0% to 4.9%, $P = .05$) greater than after the high-carbohydrate and the high-fat conditions, respectively. Relative to baseline, skin-fold thickness decreased by (mean ± SD) 5% ± 6%, 4% ± 8%, and 2% ± 7% after the high-fat, the fat with carbo-loading, and the high-carbohydrate diets, respectively. There was no clear difference between the diets in skin-fold thickness or derived body fat.

Maximal Oxygen Uptake and Peak Power

There was no clear effect of diet on $\dot{V}O_2max$ or peak power (Table 3). The within-subject error for peak power was 2.4%.

Blood Lactate During the Peak Power Test

The high-fat conditions (combined) tended to elevate power output at a blood lactate concentration of 2 mmol · L^{-1} by 22% (-1% to 44%, $P = .06$) and at 4 mmol · L^{-1} by 12% (-2% to 27%, $P = .08$) relative to the high-carbohydrate condition (Table 3).

Fuel Utilization

Protein, carbohydrate, and fat oxidation rates during the incremental test and the 100-km time trial are presented graphically as contributions to oxygen consumption in Fig 4. The percentage of energy from fat and carbohydrate during the incremental test and the 100-km time trial are shown in Fig 5.

Protein oxidation. Total protein oxidation for the incremental test was 16 to 22 g (0.27 to 0.37 g.min^{-1}), which represented 6% to 10% of total oxygen consumption, whereas,

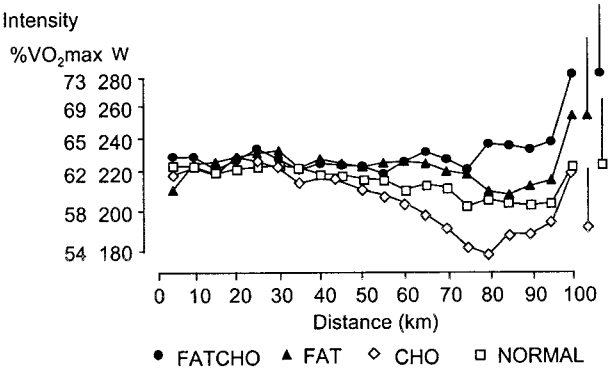

Fig 3. Power output during 100-km time trial. Data points are mean power for each 5-km split. Vertical bars are means of the between-subject standard deviations for each 5-km sample point. The sharp increase in power during the last 5 km of the time trial represents the final surge to complete the time trial.

protein oxidation over the 100-km time trial was 34 to 41 g (0.23 to 0.27 g.min^{-1}), contributing 6% to 9% of total oxygen consumption.

Fat and Carbohydrate Oxidation

The mean peak fat-oxidation rates (with the corresponding energy contribution from fat as percentage of total energy) were 0.93 g · min^{-1} (49%E) in the high-fat condition, 0.79 g · min^{-1} (38%E) in the fat with carbo-loading condition, 0.64 g · min^{-1} (33%E) in the combined normal conditions, and 0.32 g · min^{-1} (19%E) in the high-carbohydrate condition (between-subject SD for peak fat-oxidation rate: ×/÷ 1.47; for percentage energy, ± 10). The respective mean intensity at which these rates occurred was 75, 84, 82, and 68% $\dot{V}O_2$max (between-subject SD: ± 11%). Relative to the high-carbohydrate condition, the peak fat-oxidation rate was elevated 2.9-fold (2.0 to 4.3, $P = .00001$) in the high-fat condition, and 2.5-fold (1.7 to 3.6, $P = .0001$) relative to the fat with carbo-loading condition; the corresponding higher percentage contributions from fat were 30%E (21 to 39%E, $P = .00001$) and 19%E (11 to 28%E, $P = .0002$), respectively.

The peak fat-oxidation rate was positively related to 100-km performance, but the relationship was not statistically significant for the overall analysis ($P = .33$): for every 10 mg fat · kg^{-1} increase in the peak fat-oxidation rate, mean power improved by 6% (-6% to 18%). The magnitude of the effect was greater and the relationship somewhat clearer when comparing only the 3 treatment conditions at 11% (-5% to 29%, $P = .16$). With peak fat-oxidation rate as the covariate in the analysis of mechanisms, the increase in mean power relative to the high-carbohydrate was reduced to 8% (95% CL: -11% to 32%) during the high-fat condition and to 6% (-10% to 25%) in the fat with carbo-loading condition, respectively. Therefore, the peak fat-oxidation rate had a small to moderate influence on the mean 100-km power response.

Table 3. VO_2max, Peak Power, and Blood Lactate During the Peak Power Test

	Baseline	Treatment Effect (%)
VO_2max (L · min^{-1})		
CHO	5.2 ± 0.5	−3 ± 6
FATS	5.2 ± 0.5	0 ± 6
Peak power (W)		
CHO	381 ± 32	1 ± 3
FATS	378 ± 39	−1 ± 3
Peak lactate (mmol/L)		
CHO	7 ± 2	−5 ± 41
FATS	8 ± 2	−12 ± 41
Power at 2 mmol/L (W)		
CHO	309 ± 45	−6 ± 17
FATS	274 ± 50	16 ± 17
Power at 4 mmol/L (W)		
CHO	350 ± 40	−1 ± 13
FATS	312 ± 43	11 ± 13

NOTE. Baseline data are raw mean ± SD, whereas treatment effect data are least-squares mean ± 95% CI. The treatment effect is the change in performance after the treatment diet relative to the normal baseline diet.

Abbreviations: CHO, high-carbohydrate condition; FATS, high-fat and fat with carbo-loading combined.

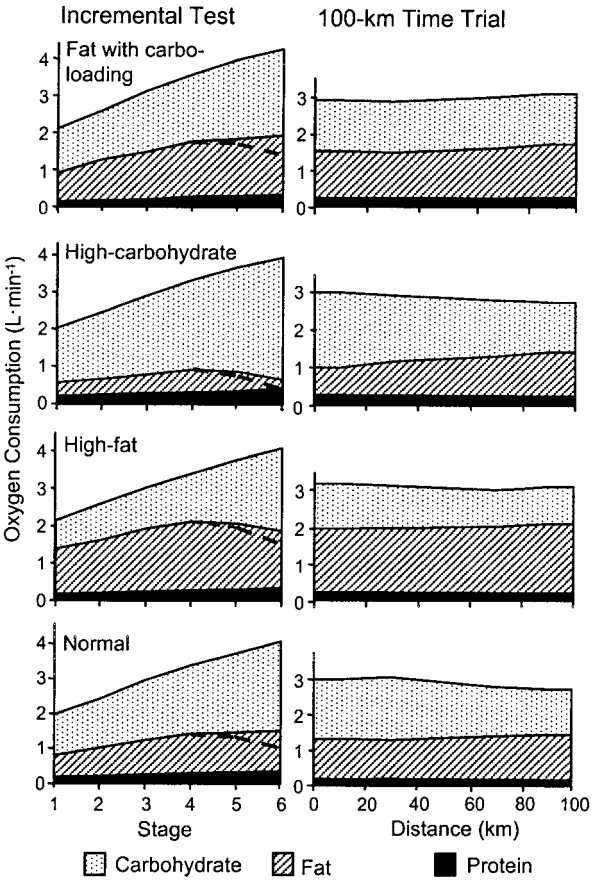

Fig 4. Effect of diet on fuel utilization during the incremental test and the 100-km time trial. Between-subject factor standard deviations (×/÷) for stages 1 to 6 of the incremental test averaged 1.7 for protein, 1.5 (stages 1 to 5) and 2.4 (stage 6) for fat, and 1.2 for carbohydrate. Corresponding standard deviations averaged for the 4 sampling points (10, 30, 70, and 90-km) during the 100-km time trial were 1.7, 1.4, and 1.6 for protein, fat, and carbohydrate, respectively. Dashed lines on the incremental test plots represent the dividing line between fat and carbohydrate oxidation calculated without the buffer co_2 correction.

The overall relative contribution of fat to total energy production during the incremental test was 49, 38, and 14%E (± 6%E) during the high-fat, the fat with carbo-loading, and the high-carbohydrate conditions, respectively, and 32 ± 12%E during the normal condition. The corresponding respiratory exchange ratios (RERs) were 0.85, 0.88, and 0.95 for the 3 respective treatment diet conditions, and 0.90 for the normal condition. The contribution of fat during the high-fat condition was 38%E (28 to 48%E, $P = .0001$), 21%E (11 to 30%E, $P = .001$), and 15%E (5 to 25%E, $P = .01$) greater than the high-carbohydrate, normal, and fat with carbo-loading conditions, respectively. During fat with carbo-loading, fat oxidation was 23%E (13 to 33%E, $P = .0002$) greater than during the high-carbohydrate condition.

The contribution of fat to total energy remained relatively stable during light to moderate work (37.5% to 60% peak

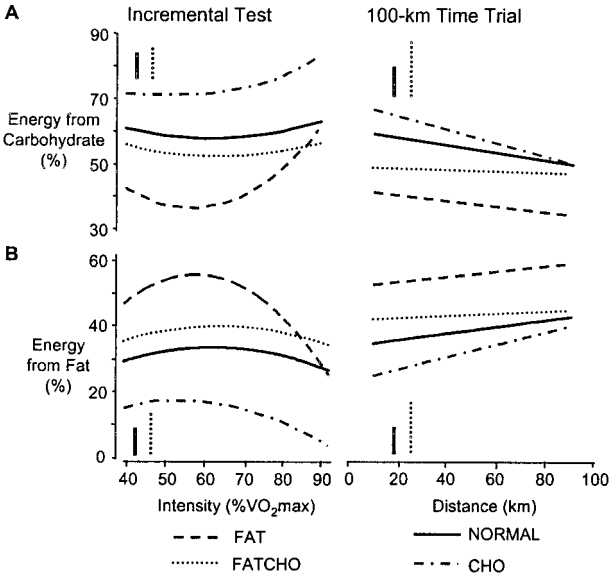

Fig 5. Effect of diet on %E contribution of carbohydrate (A) and fat (B) during the incremental test and the 100-km time trial. Data are derived from the polynomial analysis. Between-subject standard deviations for the position of the polynomial are represented by a solid vertical line for the 3 treatment conditions and by a dashed vertical line for the normal condition.

power), but declined when moving to heavy work (82.5% peak power). This decline was compensated for by an increase in the contribution of carbohydrate to total energy (Fig 5). The average effect of the buffer CO_2 correction factor was to increase fat oxidation (with a reciprocal decrease in carbohydrate oxidation) by 1 ± 2%E, 3 ± 4%E, and 10 ± 7%E at the 67.5%, 75%, and 82.5% peak-power stages, respectively.

The contribution to total energy from carbohydrate tended to decline, while the contribution from fat tended to increase as the 100-km time trial proceeded (Fig 5). The decline in carbohydrate oxidation and the increase in fat oxidation was greatest after the high-carbohydrate condition (Δ liner slope: 1.1%E · 10 km^{-1}), followed by the normal (0.6%E · 10 km^{-1}), the high-fat (0.5%E · 10 km^{-1}), and the fat with carbo-loading (0.2%E · 10 km^{-1}) conditions. The change in slope for fat and carbohydrate oxidation during the high-carbohydrate condition was greater by 0.9%E · 10 km^{-1} (0.3 to 1.4%E · 10 km^{-1}, $P = .003$) relative to the fat with carbo-loading condition, by 0.6%E · 10 km^{-1} (0.0 to 1.2%E · 10 km^{-1}, $P = .07$) relative to the high-fat condition, and by 0.5%E · 10 km^{-1} (0.7 to 1.0%E · 10 km^{-1}, $P = .01$) relative to the normal condition.

For the high-fat, the fat with carbo-loading, and the high-carbohydrate conditions, respectively, the mean contribution of carbohydrate and fat during the 100-km time trial was 38, 48, and 58%E (± 12%E) for carbohydrate and 57, 44, and 33%E (± 12 %E) for fat, representing respective RER of 0.82, 0.86, and 0.90. For the normal condition, the contribution of carbohydrate and fat was 54 ± 17%E and 39 ± 15%E, respectively, and the RER 0.88.

Circulating Substrates, Metabolites, and Hormones

Urea. The high-carbohydrate condition tended to decrease, whereas the high-fat condition tended to increase plasma-urea concentration relative to the normal and the fat with carbo-loading conditions (Fig 6A). However the only statistically significant difference was a decrease in urea by a factor of 0.79 (0.67 to 0.95, $P = .01$) in the high-carbohydrate condition relative to the high-fat condition.

Glucose. There was little difference in plasma-glucose concentration at rest before exercise between diet conditions (Fig 6B). During exercise, however, the high-carbohydrate condition lowered the overall glucose concentration by a factor of 0.88 (0.81 to 0.96, $P = .006$) and 0.88 (0.80 to 0.96, $P = .007$) relative to the fat with carbo-loading and the high-fat conditions, respectively.

Free fatty acids and glycerol. During the first approximately 3 hours of exercise, plasma free fatty-acid concentration was elevated during the high-fat condition relative to the other 3 conditions (Fig 6C and D). At the end of the 45-minute steady ride, fatty acids during the high-fat condition were increased by a factor of 2.8 (1.3 to 5.8, $P = .01$) and 1.8 (0.9 to 3.8, $P = .1$) relative to the high-carbohydrate and the fat with carbo-loading conditions, respectively; by 30 km into the 100-km time trial, the respective increases were still evident at 1.4 (0.8 to 2.4) and 1.7 (1.0 to 2.9), but not statistically significant ($P = .23$ and .07, respectively). Although the differences were not statistically significant, the free fatty acid concentration during the final 30 km of the 100-km time trial were increased in the high-carbohydrate condition relative to the other 3 conditions by factors of 1.2 to 1.5 (0.6 to 2.9, $P = .06$ to .4). Overall, the high-fat and the fat with carbo-loading conditions elevated plasma-glycerol concentration by factors of 1.39 (1.07 to 1.80, $P = .02$) and 1.29 (1.01 to 1.63, $P = .04$) relative to the high-carbohydrate condition.

Insulin. The high-carbohydrate condition raised pre-exercise plasma-insulin concentration by factors of 5.5 (3.6 to 8.5, $P = .0001$) relative to the fat with carbo-loading condition and 3.9 (2.5 to 6.2, $P = .0001$) relative to the high-fat condition (Fig 6E). Insulin declined during the first approximately 75 minutes of exercise to the point where there was little difference between any of the conditions by the end of the 45-minute steady ride.

Glucagon. There was no clear effect of diet condition on plasma glucagon concentration (Fig 6F).

Lactate. For the duration of the 15-minute test, blood-lactate concentration in the high-fat condition was lower than in the normal and high-carbohydrate conditions by factors of 0.59 (0.43 to 0.83, $P = .003$) and 0.71 (0.5 to 1.0, $P = .05$), respectively; lactate was also lower by a factor of 0.81 (0.62 to 1.11) in the fat with carbo-loading condition, but the effect was not significant ($P = .17$) (Fig 7). During the incremental test, there was no difference in the position of the lactate curve between the diet conditions. Overall, there was no difference in lactate between the diet conditions during the 100-km time trial. At the completion of the time trial, however, lactate in the fat with carbo-loading condition was elevated by a factor of 1.7 (1.1 to 2.5, $P = .03$) relative to the normal condition; the effect was similar at 2.0 (0.9 to 4.1) and 1.7 (0.9 to 3.1) relative to the

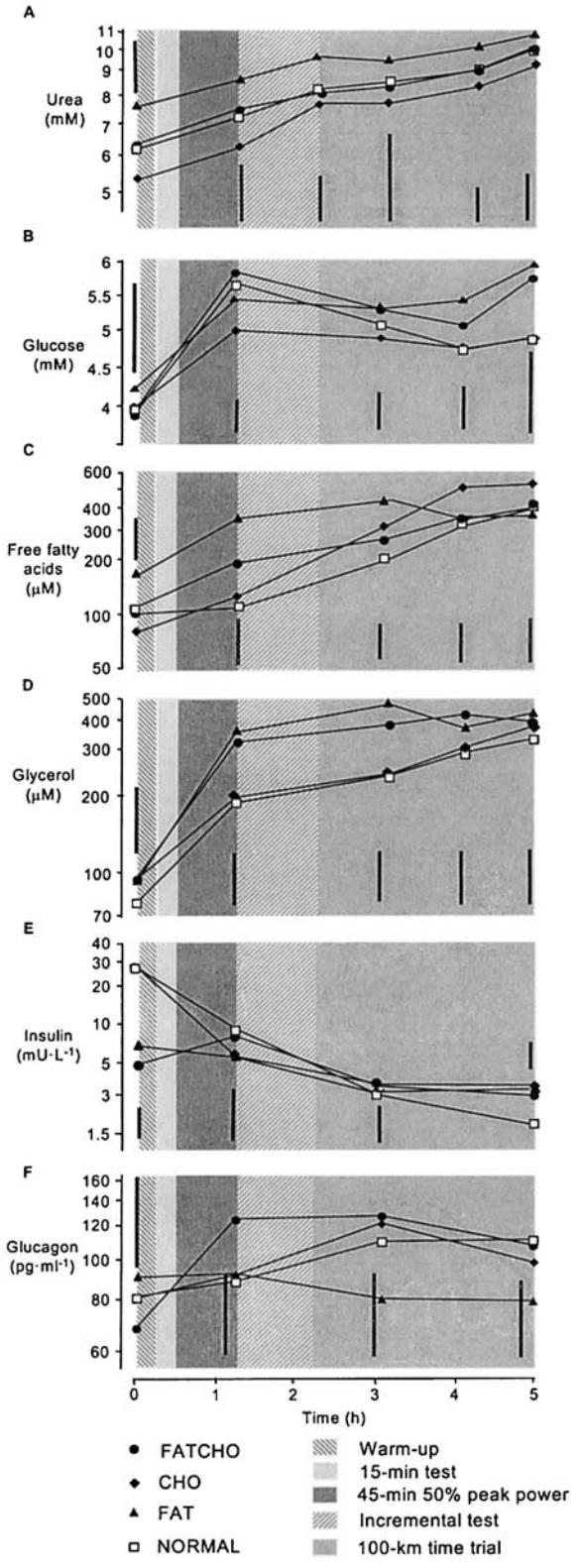

Fig 6. Effect of diet on concentrations of plasma urea (A), glucose (B), free fatty acids (C), glycerol (D), insulin (E), and glucagon (F). Bars are between-subject standard deviations.

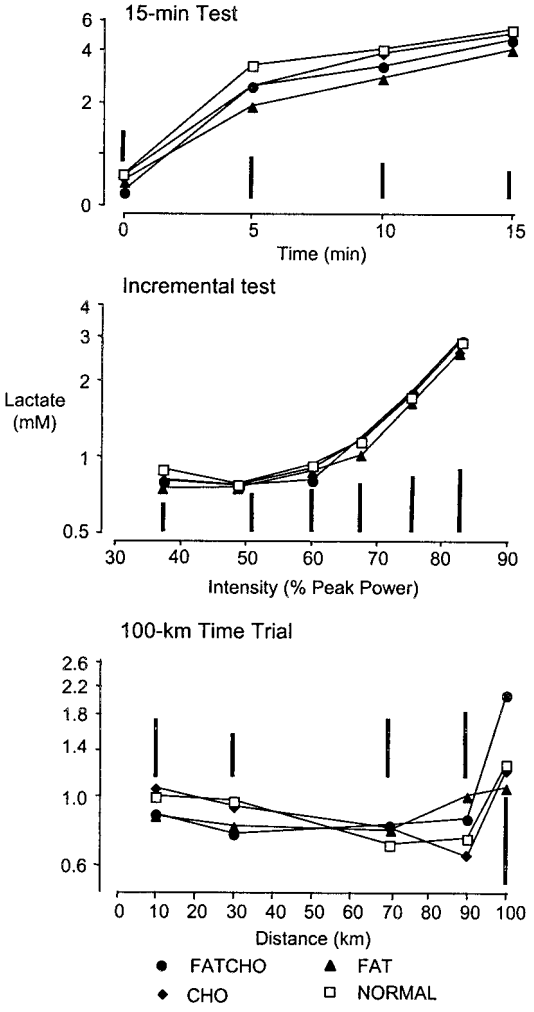

Fig 7. Blood lactate concentration during the 15-minute test, the incremental test, and the 100-km time trial. Bars are between-subject standard deviations.

high-fat and the high-carbohydrate conditions, respectively, but not statistically significant ($P = .08$ and .07, respectively).

DISCUSSION

The principle aim of this study was to investigate if enhanced fat oxidation after 2-week high-fat dietary conditioning could improve ultra-endurance performance relative to the recommended high-carbohydrate diet. Pre-exercise meals were ingested to elevate circulating fat availability in the high-fat conditions and to increase carbohydrate availability in the high-carbohydrate condition. A carbohydrate-rich supplement was ingested during exercise in all conditions to help maintain blood-glucose availability. We found a clear increase in fat oxidation, and although the differences were not statistically significant, there was some evidence for enhanced performance following high-fat conditioning. There was no apparent additional performance benefit arising from carbohydrate loading following the high-fat diet. We also investigated the effect of diet on short-duration high-intensity performance. Consistent

with the ultra-endurance outcome, there also was no statistically significant effect of diet on 15-minute performance, although there was a tendency toward impairment following the high-fat condition, which was attenuated with carbohydrate loading.

One likely reason for the lack of a statistically significant effect on 100-km performance was that the precision of the estimate was relatively low. The low precision was due to the high test error and the limited sample size. The error for mean power in a 100-km time trial is 3.6% to 4.6%.[38] Several factors may have accounted for our relatively high test error. First, the error was derived from experimental data, which means it included extra variation associated with response to the treatments. Second, the long duration of the entire exercise procedure could have compounded technical (calibration drift) or subject factors (eg, poor pace judgment). Third, preload exercise can increase the apparent error by 1.7 (1.3 to 2.3) times that for a time trial without a preload.[38] Another possible reason was individual responses to the dietary treatments, but there is an insufficient sample size to speculate any further.

Testing more subjects would probably have narrowed the confidence interval and resulted in statistical significance for the main performance outcome measures and provided some evidence for individual responses. Doubling the sample size, for example, would have likely reduced the confidence interval by a factor approximately $1/\sqrt{2}$. Unfortunately, low sample sizes are a reality for studies of this type because highly-trained ultra-endurance cyclists are difficult to recruit.

In the mechanisms analysis, we found some evidence for a positive relationship between the peak fat-oxidation rate and 100-km mean power. There is some other data to support a relationship between the capacity for fat oxidation and prolonged exercise performance in trained cyclists. During 3 hours of cycling at 55% $\dot{V}O_2$max in the fasting (placebo) condition, Rauch et al[39] found that the mean fat-oxidation rate in 2 cyclists who fatigued at 2.5 hours was about half that of the cyclists who could complete the 3-hour task ($\approx$ 0.75 g $\cdot$ min^{-1}). When given carbohydrate during exercise to increase blood-glucose availability, these 2 subjects were able to complete the exercise task.[39] In a 6-hour ride at 55% $\dot{V}O_2$max with carbohydrate supplementation, the fat-oxidation rate in 1 cyclist who exhausted at 4.5 hours was about half that of the remaining 10 cyclists who completed the ride.[40] The fat-oxidation rate in the cyclists who fatigued in these 2 studies appeared insufficient to compensate for the declining carbohydrate-oxidation rate, secondary to glycogen depletion.[39,40]

Similarly, the limited capacity for fat oxidation in the high-carbohydrate condition appeared unable to compensate for the declining carbohydrate-oxidation rate during the 100-km time trial, as reflected in the greater decline in power output in the high-carbohydrate condition relative to the other dietary conditions (Fig 3). A greater rate of carbohydrate supplementation might have attenuated this decline in power output by providing an exogenous source of carbohydrate to compensate for depleted muscle glycogen. The maximal rate for exogenous carbohydrate oxidation, however, is unlikely to be much greater than approximately 1.0 g $\cdot$ min^{-1}.[5] Since the carbohydrate-ingestion rate in the current study was near 1.0 g $\cdot$ min^{-1}, extra carbohydrate ingestion would probably have made little difference to the oxidation rate of exogenous carbohydrate in the muscle, particularly when considering that strenuous ultra-endurance exercise can impair gut function.[41] Nevertheless, the possibility exists that additional carbohydrate could increase glycogen stores in the liver and other tissues,[25,42] which might then be available for mobilization and utilization later in exercise. Even so, the maximal blood-glucose delivery from exogenous plus hepatic sources is limited (at best $\approx$ 1.1 g $\cdot$ min^{-1})[5] and therefore probably unable to provide sufficient carbohydrate to the working muscle during prolonged-strenuous exercise when glycogen is depleted. (To the best of our knowledge, carbohydrate derived from other tissue sources has not been accurately quantified.) To illustrate, we calculated that a total carbohydrate-oxidation rate of approximately 2.7 g $\cdot$ min^{-1} was required to maintain the initial power output over the duration of the 100-km time trial in the high-carbohydrate condition. By the final 30-km, the rate had reduced to 1.8 to 2.0 g $\cdot$ min^{-1}, and although fat oxidation was increasing, it apparently could not make up the energy deficit. We speculate that in athletes adapted to a high-carbohydrate diet, carbohydrate supplementation is unlikely to be able to compensate for a lower capacity for fat oxidation when muscle-glycogen stores are depleted during strenuous ultra-endurance exercise.

Relative to the high-carbohydrate condition, power output over the 100-km time trial declined less in the high-fat conditions, despite the likelihood of lower pre-exercise muscle-glycogen content.[13] Both Lambert et al[12] and Phinney et al[13] also reported that pre-exercise muscle-glycogen content did not relate to performance after high-fat diet adaptation. In the former study, mean fatigue time at 60% $\dot{V}O_2$max was increased from 43 to 80 minutes following a 2-week high-fat diet.[12] The endurance test was performed after a Wingate test and a 90% $\dot{V}O_2$max ride to exhaustion, which reduced muscle-glycogen and may have accounted for the relatively short submaximal endurance times (fatigue time at an intensity of 60% $\dot{V}O_2$max should be at least 5 hours in well-trained athletes[18]). Because these tests were performed fasted and no carbohydrate supplement was provided, reduced blood-glucose availability to the muscle secondary to liver-glycogen depletion[25] may have accounted for the premature fatigue time in the study by Lambert et al.[12] These data provide evidence to suggest that the often reported positive relationship between pre-exercise muscle-glycogen content and submaximal endurance performance observed following acute (1 to 3 days) high-fat versus high-carbohydrate dietary interventions[2-4] does not hold following a chronic high-fat diet; the increase in fat oxidation seen in this and in other studies[9,12-16,43] seems adequate to compensate for the lower pre-exercise glycogen stores. Even so, there appears to be an obligatory requirement for carbohydrate to support oxidative metabolism in the muscle.[44] This requirement was probably met in the present high-fat condition with carbohydrate derived from the supplement and any glycogen. (Any high-fat diet-induced increase in hepatic gluconeogenesis[45] was probably suppressed with the carbohydrate supplementation.[5])

In the only other ultra-endurance study, Carey et al[15] compared the effect of a 6-day high-fat diet (68%E fat) followed by 1-day carbohydrate loading versus a 7-day high-carbohydrate diet (68%E carbohydrate) on performance in a 1-hour cycling test, which followed a 4-hour preload at 63% $\dot{V}O_2$max. In both conditions a high-carbohydrate pre-exercise meal and exercise supplement were ingested. Fat oxidation was increased, and mean power was enhanced in the 1-hour test by 11% (-7% to 24%, $P = .11$) following the high-fat conditioning. Despite increased fat oxidation, there is currently no clear consensus on performance from the other high-fat dietary adaptation studies in well-trained cyclists, which employed exercise tests of 3.5 hours or less.[9,12-14,16,43] The equivocal status of the performance outcome of these shorter endurance-performance studies is difficult to explain. One possibility is that sufficient carbohydrate was available in the carbohydrate-rich dietary conditions in the form of glycogen or supplemental glucose to off-set any benefit to fuel availability derived from increased fat oxidation in the high-fat condition. For example, Goedecke et al[9] found no clear difference between high-fat and high-carbohydrate diets in the performance of a 40-km time trial that followed 150 minutes of cycling at 70% $\dot{V}O_2$max, despite an average 40% enhancement in the fat oxidation rate during exercise. The cyclists ingested a drink containing carbohydrate and medium-chain triglycerides before and during exercise. If we extrapolate from the present study, a 100-km time trial in the Goedecke study[9] might have depleted carbohydrate stores and resulted in a similar divergence of power output between the high-fat and high-carbohydrate conditions (Fig 3). Burke et al[16] found that time to complete a 7 kJ $\cdot$ kg^{-1} cycle test was 8% less (95% CI: -6% to 21%, $P = .21$) after a 5-day fat-rich diet followed by 1-day carbohydrate-loading, relative to a 6-day carbohydrate-rich diet. The work test was preceded by 2 hours of cycling at 70% $\dot{V}O_2$max. (Note the similar poor precision of the estimate in this study relative to the present study, arising at least partly from the use of a time trial after a preload[36] and the small sample size.) Despite increased fat oxidation, this performance effect was absent when the cyclists consumed a high-carbohydrate pre-exercise meal and exercise supplement,[43] suggesting sufficient carbohydrate was available in the control condition to offset any benefit to performance from enhanced fat oxidation.

It is interesting that during the high-fat and the fat with carbo-loading conditions, the fat-oxidation rate during the 100-km time trial was not substantially greater than during the incremental test, whereas during the high-carbohydrate condition, the rate increased somewhat (Fig 4). These observations suggest that, first, the power derived from fat oxidation was limited by a treatment-specific maximal physiologic capacity to oxidize fat, and second, the increased fat oxidation during the high-carbohydrate condition may have been due to a lifting of the suppressive effects of elevated pre-exercise insulin on adipose lipolysis and muscle fat metabolism.[22,23] Other factors may have limited the fat-oxidation rate in the high-carbohydrate condition: lower pre-exercise intramuscular-triglyceride content[10]; lower activity of enzymes involved in the transport and oxidation of fatty acids in the mitochondria[9-11]; and a lower capacity for sarcolemmal fatty-acid transport relative to the high-fat condition.[46] The elevated plasma fatty-acid concentration coupled with a lower rate of fat oxidation during the latter stages of the 100-km time trial in the high-carbohydrate condition does suggest impaired sarcolemmal fatty-acid transport relative to the 2 high-fat diet conditions.

The only other study to compare the effect of chronic high-fat versus high-carbohydrate diets on short-duration high-intensity performance (greater than 85% $\dot{V}O_2$max) also reported no significant difference, although there was also a trend toward impairment in the high-fat condition.[12] In the current study, blood lactate in the high-fat condition was lower during the 15-minute test relative to the other 3 conditions. A reduction in glycolytic flux secondary to low muscle-glycogen stores, therefore, is a possible explanation for the tendency towards impaired high-intensity performance relative to conditions with greater pre-exercise muscle glycogen stores. Interestingly, we found that the higher power output over the final 5-km of the 100-km time trial in the fat with carbo-loading condition (Fig 3) was related to a higher blood-lactate concentration (Fig 7) relative to the other conditions. This relationship between power output and lactate concentration might be due to greater availability of muscle glycogen for anaerobic energy production at the end of the time trial, which could be advantageous in a final sprint to the finish. Further research is required on the effect of chronic high-fat diets on high-intensity endurance performance.

Protein Metabolism

Our estimate of the contribution of protein to total energy was similar to the 7.1 to 9.5%E calculated by Brouns et al[47] during Tour de France simulation rides. Stein et al[48] reported a protein breakdown rate of 16 g $\cdot$ h^{-1} in well-trained triathletes during 8 hours of cycling and running at 53% $\dot{V}O_2$max, which is equivalent to 12%E as protein. Although the present venous-blood measurements provide only limited insight, elevated plasma-urea concentration during the high-fat condition suggests that increased amino acid catabolism was present in this condition relative to the high-carbohydrate condition. This effect might be related to low pre-exercise liver-glycogen content and higher rates of hepatic amino acid gluconeogenesis, and a low muscle-glycogen content, which promotes higher rates of amino acid metabolism in the muscle.[49] Alternatively, the higher urea concentration may be a consequence of the higher proportion of protein in the high-fat diet (Table 1), since high-protein diets increase protein metabolism and the activity of mitochondrial enzymes involved in amino acid metabolism.[50] We are unaware of any other estimates of protein oxidation in well-trained athletes during ultra-endurance exercise, and there is only sparse information on the metabolic role of protein in the high-fat condition.

Conclusion

The 2-week high-fat diet conditions increased fat availability and substantially enhanced the peak fat-oxidation rate during exercise. Despite these effects on metabolism, the main ultra-endurance performance outcomes were not statistically significant. Nevertheless, there was evidence for a substantial

enhancement of ultra-endurance cycling performance in the high-fat conditions relative to high-carbohydrate. It was not clear whether carbohydrate loading provides an additional benefit to ultra-endurance performance following high-fat conditioning. Diet had no significant effect on short-duration high-intensity performance, although a trend for impaired performance in the high-fat condition was attenuated with carbohydrate loading. Further research is required to clarify if the trend for enhancement of ultra-endurance performance following high-fat diet conditioning is real.

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REFERENCES

1. Ivy JL: Role of carbohydrate in physical activity, in Wheeler KB, Lombardo JA, (ed): Clinics in Sports Medicine: Nutritional Aspects of Exercise. Philadelphia, PA, Saunders, 1999, pp 469-484

2. Bergstrom J, Hermanssen J, Hultman E, et al: Diet, muscle glycogen and physical performance. Acta Physiol Scand 71:140-150, 1967

3. Christensen EH, Hansen O: Zur Methodik der respiratorischen Quotientbestimmung in Ruhe und bei Arbeit. III: Arbeitsfahigkeit und Ernahsrung. Scand Arch Physiol 81:160-171, 1939

4. Ahlborg G, Bergstrom J, Brohult J, et al: Human muscle glycogen content and capacity for prolonged exercise after different diets. Foersvarmedicin 3:85-99, 1967

5. Jeukendrup AE, Jentjens R: Oxidation of carbohydrate feedings during prolonged exercise: Current thoughts, guidelines and directions for future research. Sports Med 29:407-424, 2000

6. Sherman WM, Wimer GS: Insufficient dietary carbohydrate during training: Does it impair athletic performance? Int J Sport Nutr 1:28-44, 1991

7. Hawley JA, Brouns F, Jeukendrup A: Strategies to enhance fat utilisation during exercise. Sports Med 25:241-257, 1998

8. Helge JW: Adaptation to a fat-rich diet: Effects on endurance performance in humans. Sports Med 30:347-357, 2000

9. Goedecke JH, Christie C, Wilson G, et al: Metabolic adaptations to a high-fat diet in endurance cyclists. Metabolism 48:1509-1517, 1999

10. Kiens B, Essen-Gustavasson B, Gad P, et al: Lipoprotein lipase activity and intramuscular triglyceride stores after long term high fat and high carbohydrate diets in physically trained men. Clin Physiol 7:1-9, 1987

11. Fisher EC, Evans WJ, Phinney SD, et al: Changes in skeletal muscle metabolism induced by a eucaloric ketogenic diet, in Knuttgen HG, Vogel JA, Poortmans J, (ed): Biochemistry of Exercise. Champaign, IL, Human Kinetics, 1983, pp 497-501

12. Lambert EV, Speechley DP, Dennis SC, et al: Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. Eur J Appl Physiol 69:287-293, 1994

13. Phinney SD, Bistrian BR, Evans WJ, et al: The human metabolic response to chronic ketosis without caloric restriction: Preservation of submaximal exercise capability with reduced carbohydrate oxidation. Metabolism 32:769-776, 1983

14. Lambert EV, Goedecke JH, Zyle C, et al: High-fat diet versus habitual diet prior to carbohydrate loading: Effects on exercise metabolism and cycling performance. Int J Sport Nutr Exerc Metab 11:209-225, 2001

15. Carey AL, Staudacher HM, Cummings NK, et al: Effect of fat adaptation and carbohydrate restoration on metabolism and performance during 5-hr endurance exercise. J Appl Physiol 91:115-122, 2001

16. Burke LM, Angus DJ, Cox GR, et al: Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. J Appl Physiol 89:2413-2421, 2000

17. Hawley AJ, Hopkins WG: Aerobic glycolytic and aerobic lipolytic power systems: A new paradigm with implications for endurance and ultra-endurance events. Sports Med 19:240-250, 1995

18. Noakes TD, Lambert EV, Lambert MI, et al: Carbohydrate ingestion and muscle glycogen depletion during marathon and ultra-marathon racing. Eur J Appl Physiol 57:482-489, 1988

19. Ferrannini E: The theoretical bases of indirect calorimetry: A review. Metabolism 37:287-301, 1988

20. Griffiths AJ, Humphreys SM, Clark ML, et al: Forearm substrate utilization during exercise after a meal containing both fat and carbohydrate. Clin Sci 86:169-175, 1994

21. Whitley HA, Humphreys SM, Campbell IT, et al: Metabolic and performance responses during endurance exercise after high-fat and high-carbohydrate meals. J Appl Physiol 85:418-424, 1998

22. Coyle EF, Coggan AR, Hemmert MK, et al: Substrate usage during prolonged exercise following a preexercise meal. J Appl Physiol 5:429-433, 1985

23. Horowitz JF, Mora-Rodoriguez R, Byerley LO, et al: Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am J Physiol 273:E768-E775, 1997

24. Hawley JA, Burke LM: Effect of meal frequency and timing on physical performance. Br J Nutr 77:S91-S103, 1997

25. Coyle EF, Coggan AR, Hemmert MK, et al: Muscle glycogen utilisation during prolonged strenuous exercise when fed carbohydrate. J Appl Physiol 61:165-172, 1986

26. Colombani PC, Kovacs E, Frey-Rindova P, et al: Metabolic effects of a protein-supplemented carbohydrate drink in marathon runners. Int J Sport Nutr 9:181-201, 1999

27. Satabin P, Portero P, Defer G, et al: Metabolic and hormonal responses to lipid and carbohydrate diets during exercise in man. Med Sci Sport Exerc 19:218-223, 1987

28. Kuipers H, Verstappen FTJ, Keizer HA, et al: Variability of aerobic performance in the laboratory and its physiologic correlates. Int J Sports Med 6:197-201, 1985

29. Smith TBRJ: Respiratory performance during rowing, running and cycling ergometry. Masters Thesis, Physical Education, University of Otago, Dunedin, 1991

30. Livesey G, Elia M: Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 47:608-628, 1988

31. Lemon PWR, Mullin JP: Effect of initial muscle glycogen levels on protein catabolism during exercise. J Appl Physiol 48:624-629, 1980

32. Cerny F: Protein metabolism during two hour ergometer exercise, in Howald H, Poortmans JR (eds): Metabolic Adaptations to Prolonged Physical Exercise. Basel, Switzerland, Birkhauser, 1975, pp 232-237

33. Peronnet F, Massicotte D: Table of non-protein respiratory quotient: An update. Can J Sport Sci 16:23-29, 1991

34. Jones NL, Heigenhauser GJF: Getting rid of carbon dioxide during exercise. Clin Sci 90:323-335, 1996

35. Gann DS, Amaral JF: Fluid and electrolyte management, in

Sabiston DC (ed): Essentials of Surgery. Philadelphia, PA, Saunders, 1987, pp 29-61

36. Hopkins WG: Measures of reliability in sports medicine and science. Sports Med 30:1-15, 2000

37. Hopkins WG, Wolfinger RD: Estimating "individual differences" in the response to an experimental treatment. Med Sci Sports Exerc 30:S125, 1998

38. Hopkins WG, Schabort EJ, Hawley JA: Reliability of power in physical performance tests. Sports Med 31:211-234, 2000

39. Rauch HGL, Rodger I, Wilson GR, et al: The effects of carbohydrate loading on muscle glycogen content and cycling performance. Int J Sports Med 5:25-36, 1995

40. Rauch HGL, Hawley JA, Noakes TD, et al: Fuel metabolism during ultra-endurance exercise. Pflugers Arch 436:211-219, 1998

41. Jeukendrup AE, Vet-Joop K, Sturk A, et al: Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. 98:47-55, 2000

42. Kuipers H, Keizer HA, Brouns F, et al: Carbohydrate feeding and glycogen synthesis during exercise in man. Pflugers Arch 410:652-656, 1987

43. Burke LM, Angus DJ, Clark S, et al: Metabolic adaptations following fat adaptation persist despite carbohydrate availability during prolonged exercise. Med Sci Sports Exerc 32:S259, 2000

44. Spencer MK, Yan Z, Katz A: Carbohydrate supplementation attenuates IMP accumulation in human muscle during prolonged exercise. Am J Physiol 261:C71-C76, 1991

45. Podolin DA, Wei Y, Pagliassotti MJ: Effects of a high-fat diet and voluntary wheel running on gluconeogenesis and lipolysis in rats. J Appl Physiol 86:1374-1380, 1999

46. Kiens B, Helge JW: Effect of high-fat diets on exercise performance. Proc Nutr Soc 57:73-75, 1998

47. Brouns F, Saris WHM, Stroecken J, et al: Eating, drinking, and cycling. A controlled Tour de France simulation study, Part I. Int J Sports Med 10:S32-S40, 1989

48. Stein TP, Hoyt RW, O'Toole M, et al: Protein and energy metabolism during prolonged exercise in trained athletes. Int J Sports Med 10:311-316, 1989

49. Coggan AR, Mendenhall LA: Effect of diet on substrate metabolism during exercise, in Lamb D, Murry R (eds): Perspectives in Exercise Science and Sports Medicine: Prolonged Exercise. Indianapolis, IN, Benchmark, 1988, pp 435-471

50. Botwell JL, Leese GP, Smith K, et al: Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein. J Appl Physiol 85:1744-1752, 1998