# Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men

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**Abstract:** Nutrition is an important aspect of recuperation for athletes during multi-day competition or hard training. Post-exercise carbohydrate is likely to improve recovery, but the effect of protein is equivocal. The objective of this study was to determine the effect of post-exercise dietary protein content imposed over a high-carbohydrate background on subsequent performance. Using a crossover design, 12 cyclists completed 3 high-intensity rides over 4 days. Day 1 comprised 2.5 h intervals, followed by repeat-sprint performance tests on days 2 (15 h post) and 4 (60 h post), interspersed with a rest day. During 4 h recovery on days 1 and 2, cyclists ingested either 1.4 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate, 0.7 $g \cdot kg^{-1} \cdot h^{-1}$ protein and 0.26 $g \cdot kg^{-1} \cdot h^{-1}$ fat (protein-enriched) or 2.1 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate, 0.1 $g \cdot kg^{-1} \cdot h^{-1}$ protein, and equal fat (control). At other times, cyclists ingested a standardized high-carbohydrate diet. Anabolism was gauged indirectly by nitrogen balance, stress and inflammation via cortisol and cytokines, skeletal-muscle membrane disruption by creatine kinase, and oxidative stress by malonyl dealdehyde. Sprint mean power was not clearly different on day 2 (0.0%; 95%CL: ±3.9%), but on day 4 it was 4.1% higher (±4.1%) in the protein-enriched condition relative to control. Reduced creatine kinase was possible (26%; ±30%) but effects on oxidative stress, inflammatory markers, and cortisol were inconclusive or trivial. Overnight nitrogen balance was positive in the protein-enriched condition on day 1 (249 ± 70 mg $N \cdot kg\ FFM^{-1}$; mean ± SD), but negative (–48 ± 26 mg $N \cdot kg\ FFM^{-1}$) in the control condition. A nutritive effect of post-exercise protein content was not discernible short term (15 h), but a delayed performance benefit (60 h) was observed following protein-enriched high-carbohydrate ingestion.

*Key words:* muscle damage, interleukin-6, tumor necrosis factor-α, C-reactive protein, nitrogen balance, oxidative stress.

**Résumé :** L'alimentation constitue un facteur important dans la récupération chez les athlètes lors d'une compétition sur plusieurs jours ou au cours d'une période intensive d'entraînement. L'apport de sucres à la fin des activités facilite vraisemblablement la récupération, mais l'effet des protéines demeure équivoque. Le but de cette étude est d'analyser l'effet de la quantité de protéines ajoutée à une riche portion de sucres sur une performance subséquente. On opte pour un protocole de nature expérimentale avec inversion des groupes ; sur une période de 4 jours, 12 cyclistes participent à 3 séances de forte intensité. Le jour 1 consiste en des séances d'effort par intervalles d'une durée de 2,5 h suivies le jour 2 (15 h post) et le jour 4 (60 h post) d'épreuves répétées de sprint, le jour 3 étant dédié au repos. Au cours des 4 h de récupération les jours 1 et 2, les athlètes consomment 1,4 $g \cdot kg^{-1} \cdot h^{-1}$ de sucres, 0,7 $g \cdot kg^{-1} \cdot h^{-1}$ de protéines et 0,26 $g \cdot kg^{-1} \cdot h^{-1}$ de gras (enrichi de protéines) ou 2,1 $g \cdot kg^{-1} \cdot h^{-1}$ de sucres, 0,1 $g \cdot kg^{-1} \cdot h^{-1}$ de protéines et la même quantité de gras (contrôle). Au cours des autres repas, les athlètes consomment un régime hyperglucidique standard. On évalue indirectement l'anabolisme au moyen du bilan azoté, le stress et l'inflammation au moyen des concentrations de cortisol et de cytokines, les lésions des membranes musculaires au moyen de la concentration de créatine kinase et le stress oxydatif au moyen de la concentration de malonaldéhyde. Le jour 2, la puissance moyenne produite ne diffère pas nettement de celle du jour 1 (0,0 %; Intervalle de confiance de 95 %: ± 3,9 %), mais elle est 4,1 % (±4,1 %) plus élevée le jour 4 dans le groupe ayant consommé plus de protéine comparativement au groupe de contrôle. On observe une diminution de la concentration de créatine kinase (26 % ± 30 %), mais ses effets sur le stress oxydatif, sur les marqueurs de l'inflammation et sur la concentration de cortisol sont non concluants ou minimes. Le bilan azoté en une nuit est positif le jour 1 de l'apport enrichi en protéines (249 ± 70 mg $N \cdot kg\ FFM^{-1}$; moyenne ± É-T), mais négatif en période de contrôle (–48 ± 26 mg $N \cdot kg\ FFM^{-1}$). Un apport riche en protéines à la suite d'un effort physique ne donne pas d'effet notable à court terme (15 h), mais procure des gains au niveau de la performance dans les 60 h suivant un repas hyperglucidique enrichi de protéines.

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## Introduction

Nutrition has been established as one of the key factors supporting recuperation from heavy training or competition (Ivy 2004). Prolonged high-intensity endurance exercise reduces glycogen stores, which require restoration with dietary carbohydrate to support subsequent efforts (Ivy 2004). Intramyocellular triacylglycerol is utilized and its restoration during recovery may play an important role in fuel supply for subsequent endurance exercise (van Loon et al. 2003). In addition, prolonged-strenuous exercise generally results in trauma to the contracting skeletal muscle, free-radical damage to cellular components, and mobilization and catabolism of some protein (Belcastro et al. 1998; Tidball 1995). To compensate for these effects, athletes have a greater caloric requirement and a 1.5- to 2.2-fold increased dietary protein requirement to maintain nitrogen balance, compared with sedentary persons (Tipton and Wolfe 2004).

The immediate hours following exercise appear to be the best time, or "critical window" for the ingestion of nutrients with the aim of facilitating myocellular recovery. Glycogen restoration is most rapid immediately after exercise, declining with time to return to normal resting levels after about 8 h (reviewed by Ivy 2004). When carbohydrate ingestion is not optimal (i.e., 1.2–1.4 $g \cdot kg^{-1} \cdot h^{-1}$) then protein co-ingestion can facilitate glycogen recovery (Burke et al. 2004; Ivy et al. 2002). Similarly, muscle amino-acid uptake appears to be greatest immediately following exercise after a meal that raises blood amino-acid concentrations (Levenhagen et al. 2001, 2002), and increased intracellular amino-acid concentrations, particularly that of leucine, stimulate protein synthesis via mechanisms involving activation of translation–initiation factors (Kimball and Jefferson 2004). For some hours immediately after exercise there is increased blood flow to the exercised muscle, as well as enhanced insulin sensitivity (Goodyear et al. 1990; Ivy and Kuo 1998), amino-acid uptake (Miller et al. 2003), and protein synthesis, which makes the tissue most responsive to nutrient supplementation during that time (Borsheim et al. 2002).

Despite the physiological rationale and growing understanding of the role of nutrition in the recovery processes, only a handful of studies have been aimed at quantifying the interaction of protein and carbohydrate on the recovery of performance ability in endurance-trained people. Improvements in time-to-exhaustion during subsequent exercise in runners and cyclists were observed with protein-plus-carbohydrate beverages consumed either in the hours following exhausting exercise (Niles et al. 2001; Williams et al. 2003) or both during and following exercise (Saunders et al. 2004) relative to carbohydrate only. However, only in 1 of the aforementioned studies (Niles et al. 2001) were the beverages isocaloric. In contrast, 3 other studies employing isocaloric designs and carbohydrate ingestion doses nearer to the likely saturation rates for glycogen resynthesis (1.0–1.4 $g \cdot kg^{-1} \cdot h^{-1}$ (Ivy 2004)) found that protein conferred no clear additional benefit to subsequent endurance performance (Betts et al. 2005; Millard-Stafford et al. 2005; Rowlands et al. 2007). Nevertheless, there was evidence for attenuated muscle damage or faster repair as reflected by lower blood creatine kinase (CK) and less tiredness and muscle soreness in the protein condition (Millard-Stafford et al. 2005; Rowlands et al. 2007; Saunders et al. 2004), effects that over a longer time period could contribute to improved recovery and subsequent high-intensity performance ability.

Therefore, the purpose of this study was to examine the effect of a protein-enriched, high-carbohydrate, post-exercise recovery diet on next-day high-intensity cycling performance. We then broadened our investigation to 60 h to investigate if a delayed protein–nutrient effect on recovery was present. We hypothesized that indicative mechanisms implicate attenuated muscle damage or facilitated repair and adaptation processes that would lead to a delayed performance benefit not logically expected with limited same-day or overnight recovery periods. The nature of this study precluded muscle biopsies for primary-level evidence, but we were able to examine nitrogen balance and the possibility that the protein content of the recovery food may reduce tissue oxidative stress and inflammation.

## Materials and methods

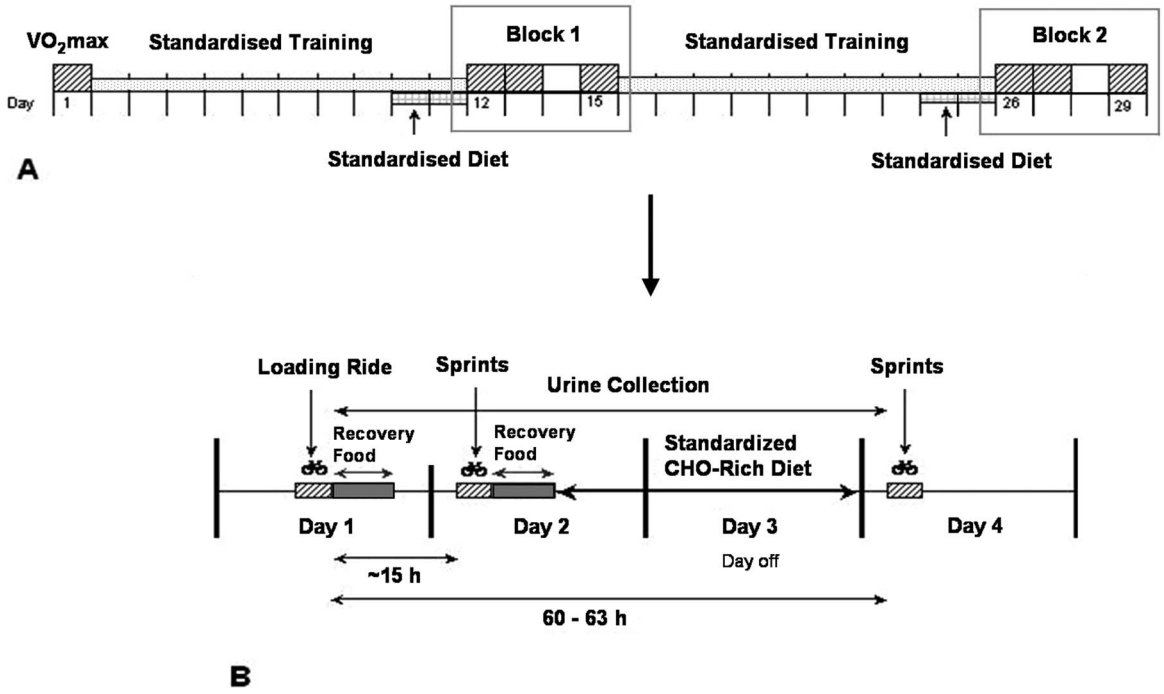
### Participants

Twelve endurance-trained male cyclists (mean ± SD: age, 34 ± 10 y; height, 179 ± 4 cm; body mass, 75.9 ± 4.3 kg) completed the study. Estimated fat-free mass (FFM) was 68.2 ± 2.3 kg and body fat mass was 10% ± 4.0% of total mass. Maximal oxygen uptake ($\dot{V}O_{2\,max}$) was 4.9 ± 0.6 $L \cdot min^{-1}$ and the corresponding peak power output ($W_{max}$) was 362 ± 29 W. The cyclists had been training for 7.5 ± 3.5 y, with a reported weekly average of 12.0 ± 3.5 h spent training over the 6 months prior to the study, which included high-intensity training and competition. Before beginning experimentation, all subjects read the study information sheet, were informed of their rights, were screened for precluding health conditions, and signed a consent form. The study was approved by the Massey University Human Ethics Committee.

### General design

Cyclists participated in a double-blind, randomized, crossover design, with a protein-enriched recovery feeding intervention and a low-protein control condition. There were 2 experimental blocks spaced 14 d apart, the details of which are illustrated in Fig. 1 and described below (see Experimental protocol). Before the first testing block, cyclists recorded their training and diet for 10 and 2 d, respectively, in their provided diaries. Training was kept light for 3 d before the testing block. This training and dietary regime was

**Fig. 1.** Experimental design (A) and the 4 d experimental block (B, inset). Within each block (B), cyclists completed a loading ride on day 1, followed by a 4 h recovery feeding period and 2 subsequent performance tests (sprints) on the mornings of day 2 and 4. Recovery feeding was repeated following exercise on day 2. The recovery duration from day 1 loading ride to the respective performance rides is shown.




repeated preceding the second block to standardize preconditioning.

### Preliminary testing

One to 2 weeks before start of the first experimental block, cyclists reported to the laboratory for measurement of body composition, a test to determine $VO_{2\ max}$ and $W_{max}$, and a practice ride using the experimental exercise protocols. Body composition was compartmentalized into fat and FFM using extrapolation from body density (BD) estimated from the sum of 7 skin folds (triceps, subscapular, biceps, supraspinale, abdominal, front thigh, medial calf) measured with Harpenden callipers using the following formula:

[1] $$BD = 1.09888 - 0.0004X_1$$

where $X_1$is the sum of 7 skinfolds (in millimetres) (Norton and Olds 1996). The fat compartment, expressed as percent body fat (%BF), was calculated from the standard regression (Siri 1956):

[2] $$\%BF = (495/BD) - 450$$

Fat-free mass was calculated by subtracting fat compartment mass from total mass. During the period between the preliminary test and the experiment a dietary recall diary was completed for assessment of habitual normal diet and energy intake.

$VO_{2\ max}$ was determined on an electromagnetically braked cycle ergometer (Velotron, Version 1.9 Software, Racer Mate, Seattle, Wash.) using a continuous, graded exercise protocol as described previously (Thorburn et al. 2006). Following 5 min of rest, the cyclists performed a familiarization trial of the performance test protocol and the psychometric sampling procedures. All testing occurred in a laboratory with air conditioning set at 19 °C and 50% relative humidity.

### Experimental protocol and procedures

On day 1, cyclists consumed a standardized low-protein, animal flesh-free diet, with the final meal taken 4 h before coming into the laboratory at ~1500 h (Fig. 1). In the lab, the cyclists completed a ride comprising 2.5 h of intervals to induce fatiguing exercise stress; e.g., reduced glycogen stores, muscle damage (Jentjens et al. 2001) (“loading ride”, Fig. 1). For each cyclist, the protocol was programmed into the ergometer software and consisted of a warm-up period comprising 12 min at 30%, 5 min at 40%, 5 min at 50% $W_{max}$, 10 × 2 min intervals at 90% $W_{max}$, and 12 × 2-min intervals at 80% $W_{max}$ alternated with 2 min recovery periods at 50% $W_{max}$, and finishing with three 5 min intervals at 70% $W_{max}$ interspersed with three 5 min recovery periods at 50% $W_{max}$. Three cyclists completed the testing procedure at once and this same group completed the second experimental block to repeat any effects of personality-induced psychostimuli. Average total internal work performed assuming 18.8% efficiency (Moseley et al. 2004) was 10.5 MJ.

Shortly after the exercise, cyclists toileted, then a 20 GA cannula (Beckton Dickinson, Medical Pte. Ltd., Singapore) was placed into an antecubital vein in suitable position. A 2-way stopcock valve (Connecta Plus 3, Beckton Dickinson, Medical Pte. Ltd., Singapore) was secured to the end of the cannula. A 10 mL blood sample was drawn at the time of placement (~5 min post-exercise). Additional samples were collected every 30 min for the following 120 min recovery period, with one final sample collected at 180 min. The

cannula was kept patent with isotonic saline (Pharmacia & Upjohn, Peapack, N.J.).

Immediately after the first draw of blood, cyclists consumed their first unit of the recovery food (see Recovery feeding intervention). Subsequent units were consumed at 30 min intervals immediately following blood collection. Cyclists remained in the laboratory for the first 3 h of the 4 h recovery feeding period during which time they showered and rested. After the 3 h, cyclists were asked to consume the last unit within 1 h after leaving the laboratory, prepare for the following morning, and go to bed. Cyclists then fasted until the next morning.

On day 2 of the experimental protocol, cyclists reported to the laboratory between 0630 and 0730 h. Start time was standardized for each rider. Upon arrival a cannula was placed as described previously and a 10 mL fasting blood sample was drawn. After blood collection, the cyclists consumed a small breakfast to simulate real-life normal practice and metabolic response and sport nutrition guidelines (replenish liver glycogen, reduce hunger) comprising a 50 g cereal bar containing 3 g protein (from oats and wheat), 6.8 g fat, 29.4 g carbohydrate, and 780 kJ (Baked Oaty Slice, Mother Earth, Auckland, New Zealand), as well as 250 mL of water. Fifteen minutes later, a blood sample was taken and the subjects started the repeated-sprint performance test, which has been described and validated previously (Rowlands et al. 2007; Thorburn et al. 2006). Following a warm-up that included 6 × 2 min priming intervals at 80% $W_{max}$, cyclists completed 10 maximal sprints interspersed and beginning with a recovery interval at 40% $W_{max}$. The internal work to be done (kilocalories) during the sprint (2–3 min) and recovery (5–6 min) periods was determined by individual $W_{max}$ (kilocalories = 0.125 × $W_{max}$). Fixed linear workloads approximately equivalent to riding a 39- or 48-tooth front chainring and a 10-sprocket 21- to 11-tooth rear cluster were programmed into the Velotron software. A gear switch was positioned on the end of the right handlebar break hood to allow linear resistance to be changed conveniently. Cyclists self-selected cadence and gearing, but were instructed to sprint as fast as possible until the required kilocalories were achieved. No verbal encouragement was provided to the participants; the only information provided during the sprints was elapsed work completed (kilocalories), which was shown on the computer screen. Participants were given a verbal countdown in preparation for the start of each sprint and at 20, 10, 5 and 2 kilocalories to go in preparation for the end of each sprint. The average total internal work performed during a sprint test was 5.0 MJ (7.7 MJ including warm-up). During all exercise procedures the cyclists were cooled with fans to minimize thermal distress.

Following the performance test, the cyclists repeated the ingestion of the recovery food for 4 h in the same experimental condition as the previous day. This time, however, cyclists remained in the laboratory and blood was collected every 30 min for only the first 90 min into recovery to accommodate work or study commitments. Cyclists ingested the remaining units at 30 min intervals at work or at home, and then moved on to a standardized carbohydrate-rich diet until the late evening of the following day (day 3; Fig. 1). The diet was provided to the cyclists and consisted of carbohydrate-rich foods (oats, rice, pasta, bread, vegetables, fruit, milk, yogurt, cheese, sports drink, fruit juice, and cereal bars). Participants were instructed to ingest a minimum of the food items to provide at least 10 and 8 g carbohydrate·kg $FFM^{-1}$ on days 2 and 3, respectively; to accommodate unknown variation in hunger and metabolic rate, they could consume above the minium amounts if still hungry, but had to record all portions and replicate their consumption during the second block of the crossover. The standardized high-carbohydrate diet was designed to further replensh intramuscular glycogen concentrations before exercise on day 4 and to be consistent with dietary macronutrient recommendations for endurance athletes for carbohydrate. The diet was identical between conditions, and combined with the energy contribution from the exercise supplement and recovery food provided total energy to approximate the normal habitual average daily energy intake for the cyclist sample (17.3 $MJ \cdot d^{-1}$).

On the morning of day 4, cyclists reported to the laboratory, were fitted with a cannula, and repeated the performance test in exactly the same manner as on day 2. Urine was collected throughout each experimental block from the completion of exercise on day 1 until the beginning of exercise on day 4 (Fig. 1).

### Blood handling

Collected blood was immediately transferred into evacuated containers (Becton Dickinson & Co., Franklin Lakes, N.J.) with EDTA or lithium heparin. These tubes were then immediately centrifuged at 2500*g* for 12 min (Centrifuge, Haraeus Sepatech Medifuge, Germany). The plasma was aspirated into eppendorf tubes, then immediately frozen in liquid nitrogen and stored at –80 °C for later analysis.

### Recovery feeding intervention

The protein-enriched condition was a commercially available milk chocolate-coated recovery bar and milk-like drink formulation (chocolate or vanilla flavored). The low-protein control condition was an isocaloric bar formulated by the same food technologist who made the protein-enriched bar and a milk-like drink to resemble as close as possible the intervention bar and drink (chocolate coating (bar), similar taste, color and texture, non-distinguishable serving volume and size). The protein-enriched condition was designed to provide 1.6 g carbohydrate, 0.8 g protein, and 0.29 g fat per kilogram FFM per hour, whereas the control provided 2.35 g carbohydrate, 0.12 g protein, and 0.29 g fat. One hundred grams of the bar used in the protein-enriched condition contained 27.4 g carbohydrate, 20.1 g protein, and 4 g fat. Ingredients were protein blend (whey protein isolate, calcium caseinate, soy protein isolate), glucose, honey, milk chocolate (14%) (sugar, cocoa butter, cocoa liquor, whole milk powder, skim milk powder, emulsifier), apple juice concentrate, crisped soy nuggets (isolated soy protein, rice flour, malt, salt), glycerin, cocoa powder, emulsifier, canola oil, flavor, corn syrup solids, vitamins, and minerals. The control bar contained 5.1 g protein, 41 g carbohydrate, and 7.2 g fat per 100 g. Ingredients for the control bar were exactly the same as above for the protein-enriched bar, except that the protein-blend and the soy nuggets were replaced with waxy maize starch and rolled oats, respectively. The protein-enriched beverage contained 4.5% sucrose, 6.3%

maltodextrin, 1.85% whey protein isolate, 3.7% milk protein concentrate, 3.2% cream powder, 0.1% salt, flavoring, and water. The control beverage was made of 4.5% sucrose, 12.2% maltodextrin, 2.45% cream powder, 0.12% salt, flavoring, and water. Bar and drink servings were given every 30 min, starting immediately after exercise on day 1, for 4 h totaling 8 bars and drinks during this period. The protein in the control bar and drink was contained within the chocolate coating, rolled oats, and in trace amounts in the cream powder used in the drinks to assist placebo purposes, energy balance between conditions, and provision of a quantity of fat normally present in the diet of athletes.

Four cyclists on day 1 and two on day 2 did not ingest 1–2 of the 8 units of the recovery food complaining of fullness; these servings were also removed from the second block of the crossover to retain isocaloric conditions. These cyclists were among those who had expended the least amount of energy during the preceding ride. In contrast, 3 other cyclists, 2 of whom completed the highest amount of work of the group, complained of overnight and morning hunger on day 2.

### Fluid and carbohydrate replacement during exercise

During all exercise procedures the cyclists were provided with a 6.8% carbohydrate–electrolyte solution to provide carbohydrate at the rate of 0.8 g·kg $FFM^{-1}·h^{-1}$. The cyclists consumed on average 802 ± 27 $mL·h^{-1}$. On days 1 and 2, the cyclists were weighed immediately before and after exercise after they had gone to the toilet. If weight loss occurred, a mass of tap water equivalent to the deficit was provided during the 4 h recovery period to assist rehydration.

### Ratings of fatigue and exertion

On days 2 and 4, during exercise, cyclists were asked to rate sensation perception on psychometric charts immediately after priming intervals 2 and 5, and sprints 1, 4, 7, and 10. The measured categories were sensations of tiredness, leg soreness, ability to sprint, level of exertion, and nausea. These responses were quantified using linear interval Likert scales from 1 (nothing) to 9 (maximal). The categorical coefficients of variance (CVs) were determined previously and ranged between 0.20 for tiredness and 0.96 for nausea (Rowlands et al. 2007).

### Biochemistry

Plasma lactate and glucose concentrations were measured using an automated analyzer (Bayer Rapidlab 800 system, Corning & Diagnostics Corp., Medfield, Mass.). Creatine kinase (CK) as a marker for skeletal muscle membrane damage was detected by enzymatic assay (Roche Diagnostics NZ Ltd., Auckland, NZ) on a Roche Cobas Fara II analyzer in accordance with the kit manufacturer's recommendations. To assess systemic stress and inflammation, cortisol, interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and C-reactive protein (CRP) concentrations were determined from the appropriate blood samples collected on day 1 at 90 min of recovery, day 2 after fasting, day 2 at 90 min of recovery, and on day 4 after fasting. More extensive analysis of stress and inflammation markers was not undertaken because of limited funds. Cortisol was measured using double antibody radioimmunoassay (Linco Research Inc., St. Charles, Mo.). Enzyme-linked immunosorbent assay (ELISA) kits were used to determine IL-6 (Cat. No. HS600B, R&D Systems, Minneapolis, Minn.), TNF-α (Cat. No. HSTA00C, R&D Systems), and CRP (Cat. No. 1000, Alpha Diagnostic, San Antonio, Tex.). The lipid peroxide malonyl dealdehyde (MDA) was measured in the plasma as a marker of oxidative stress by spectrophotometry (Varian bio 300, Paulo Alto, Calif.) using an LPO-586 kit from Bioxytech (Foster City, Calif.). Urinary urea was measured by spectrophotometry (Jenway 6505, Jenway, UK) using enzymatic urea slow rate reagent kit (Thermo Electron, Medica Pacific, Auckland, New Zealand). Measurement of urinary creatine and creatinine was by high-performance liquid chromatography (Agilent-Hewlett Packard, 1100 Series, Waldbronn, Germany).

### Nitrogen balance

Net nitrogen balance (dietary intake minus loss) was calculated over 3 periods during the experimental period to total 60 h as follows: (*i*) from the completion of cycling on day 1 to the final urine collection shortly before the pre-exercise meal on day 2; (*ii*) during exercise on day 2; and (*iii*) from the end of exercise on day 2 to the final urine collection on the morning of day 4 preceding the pre-exercise meal. Nitrogen intake was calculated from total dietary protein intake in grams divided by the amino acid-nitrogen constant 6.25. Nitrogen outputs were urinary urea, creatine, and creatinine (measured). Additional (estimated) nitrogen losses were from sweat at rest, sweat during exercise on day 2, and from feces and miscellaneous loss throughout the 60 h collection period (based on the urine to sweat loss ratios observed previously in endurance athletes on low- or high-protein diets for the protein-enriched or control conditions, respectively (Tarnopolsky et al. 1988)). Estimated additional nitrogen loss accounted for 22% and 17% of total loss in the control and protein-enriched condition, respectively.

### Statistical analysis

#### *General method*

The effects of the protein-enriched intervention relative to the control condition on dependent outcome variables was estimated from mixed modeling (proc mixed) in the Statistical Analysis System (version 9.1, SAS Institute Inc. Cary, N.C.). Most dependent variables were analyzed after log transformation to reduce or eliminate effects of heteroscedastic error apparent after preliminary visual assessment of the residuals; the exception was perceptual data and data sets with negative values where log-transformation is not appropriate because of interval scaling and mathematics, respectively (Nevill and Lane 2007). Appropriate mixed (fixed and random effects) linear models were applied to all data sets. To fully account in the analysis for the multiple known experimental design effects, the sprint performance outcome was determined from the modeled interaction between the following fixed effects: treatment, order of treatment (1 or 2), and day (2 or 4). The order effect was included in the analysis of power and perceptual responses to account for the familiarization effect between consecutive

trials common to most physical and psychological measures, while the day effect was included to derive and account for the day difference. For the analysis of sprint performance, lactate and glucose, and the psychometric responses, sprint number or time was a coded as a quantitative numeric predictor (as in standard linear regression) to model the effect of time on outcomes (slope and fatigue effects). For the analysis of CK, cytokines, MDA, and nitrogen balance, overall average and sample point effects were estimated from repeated-measures linear models (sample time or sprint number coded as qualitative fixed classification variables as in traditional analysis of variance).

Appropriate random effects (variation) were included in the models to account for clustering effects, individual responses to treatment, and multiple-day outcomes. In all data sets, subject identity and the identity–condition interaction were within-subject random effects. In the models for sprint performance, perceptual data, and glucose and lactate concentrations, the variation associated with moving between sprints (fatigue) or progression along a time series and the unique by-day variation associated with treatment, order, and condition were included in the model. The within-subject standard deviation was calculated as the root of the remaining residual variance.

### *Presentation of data*

Subject descriptive and dietary variables are raw means and standard deviations (SD). Means derived from the analysis of log-transformed variables are back log-transformed least-squares means, or adjusted means. The associated spread around these means is represented by percentage (geometric) standard deviations ($SD_{\%}$), which can be converted to a unit value by conversion to a factor. For example, for a plasma glucose concentration of 5 $mmol \cdot L^{-1}$ with a between-subject standard deviation of 20% ($SD_{\%}$ 20), the typical variation is 5 × 1.20 to 5 ÷ 1.20, or 6 to 4.16 $mmol \cdot L^{-1}$. Data and statistics are expressed in the International System of Units (SI) or otherwise the more common unit and are rounded to 2 significant digits, or in some cases 3 where the first digit is "1".

### *Estimate precision and statistical inference*

In keeping with recent trends in methods of inferential statistics (e.g., Batterham and Hopkins 2006; Sterne and Smith 2001), we report uncertainty of outcomes as 95% confidence limits (CL) or intervals (CI) and make probabilistic magnitude-based inferences about true (large-sample) values of outcomes using methods described below and in more detail elsewhere (Batterham and Hopkins 2006). The advantages of the confidence-limit magnitude-based approach over traditional null-hypothesis significance testing based on an arbitrary $p$ value of 0.05, are that it emphasizes effect magnitudes and estimate precision rather than an absolute effect vs. non-effect interpretation (recognizing sampling variability, that a range of feasible values is possible, and that what scientists are really interested in is how large an effect is — the hypothesis test and $p$ value alone provide for none of these). It also necessarily defines the smallest important biological or practical effect, and bases the interpretation of outcomes relative to this quantity; in doing so, it provides a method to readily identify a likely true null or trivial effect (null-hypothesis testing fails to deal with the real world significance of an outcome). Finally, it qualifies (and quantifies if required) the probability of an important effect with appropriate inferential descriptors to facilitate interpretation.

To this end, the smallest worthwhile or important (substantial) performance effect was defined as a reduction or increase in mean power of >1.1%. (for derivation see Hopkins et al. 1999; Thorburn et al. 2006), while the threshold value for a substantial effect in the biochemical and psychometric variables was the conventional smallest standardized (Cohen) change in the mean: 0.20 times the between-subject SD for the control value (Batterham and Hopkins 2006; Hopkins et al. 1999). Further qualification of effect magnitudes was made in places using modified Cohen effect-size (ES) thresholds: <0.2, trivial; <0.6, small; <1.2, moderate; <2.0, large; >4.0, very large (Hopkins 2002). To provide probability-based practical inference, an effect was described as unclear if its confidence interval overlapped both the upper and lower thresholds for substantiveness by >2.5%; that is, if the effect could be substantially positive and negative (increase and decrease, etc). Otherwise, the probabilities of a substantial effect were calculated from the 2-tailed Student's $t$ distribution using a published spreadsheet (Hopkins, 2001*a*) and inferred modified from Batterham and Hopkins (2006) as follows: <0.5%, almost certainly not; 0.5%–2.5%, very unlikely; 2.5%–12.5%, unlikely; 12.5%–87.5%, possible; 87.5%–97.5%, likely; 97.5%–99.5%, very likely; >99.5%, almost certain. In the case where the majority of the confidence interval lies between the threshold for a substantially positive and negative effect, the likelihood the effect being trivial is qualified. Because the inferential method is new to this journal, in this paper, we have provided $p$ values for the main experimental outcomes so that readers may make an inferential comparison.

## Results

### Performance

Performance outcomes on days 2 and 4 are shown in Fig. 2. A summary of the statistical analysis is provided in Table 1. On day 2, there was no clear effect of the protein-enriched condition on overall sprint mean power. On day 4, however, mean power was substantially higher in the protein-enriched condition, relative to control. The decline in mean power from sprint 1 to 10 (fatigue effect) was greater in the control condition on day 2, relative to the protein-enriched condition; however, on day 4, the difference in fatigue between conditions was unclear (Table 1).

With the treatment effect factored out, overall sprint mean power on day 4 compared with day 2 was 8.7% (95%CI: 4.7%–13%, $p < 0.0001$) and 7.9% (3.5%–12.4%, $p < 0.0001$) higher during the first and second experimental blocks, respectively. The second order of trial increased overall sprint mean power on days 2 and 4 relative to the first by an insubstantial 1.9% (–2.1%–6.0%, 0.36) and 1.0% (–3.0%–5.2%, 0.62), respectively.

### Fatigue and exertion

There were mainly likely trivial effects of the protein-

**Fig. 2.** Mean sprint power during the performance tests on days 2 and 4. Data are least-squares mean power from the analysis presented on a logarithmic *y* axis (left) and as relative to $W_{max}$ (right). Bars are between-subject standard deviations for the respective conditions. The single point (circle) error bars are the within-subject standard deviation.

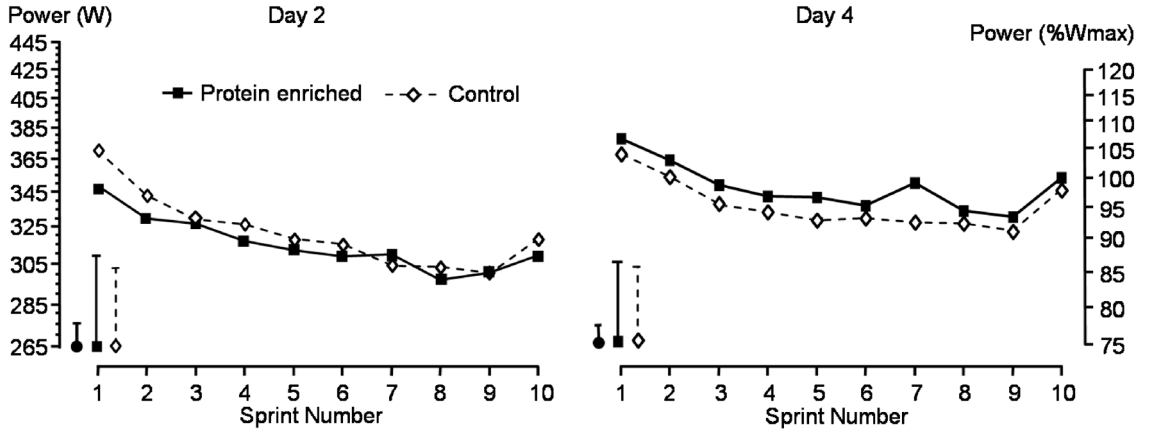



**Table 1.** Effect of the protein-enriched condition on performance.

| | Diet condition[a] | | Outcome | | |
|---|---|---|---|---|---|
| Performance measure | Control ($SD_{\%}$) | Protein-enriched ($SD_{\%}$) | Mean effect[b]; 95%CL[c] | *p* value[d] | Qualitative inference |
| **Day 2** | | | | | |
| Overall sprint mean power (W) | 318 (16) | 318 (17) | 0.0; ±3.9 | 0.99 | Unclear |
| Fatigue[e] | –18.5 (6.9) | –14.8 (8.4) | 4.5; ±5.3 | 0.088 | Reduction likely |
| **Day 4** | | | | | |
| Overall sprint mean power (W) | 338 (16) | 351 (17) | 4.1; ±4.1 | 0.048 | Increase likely |
| Fatigue[e] | –11.8 (6.9) | –10.4 (8.4) | 1.6; ±5.3 | 0.56 | Unclear |

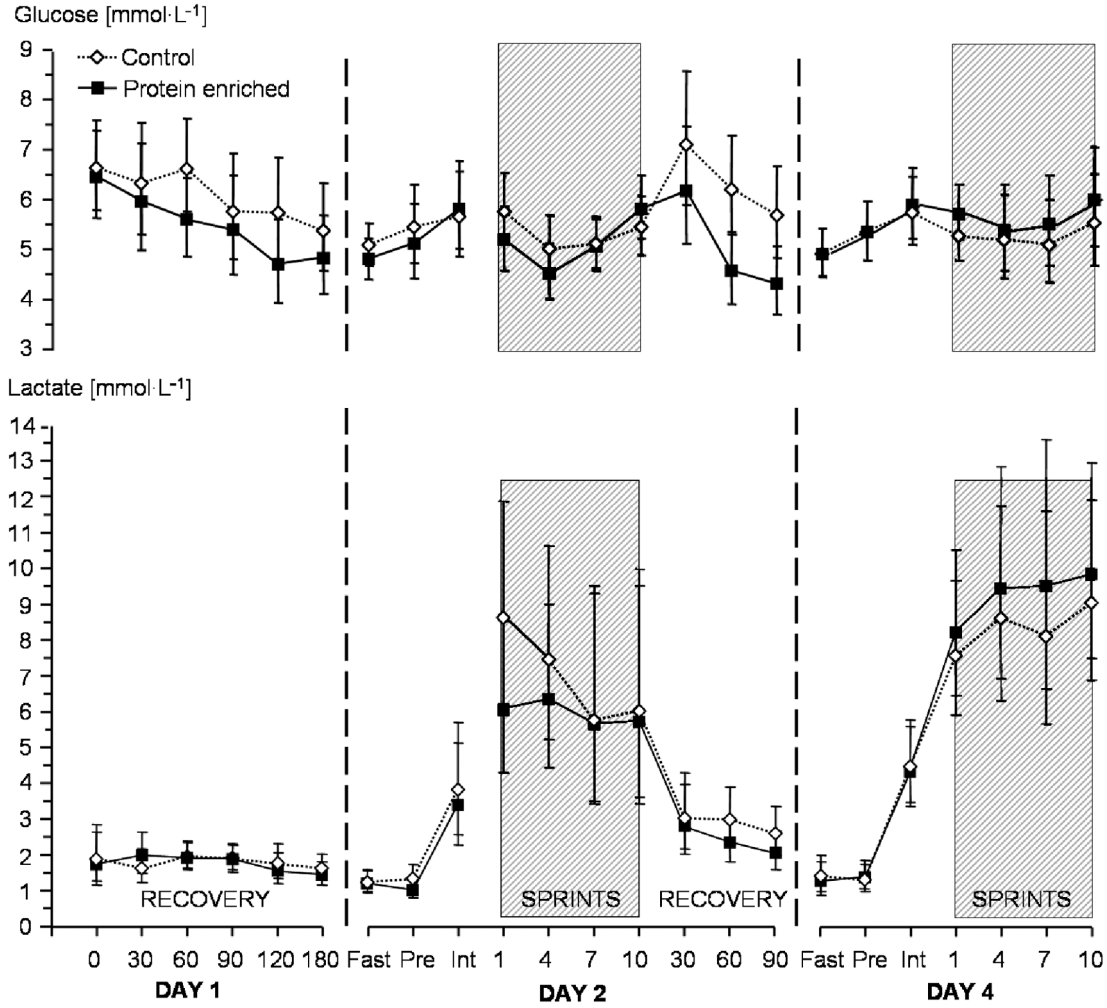
[a]Data are least-squares means expressed as percent. Fatigue is the percentage reduction in the absolute value for mean power from sprint 1 to 10.
[b]Mean effect refers to the protein-enriched effect minus control.
[c]±95%CL: add and subtract this number to the mean effect to obtain the 95% confidence limits for the effect.
[d]Rounded to 2 significant digits.
[e]The smallest worthwhile effect of treatment on fatigue is 1.1%.

**Table 2.** Effect of the protein-enriched condition on the perception of fatigue and exertion during the sprints.

| | | Outcome | |
|---|---|---|---|
| Perception rating | Mean effect[a]; ±95%CL[b] | *p* value[c] | Qualitative inference |
| **Sprints day 2** | | | |
| Tiredness | –0.3; ±0.3 | 0.016 | Decrease likely |
| Leg soreness | –0.2; ±0.5 | 0.30 | Likely trivial |
| Leg strength | 0.2; ±0.3 | 0.12 | Increase possible |
| Perceived effort | –0.1; ±0.2 | 0.50 | Likely trivial |
| Nausea | –0.1; ±0.2 | 0.49 | Very likely trivial |
| **Sprints day 4** | | | |
| Tiredness | 0.1; ±0.3 | 0.34 | Unclear |
| Leg soreness | 0.2; ±0.3 | 0.086 | Unclear |
| Leg strength | 0.2; ±0.4 | 0.26 | Increase possible |
| Perceived effort | 0.4; ±0.2 | 0.001 | Increase very likely |
| Nausea | –0.5; ±0.4 | 0.015 | Decrease possible |

[a]Mean effect refers to the protein-enriched effect minus control.
[b]±95%CL: add and subtract this number to the mean effect to obtain the 95% confidence limits for the true difference.
[c]Rounded to 2 significant digits.

**Fig. 3.** Plasma glucose and lactate concentrations during the post-exercise recovery feeding periods on days 1 and 2 (Recovery) and during the repeated-sprint performance tests on days 2 and 4 (Sprints). Data are least-squares means and bars are between-subject standard deviations. Fast, before pre-exercise meal; Pre, before exercise; Int, end of priming intervals.




enriched condition on psychometric measures on day 2, except for a likely decrease in the perception of tiredness (Table 2). On day 4, possible small increases in strength, decreases in nausea, and a very likely increase in perceived effort were observed in the protein-enriched condition, relative to control (Table 2). The change in perception ratings from sprint 1 to 10 (slope) was unclear (effects not shown), with the exception in day 2 of the rating of leg soreness increasing by 0.13 units (95%CI: 0.04–0.22, $p$ = 0.01) more per sprint and the rating of leg strength decreasing 0.08 units (0.00–0.15, $p$ = 0.05) more per sprint in the protein-enriched condition compared with the control condition.

## Physiological parameters

### *Glucose and lactate*

The plasma glucose and lactate concentrations are shown in Fig. 3. Overall, glucose was 15% (95%CI: 9%–21%, $p$ = 0.0001) and 24% (15%–33%, $p < 0.0001$) lower in the protein-enriched condition relative to the control condition during the day 1 and 2 recoveries, respectively. In addition, the rate of decline (suggesting clearance) in glucose concentration in the protein-enriched condition was 23% (7%–40%, $p$ = 0.015) and 75% (37%–121%, $p$ = 0.0003) greater on days 1 and 2 relative to control. On day 2 no clear difference in glucose concentration was observed following the sprints; on day 4, however, concentrations were 8% (5%–11%, $p < 0.0001$) higher in the protein-enriched condition.

There was no clear difference in plasma lactate concentrations during recovery on days 1 or 2. On day 2 during the sprints, however, lactate was 15% higher (–2%–35%, $p$ = 0.15) in the control, and due to the tendency for higher lactate during the first half of the test, the decline from sprint 1 to 10 was 34% steeper (9%–67%, $p$ = 0.022) in the control condition relative to protein-enriched condition. On the contrary, lactate concentrations during the sprints on day 4 were 13% (4%–22%, $p$ = 0.013) higher during the protein-enriched condition relative to the control condition, but there was no difference in slope.

### *Membrane damage, oxidative stress, cortisol, and cytokines*

Plasma CK activity, cortisol, and cytokine concentrations during the recovery from exercise on days 1 and 2, and in the morning before the 2 performance tests, are shown in

**Table 3.** Effect of the protein-enriched condition on plasma markers of stress, inflammation, and skeletal muscle membrane damage during the recovery periods and in the morning before the performance tests.

| | Diet condition | | Outcome | | |
|---|---|---|---|---|---|
| Biomolecule | Control ($SD_{\%}$) | Protein-enriched ($SD_{\%}$) | Mean effect[a]; ±95%CL[b] (%) | *p* value | Qualitative inference |
| **Day 1 recovery 90 min** | | | | | |
| Cortisol ($nmol \cdot L^{-1}$) | 243 (52) | 190 (52) | –28; ±40 | 0.23 | Unclear |
| IL-6 ($pg \cdot mL^{-1}$) | 4.06 (60) | 4.14 (51) | 2.0; ±52 | 0.95 | Unclear |
| TNF-α ($pg \cdot mL^{-1}$) | 1.25 (40) | 1.32 (35) | 5.6; ±19 | 0.61 | Unclear |
| **Day 2 AM Fasting** | | | | | |
| Cortisol ($nmol \cdot L^{-1}$) | 482 (36) | 397 (36) | –22; ±25 | 0.15 | Decrease possible |
| CK ($units \cdot L^{-1}$) | 155 (76) | 127 (56) | –20; ±37 | 0.30 | Decrease possible |
| CRP ($\mu g \cdot mL^{-1}$) | 0.96 (85) | 1.03 (88) | 7.0; ±70 | 0.83 | Unclear |
| IL-6 ($pg \cdot mL^{-1}$) | 0.70 (132) | 0.65 (199) | –8; ±54 | 0.77 | Unclear |
| TNF-α ($pg \cdot mL^{-1}$) | 1.35 (44) | 1.11 (54) | –21; ±20 | 0.055 | Decrease likely |
| **Day 2 recovery 90 min** | | | | | |
| Cortisol ($nmol \cdot L^{-1}$) | 496 (36) | 530 (36) | 7.0; ±23 | 0.57 | Unclear |
| CK ($units \cdot L^{-1}$) | 177 (84) | 142 (54) | –22; ±38 | 0.25 | Decrease possible |
| CRP ($\mu g \cdot mL^{-1}$) | 1.07 (93) | 0.84 (120) | –18; ±75 | 0.63 | Unclear |
| IL-6 ($pg \cdot mL^{-1}$) | 4.28 (92) | 2.56 (63) | –67; ±65 | 0.055 | Decrease likely |
| TNF-α ($pg \cdot mL^{-1}$) | 1.22 (51) | 1.27 (49) | 4.0; ±21 | 0.67 | Unclear |
| **Day 4 morning fasting** | | | | | |
| CK ($units \cdot L^{-1}$) | 148 (68) | 109 (46) | –35; ±38 | 0.086 | Decrease likely |
| CRP ($\mu g \cdot mL^{-1}$) | 0.58 (111) | 0.39 (120) | –48; ±79 | 0.23 | Decrease possible |
| IL-6 ($pg \cdot mL^{-1}$) | 0.56 (94) | 0.46 (92) | –22; ±56 | 0.44 | Unclear |
| TNF-α ($pg \cdot mL^{-1}$) | 1.07 (47) | 1.10 (55) | 3; ±20 | 0.77 | Unclear |
| **Overall average effect[c]** | | | | | |
| Cortisol ($nmol \cdot L^{-1}$) | 416 (51) | 374 (51) | –11; ±23 | 0.39 | Unclear |
| CK ($units \cdot L^{-1}$) | 158 (70) | 126 (100) | –26; ±30 | 0.16 | Decrease possible |
| CRP ($\mu g \cdot mL^{-1}$) | 0.82 (97) | 0.71 (110) | –17; ±61 | 0.57 | Unclear |
| IL-6 ($pg \cdot mL^{-1}$) | 1.56 (127) | 1.30 (113) | –20; ±26 | 0.098 | Possibly trivial |
| TNF-α ($pg \cdot mL^{-1}$) | 1.23 (45) | 1.21 (44) | –1.8; ±9.2 | 0.69 | Likely trivial |

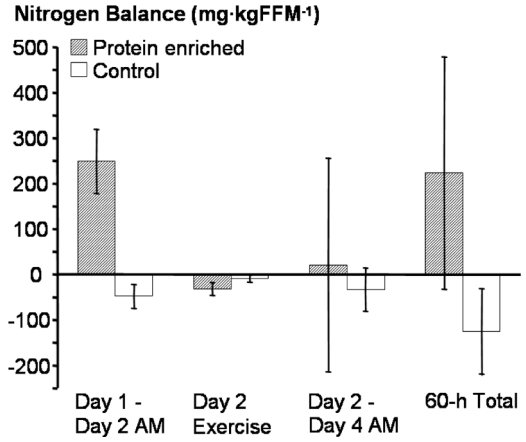
[a]Mean effect refers to the protein-enriched effect minus control.
[b]±95%CL: add and subtract this number to the mean effect to obtain the 95% confidence limits for the true difference.
[c]Overall average for the measured time points.

Table 3. During the 4 h recovery on day 1, the protein-enriched condition had no clear effect on cortisol or cytokines. On day 2 in the morning before exercise, TNF-α was reduced in the protein-enriched condition; however, the effects of treatment on these markers at the other time points were unclear or most likely trivial. Interleukin-6 was lower in the protein-enriched condition 90 min into the recovery on day 2, but at other time points it was not clearly different. There was a substantial reduction in CK activity on day 4 before exercise in the protein-enriched condition, but overall the reduction could only be inferred as possible. Overall there was no clear effect on CRP. Malonyl dealdehyde was measured on day 2 at the following time points: before exercise, at the completion of exercise, and 60 min into recovery. Although exercise increased MDA by 21 ± 36 $mg \cdot L^{-1}$ and 21 ± 24 $mg \cdot L^{-1}$ in the protein-enriched and control conditions, respectively, there was no clear effect of treatment at any time point and further investigation on oxidative-stress markers was abandoned.

### *Nitrogen balance*

Nitrogen balance is shown in Fig. 4. For the sampling period from day 1 post exercise to the morning of day 2, nitrogen balance was substantially positive in the protein-enriched condition, but negative in the control condition; as a result, the net difference between conditions was

**Fig. 4.** Effect of treatment on nitrogen balance. Data are means and standard deviations.



297 mg N·kg FFM$^{-1}$ (95%CI: 207–386). In contrast, there was no clear effect of treatment on nitrogen balance between conditions during exercise on day 2 and from day 2 to the morning of day 4, and the overall 60 h net gain in the protein-enriched condition relative to the net loss in the control condition was 349 mg N·kg FFM$^{-1}$ (191–507), which was mostly due to the overnight differential following exercise and recovery feeding on day 1.

## Discussion

The aim of our study was to determine if there is a protein-sensitive recovery feeding effect on subsequent high-intensity cycling performance 1 (15 h) and 3 days (60 h) after interval riding, relative to an isocaloric control. There was no difference in performance 15 h after the initial loading exercise. However, the interesting and novel finding of this research was that high-protein–carbohydrate (with lipid) recovery feeding substantially enhanced performance after 60 h. This delayed performance benefit was linked to substantially higher plasma lactate and perceptions of effort during the sprints and relative net positive nitrogen balance during recovery. Some inflammatory and muscle damage markers were lower at some time points, notably CK on day 4 when performance was improved, but on the whole we could find little firm evidence in support of an explanation for the delayed performance effect.

The present research was spurred by recent attention to the effects of ingesting commercially oriented protein and carbohydrate exercise and recovery beverages on the performance of subsequent exercise (Betts et al. 2005; Millard-Stafford et al. 2005; Niles et al. 2001; Saunders et al. 2004; Williams et al. 2003). Until now, investigation of the subsequent performance outcome has been restricted to the immediate 1.5–4 h or 12–15 h (overnight) after the initial bout of fatiguing exercise. In a study of recreational cyclists, Saunders et al. (2004) added 1.8% protein to a 7.3% carbohydrate solution during exhaustive cycling at 75% $VO_{2\ max}$ (82–102 min). From 0–30 min after exercise an additional 13 g protein was ingested with the control-level 54 g carbohydrate. The addition of protein to the carbohydrate solution improved endurance at 85%$VO_{2\ max}$ the following morning by 40%, which is roughly equivalent to an improvement in mean power of 3.0% ($\Delta$ mean power = $\Delta$ time$\left(\frac{6.4}{\text{power}}\right)$), where time is expressed as a percentage, and power is expressed as a percentage of $VO_{2\ max}$; Hopkins et al. 1999). The results implicate a role for protein, but the recovery energy provided by Saunders et al. (2004), 1076 kJ, was likely to have left the cyclists hungry and in a state of overnight negative energy and nitrogen balance, and there may have been insufficient carbohydrate for adequate glycogen restoration. Similarly, others have demonstrated improved same-day subsequent endurance performance with similar protein–carbohydrate exercise and recovery beverages vs. isocaloric (Niles et al. 2001) and non-isocaloric carbohydrate-only controls (Williams et al. 2003). Such improvements in subsequent performance were suggested as due to greater muscle-glycogen restoration secondary to a protein-induced relative hyperinsulinemia driving more glucose into the muscle (Ivy and Kuo 1998; Jentjens and Jeukendrup 2003); however, in the non-isocaloric designs, the performance benefit could be attributable to the additional energy provided in the protein-carbohydrate solutions and have little to do with protein.

In contrast, where protein–carbohydrate post-exercise beverages (Betts et al. 2005; Millard-Stafford et al. 2005) or protein–carbohydrate–lipid food and beverages (Rowlands et al. 2007) were compared relative to isocaloric quantities of carbohydrate or carbohydrate–lipid only, respectively, no clear effects of protein on subsequent performance were evident. In these studies, greater energy was provided post exercise. In the recovery drinks, Millard-Stafford et al. (2005) and Betts et al. (2005) provided 1.0 or 1.2 g·kg$^{-1}$·h$^{-1}$ carbohydrate, for 2 × 2 h and 4 h post-exercise, respectively, but observed no clear advantage in subsequent same-day or next-day run performance relative to isocaloric protein–carbohydrate beverages. Our cyclists did more absolute work under intermittent high-intensity loading conditions, so we provided carbohydrate during the 4 h recovery period at a higher rate (1.4 g·kg$^{-1}$·h$^{-1}$), which should have saturated glycogen resynthesis processes in the intervention condition, negating glycogen content as the principle explanation for a protein-induced performance effect. Additionally, in the present study, we provided the recommended high-carbohydrate recovery diet (8–10 g·kg$^{-1}$·d$^{-1}$) for endurance athletes to further ensure a high pre-exercise muscle-glycogen content in both the protein-enriched and control conditions (Ivy 2004). We also wanted to ensure that cyclists were provided with sufficient kilojoules to minimize deficit-induced metabolism. By saturating muscle-glycogen restoration in this way and by providing adequate energy, the design revealed the effect of protein on subsequent performance. Consequently, the present delayed performance benefit is consistent with a specifically protein-mediated effect. Nevertheless, enhanced carbohydrate metabolism should not be ruled out as a possible mechanism for the protein-mediated effect, as evidenced by the substantially higher blood lactate and glucose concentrations, and also higher perceived exertion in the protein-enriched condition on day 4 (Fig. 3; Table 2).

Although not clear cut, plasma CK activity trended lower (ES 0.37) in the protein-enriched condition during the intervention period implicating the possibility of mildly attenuated sarcoplasmic damage over the 60 h measurement period. Morning fasted CK activity was substantially (ES ~3.7) reduced in cyclists (Saunders et al. 2004) and a moderate effect (ES ~0.7) was possible in the plasma of runners with protein plus carbohydrate recovery feeding (Millard-Stafford et al. 2005). Similarly, with the addition of antioxidants, Romano-Ely et al. (2006) and Luden et al. (2007) found CK elevation to be substantially reduced with the addition of protein (ES 2.45 and ~0.25, respectively). Although we observed likely small- and moderate-sized reductions in TNF-$\alpha$ (ES 0.48) and IL-6 (ES 0.73) on day 2 before exercise and during recovery, respectively, the overall effect of high-protein–carbohydrate feeding during recovery from high-intensity cycling on systemic markers of stress, inflammation, and lipid peroxidation was of trivial or uncertain magnitude (Table 3). We could find no other published information on the effect of protein ingested post intermittent high-intensity endurance exercise on markers of cell stress or inflammation.

Previous workers in this field have used sub-maximal, constant-load, time-to-exhaustion cycle or run tests to measure performance. Although reliable (Hopkins et al. 2001*b*), these tests arguably do not best simulate the intermittent high-intensity nature of competitive endurance cycling (and many other sports) and for this reason we used a repeated maximal-effort constant-work sprint procedure. A second reason was that although also likely limited by glycogen, performance in the repeated-sprint test is a measure of the capacity of the muscle to produce power, whereas performance in sub-maximal exhaustion tests is a measure of endurance capability most likely limited by carbohydrate availability. The disparate physiology relating to test selection between our studies and others in this area (most others used exhaustion tests) may be a factor in our observed performance effect; however, it appears more likely to be due to the delayed time course because we also observed no clear evidence for a protein plus carbohydrate benefit to performance measured at 12–15 h post prior exhausting exercise.

The quantity of protein provided in the protein-enriched condition was intended to saturate protein synthesis processes during recovery, and therefore maximize this specific nutrient effect on the cellular response to exercise. Information is limited and based mostly on resistance exercise in untrained people, but maximal net muscle protein synthesis in response to single or double post-exercise feedings may occur with the ingestion of somewhere between 6 and 20 g of essential amino acids, and is greater with the co-ingestion of carbohydrate (Biolo et al. 1999; Borsheim et al. 2002; Miller et al. 2003; Tipton et al. 1999). Leucine is the most important amino acid stimulating protein synthesis (Kimball and Jefferson 2004). Hence, a sustained high leucine signal may promote a greater translation of new exercise response mRNA transcripts during recovery, compared with a low leucine signal. The milk proteins selected for the present study were high in leucine relative to other proteins, and essential amino acids comprised approximately half of the ingested protein. Therefore, cyclists were ingesting on average about 25 g of essential amino acids (~5 g leucine) per hour in the protein-enriched condition, but about 4 g (~0.8 g leucine) in the control condition.

Finally, the nitrogen-balance outcome may have some implications for nutritional strategies for recovery from prolonged high-intensity endurance exercise. We cannot determine the fate of the nitrogen retained in the protein-enriched condition, but the average 50 mg·kg $FFM^{-1}$ net loss in the control condition suggests that the 30 g (0.4 $g \cdot kg^{-1}$) of protein ingested in the 4 h following exercise on day 1 was insufficient to meet nitrogen requirements for the overnight recovery period. This difference carried through over the 4 day experimental period to an estimated average 54 g of protein lost in the control condition, compared with a net 95 g gain in the protein-enriched condition. Chronic high-protein diets substantially increase protein oxidation (Bowtell et al. 1998) but this effect, and a reduction in the post-exercise mixed-muscle protein fractional synthetic rate (Bolster et al. 2005), seems unlikely to have occurred in the present limited post-exercise exposure. Although a correction for sweat, feces, and other nitrogen losses was added, nitrogen loss in the control condition may have been greater because the nitrogen balance method tends to underestimate nitrogen excretion either by lack of complete sample collection or through unmeasured losses (skin desquamation, hair, nails, mucous, semen). We measured only urinary nitrogen loss, which accounts for >75% of total loss (Tarnopolsky et al. 1988), so 25% of our loss estimate was subject to unknown error, although an error likely to be similar within subjects making the observed treatment effect plausible. The average protein intake in the standardized diet was 50% above the RDI (0.8 $g \cdot kg^{-1} \cdot d^{-1}$); combined with the control recovery feeding; however, it appeared insufficient to maintain protein balance and imposed an inferior performance outcome relative to the conditions provided by the protein-enriched recovery feeding condition. The literature range for average daily protein intake recommended for endurance athletes is 1.2–1.7 $g \cdot kg^{-1} \cdot d^{-1}$ (Tarnopolsky et al. 1988; Tipton and Wolfe 2004) and the protein-enriched condition overshot this; as a comparison, endurance cyclists in competition and training habitually consume more than recommend levels. For example, during a 3-week tour, cyclists consumed around 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ in a diet comprising 14.5% energy from protein and 23.5 $MJ \cdot d^{-1}$ (Garcia-Roves et al. 1998) — the average for our present sample was 1.9 $g \cdot kg^{-1} \cdot d^{-1}$. Further research is required to determine the recovery and performance responses to dietary protein and carbohydrate dose under high-intensity competition or heavy training loads. Sport nutritionists and others also do not adequately understand the optimal competition- and training-load calibrated quantity of protein required in the peri-training period to stimulate optimal training adaptations.

In conclusion, the ingestion of a protein-enriched, high-carbohydrate, mixed diet for 2 days during the 4 h recovery period after high-intensity cycling provided a substantial enhancement to subsequent performance 60 h after the first bout of interval cycling. The ergogenic effect was linked to a positive nitrogen balance during recovery on day 1, but the specific nutrient-mediated effect appears delayed and the mechanisms yet to be determined.

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## References

Belcastro, A.N., Shewchuk, L.D., and Raj, D.A. 1998. Exercise-induced muscle injury; a calpain hypothesis. Mol. Cell. Biochem. **179**: 135–145. doi:10.1023/A:1006816123601. PMID:9543356.

Batterham, A.M., and Hopkins, W.G. 2006. Making meaningful inferences about magnitudes. Int. J. Sport. Physiol. Perf. **1**: 50–57.

Betts, J.A., Stevenson, E., Williams, C., Sheppard, C., Grey, E., and Griffin, J. 2005. Recovery of endurance running capacity: effect of carbohydrate-protein mixtures. Int. J. Sport Nutr. Exerc. Metab. **15**: 590–609. PMID:16521845.

Biolo, G., Williams, B.D., Fleming, R.Y., and Wolfe, R.R. 1999. Insulin action on muscle protein kinetics and amino acid trans-

port during recovery after resistance exercise. Diabetes, **48**: 949–957. doi:10.2337/diabetes.48.5.949. PMID:10331397.

Bolster, D.R., Pikosky, M.A., Gaine, P.C., Martin, W., Wolfe, R.R., Tipton, K.D., et al. 2005. Dietary protein intake impacts human skeletal muscle protein fractional synthetic rates after endurance exercise. Am. J. Physiol. Endocrinol. Metab. **289**: E678–E683. doi:10.1152/ajpendo.00060.2005. PMID:15914508.

Borsheim, E., Tipton, K.D., Wolf, S.E., and Wolfe, R.R. 2002. Essential amino acids and muscle protein recovery from resistance exercise. Am. J. Physiol. Endocrinol. Metab. **283**: E648–E657. PMID:12217881.

Bowtell, J.L., Leese, G.P., Smith, K., Watt, P.W., Nevill, A., Rooyackers, O., Wagenmakers, A.J.M., and Rennie, M.J. 1998. Modulation of whole-body protein metabolism, during and after exercise, by variation of dietary protein. J. Appl. Physiol. **85**: 1744–1752. PMID:9804577.

Burke, L.M., Kiens, B., and Ivy, J. 2004. Carbohydrates and fat for training and recovery. J. Sports Sci. **22**: 15–30. doi:10.1080/02640410310001405 27. PMID:14971430.

Garcia-Roves, P.M., Terrados, N., Fernandez, S.F., and Patterson, A.M. 1998. Macronutrients intake of top level cyclists during continuous competition–change in the feeding pattern. Int. J. Sports Med. **19**: 61–67. doi:10.1055/s-2007-971882. PMID: 9506803.

Goodyear, L.J., Hirshman, M.F., King, P.A., Horton, E.D., Thompson, C.M., and Horton, E.S. 1990. Skeletal muscle plasma membrane glucose transport and glucose transporters after exercise. J. Appl. Physiol. **68**: 193–198. PMID:2312459.

Hopkins, W.G. 2002. Effect statistics: a scale of magnitudes for effect statistics. [online] Available from http://www.sportsci.org/resource/stats/effectmag.html [Accessed on 13 March 2007].

Hopkins, W.G., Hawley, J.A., and Burke, L.M. 1999. Design and analysis of research on sport performance enhancement. Med. Sci. Sports Exerc. **31**: 472–485. doi:10.1097/00005768-199903000-00018. PMID:10188754.

Hopkins, W.G. 2001a. Calculating likely (confidence) limits and likelihoods for true values (Excel spreadsheet). *In* A new view of statistics [on-line]. Available from www.sportsci.org/resource/stats/xcl.xls [Accessed March 2007].

Hopkins, W.G., Schabort, E.J., and Hawley, J.A. 2001*b*. Reliability of power in physical performance tests. Sports Med. **31**: 211–234. doi:10.2165/00007256-200131030-00005. PMID:11286357.

Ivy, J.L. 2004. Regulation of muscle glycogen repletion, muscle protein synthesis and repair following exercise. J. Sports Sci. Med. **3**: 131–138.

Ivy, J.L., Goforth, H.W., Jr., Damon, B.M., McCauley, T.R., Parsons, E.C., and Price, T.B. 2002. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J. Appl. Physiol. **93**: 1337–1344. PMID:12235033.

Ivy, J.L., and Kuo, C.H. 1998. Regulation of glut4 protein and glycogen synthase during muscle glycogen synthesis after exercise. Acta Physiol. Scand. **162**: 295–304. doi:10.1046/j.1365-201X.1998.0302e.x. PMID:9578375.

Jentjens, R., and Jeukendrup, A. 2003. Determinants of post-exercise glycogen synthesis during short-term recovery. Sports Med. **33**: 117–144. doi:10.2165/00007256-200333020-00004. PMID: 12617691.

Jentjens, R.L., van Loon, L.J., Mann, C.H., Wagenmakers, A.J., and Jeukendrup, A.E. 2001. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. J. Appl. Physiol. **91**: 839–846. PMID:11457801.

Kimball, S.R., and Jefferson, L.S. 2004. Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. Biochem. Biophys. Res. Commun. **313**: 423–427. doi:10.1016/j.bbrc.2003.07.014. PMID: 14684179.

Levenhagen, D.K., Carr, C., Carlson, M.G., Maron, D.J., Borel, M.J., and Flakoll, P.J. 2002. Postexercise protein intake enhances whole-body and leg protein accretion in humans. Med. Sci. Sports Exerc. **34**: 828–837. doi:10.1097/00005768-200205000-00016. PMID:11984302.

Levenhagen, D.K., Gresham, J.D., Carlson, M.G., Maron, D.J., Borel, M.J., and Flakoll, P.J. 2001. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am. J. Physiol. Endocrinol. Metab. **280**: E982–E983.

Luden, N.D., Saunders, M.J., and Todd, M.K. 2007. Postexercise carbohydrate–protein– antioxidant ingestion decreases plasma creatine kinase and muscle soreness. Int. J. Sport Nutr. Exer. Metab. **17**: 109–123.

Millard-Stafford, M., Warren, G.L., Thomas, L.M., Doyle, J.A., Snow, T., and Hitchcock, K. 2005. Recovery from run training: efficacy of a carbohydrate–protein beverage? Int. J. Sport Nutr. Exer. Metab. **15**: 610–624.

Miller, S.L., Tipton, K.D., Chinkes, D.L., Wolf, S.E., and Wolfe, R.R. 2003. Independent and combined effects of amino acids and glucose after resistance exercise. Med. Sci. Sports Exerc. **35**: 449–455. doi:10.1249/01.MSS.0000053910.63105.45. PMID:12618575.

Moseley, L., Achten, J., Martin, J.C., and Jeukendrup, A.E. 2004. No differences in cycling efficiency between world-class and recreational cyclists. Int. J. Sports Med. **25**: 374–379. doi:10.1055/s-2004-815848. PMID:15241718.

Nevill, A., and Lane, A. 2007. Why self-report ''Likert'' scale data should not be log-transformed. J. Sports Sci. **25**: 1–2. doi:10.1080/02640410601111183. PMID:17127576.

Niles, G.S., Lachowetz, T., Garfi, J., Sullivan, W., Smith, J.C., Leyh, B.P., and Headley, S.A. 2001. Carbohydrate-protein drink improves time to exhaustion after recovery from endurance exercise. J. Exerc. Physiol. **4**: 45–52.

Norton, K., and Olds, T. 1996. Antrhopometrica — a textbook of body measurement for sports and health courses. University of New South Wales Press, Sydney, Australia.

Romano-Ely, B.C., Todd, M.K., Saunders, M.J., and St.Laurent, T. 2006. Effect of an isocaloric carbohydrate–protein–antioxidant drink on cycling performance. Med. Sci. Sports Exerc. **38**: 1608–1616. doi:10.1249/01.mss.0000229458.11452.e9. PMID: 16960522.

Rowlands, D.S., Thorp, R.M., Rossler, K., Graham, D., and Rockell, M. 2007. Effect of protein-rich feeding on recovery following intense exercise. Int. J. Sport Nutr. Exer. Metab. **17**: 541–543.

Saunders, M.J., Kane, M.D., and Todd, K.M. 2004. Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. Med. Sci. Sport Exerc. **36**: 1233–1238. doi:10.1249/01.MSS.0000132377.66177.9F.

Siri, W.E. 1956. The gross composition of the body. *In* Advances in biological and medical physics. *Edited by* J.H. Lawrence and C.A. Tobias. Academic Press, New York, N.Y. pp. 239–280.

Sterne, J.A.C., and Smith, G.D. 2001. Sifting the evidence — what's wrong with significance tests? BMJ, **322**: 226–261. doi:10.1136/bmj.322.7280.226. PMID:11159626.

Tarnopolsky, M.A., MacDougall, J.D., and Atkinson, S.A. 1988. Influence of protein intake and training status on nitrogen balance and lean body mass. J. Appl. Physiol. **64**: 187–193. PMID:3356636.

Thorburn, M.S., Vistisen, B., Thorp, R.M., Rockell, M., Jeukendrup, A.E., Xuebing, X., and Rowlands, D.S. 2006. Attenuated gastric tolerance but no benefit to performance with

adaptation to octanoate-rich esterified oils in well-trained male cyclists. J. Appl. Physiol. **101**: 1733–1743. doi:10.1152/japplphysiol.00393.2006. PMID:16840580.

Tidball, J.G. 1995. Inflammatory cell response to acute muscle injury. Med. Sci. Sport Exerc. **27**: 1022–1032. doi:10.1249/00005768-199507000-00011.

Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D., Jr., and Wolfe, R.R. 1999. Post-exercise net protein synthesis in human muscle from orally administered amino acids. Am. J. Physiol. **276**: E628–E634. PMID:10198297.

Tipton, K.D., and Wolfe, R.R. 2004. Protein and amino acids for athletes. J. Sports Sci. **22**: 65–79. doi:10.1080/0264041031000140554. PMID:14971434.

van Loon, L.J.C., Schrauwen-Hinderling, V.B., Koopman, R., Wagenmakers, A.J.M., Hesselink, M.K.C., Schaart, G., Kooi, M.E., and Saris, W.H.M. 2003. Influence of prolonged endurance cycling and recovery diet on intramuscular triglyceride content in trained males. Am. J. Physiol. Endocrinol. Metab. **285**: E804–E811.

Williams, M.B., Raven, P.B., Fogt, D.L., and Ivy, J.L. 2003. Effects of recovery beverages on glycogen restoration and endurance exercise performance. J. Strength Cond. Res. **17**: 12–19. doi:10.1519/1533-4287(2003)017<0012:EORBOG>2.0.CO;2. PMID:12580650.