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Carbohydrate-Restricted Exercise With Protein Increases Self-Selected Training Intensity in Female Cyclists but Not Male Runners and Cyclists

Tanja Oosthuyse,[1] Gabriella E. Florence,[2] Arron Correia,[2] Camilla Smyth,[2] and Andrew N. Bosch[2]

[1]School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Medical School, Johannesburg, South Africa; and [2]Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town, South Africa

Abstract

Oosthuyse, T, Florence, GE, Correia, A, Smyth, C, and Bosch, AN. Carbohydrate-restricted exercise with protein increases self-selected training intensity in female cyclists but not male runners and cyclists. *J Strength Cond Res* 35(6): 1547–1558, 2021—Carbohydrate-restricted training challenges preservation of euglycemia and exercise intensity that precludes ergogenic gains, necessitating countering strategies. We investigated the efficacy of ingesting casein protein hydrolysate in overnight-fasted male runners, male cyclists, and female cyclists. Twenty-four overnight-fasted athletes ingested 15.8 $g \cdot h^{-1}$ casein hydrolysate or placebo-water during exercise (60–80 minutes) comprising an incremental test to exhaustion, steady-state exercise (70% Vmax or 60% peak power output, 87 ± 4% HRmax), and 20-minute time trial (TT) in a double-blind randomized crossover design, with $p < 0.05$ accepted as significant. Ingesting protein vs. placebo increased metabolic demand {oxygen consumption, +4.7% (95% confidence interval [CI] ± 4%), $p = 0.0297$; +3.2% (95% CI ± 3.4%), $p = 0.061$}, heart rate ($p = 0.0083$; $p = 0.007$) and rating of perceived exertion (RPE) ($p = 0.0266$; $p = 0.0163$) in male cyclists and runners, respectively, but not female cyclists. Protein vs. placebo increased carbohydrate oxidation (+0.26 [95% CI ± 0.13] $g \cdot min^{-1}$, $p = 0.0007$) in female cyclists alone. Cyclists reported +2 ± 1 higher RPE than runners ($p = 0.0062$). Glycemia was maintained only in runners and increased with protein vs. placebo after 20 minutes of steady-state exercise (+0.63 [95% CI ± 0.56] $mmol \cdot L^{-1}$, $p = 0.0285$). TT performance with protein vs. placebo ingestion was modestly compromised in runners (−2.8% [95% CI ± 2.2%], $p = 0.0018$), unchanged in male cyclists (+1.9% [95% CI ± 5.6%], $p = 0.5794$), and modestly improved in female cyclists (+2.5% [95% CI ± 1.8%], $p = 0.0164$). Casein hydrolysate ingestion during moderate to hard carbohydrate-restricted exercise increases glycemia in runners, but not cyclists. Casein hydrolysate increases metabolic demand in male athletes and carbohydrate oxidation in female cyclists and is suitable for improving carbohydrate-restricted training intensity in female but not male endurance athletes.

Key Words: carbohydrate-restricted training, euglycemia, protein supplement, exercise performance, sex-differences, sport differences

Introduction

Overnight-fasted training is routinely practiced by amateur athletes who train in the early mornings before working hours and is also the simplest model of periodized carbohydrate-restricted training cliched, "training low" purposely practiced by elite athletes (2). Periodized "training low" with critically low muscle glycogen content is widely advocated for endurance athletes to achieve a maximal metabolic signaling response that enhances beneficial cellular training adaptations (2). Recent evidence shows that muscle glycogen depletion to ~300 $mmol \cdot kg \cdot dw^{-1}$, which is typically achieved during overnight-fasted training (9,42), is sufficient to produce the same metabolic signaling stimulus as when training with more extreme muscle glycogen depletion (<200 or <100 $mmol \cdot kg^{-1}$ dw) (17,18). Despite cellular metabolic gains with periodized carbohydrate-restricted training, it has become apparent that corresponding performance gains are not realized (43,45) without a concerted effort to ensure that training intensity is not compromised (29). Critically low muscle glycogen stores may provide direct peripheral sensory feedback to regulate perceived exertion and exercise intensity (27). However, waning blood glucose concentrations that occur during carbohydrate-restricted training (43) likely also contribute toward the increased perceived exertion and reduced training intensity/capacity typically reported (18,20,45) and that jeopardizes ergogenic gains even in the less severe overnight-fasted model (43). Therefore, strategies to promote maintenance of euglycemia without carbohydrate replacement during overnight-fasted training should be considered.

During carbohydrate-restricted training, euglycemia is maintained by hepatic glycogenolysis and gluconeogenesis (11). In an overnight-fasted state, gluconeogenesis becomes increasingly important as exercise duration persists and is fueled by lactate, glycerol derived from lipolysis, and amino acids from proteolysis. For this reason, hepatic glucose production during frequent overnight-fasted training may lead to unwanted loss of muscle mass with carbohydrate-restricted training and therefore increases protein requirements of endurance athletes (14). Provision of an exogenous gluconeogenic source during fasted exercise increases the rate of hepatic glucose production by upregulating gluconeogenesis while decreasing hepatic glycogenolysis (11) and is expected to spare endogenous protein stores (19). Previously, ingesting a quickly absorbed protein supplement (casein protein hydrolysate) before and during exercise was found to not compromise the enhanced cellular

Address correspondence to Dr. Tanja Oosthuyse, oosthuyse@polka.co.za.

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signaling or metabolic response of carbohydrate-restricted training (41). Casein hydrolysate provides all essential amino acids of which 18 are glucogenic and 6 ketogenic (39). Certain amino acids, namely alanine, arginine, cysteine, glycine, lysine, and proline, stimulate glucagon secretion from pancreatic alpha cells (13). Glucagon binds hepatic glucagon receptors increasing hepatic uptake of amino acids (13) and stimulates a hepatic signaling cascade essential to promote gluconeogenic flux (34). For this reason, provision of casein hydrolysate during carbohydrate-restricted exercise is expected to support hepatic glucose production and maintenance of euglycemia without compromising the low muscle glycogen state required for enhancing metabolic signaling in response to fasted exercise. In the previous study that used a more extreme "sleep low-train low" carbohydrate-restriction model and where the ingested casein hydrolysate dose during exercise was low (10 $g \cdot h^{-1}$ during 50% of peak power output [PPO]), glycemia was not maintained, and subsequent high-intensity exercise capacity did not improve when ingesting protein compared with placebo (41). Likewise, ingesting 11 $g \cdot h^{-1}$ dose of whey isolate (split into 30-minute aliquots) compared with placebo during fasted exercise (2 hours, 60% PPO) did not alter metabolism or glycemia (21). We hypothesize that ingesting a higher dose of rapidly absorbed casein hydrolysate in a greater aliquot frequency during the less extreme overnight-fasted model may provide a more adequate exogenous glucogenic substrate to support maintenance of euglycemia during moderate to high intensity exercise and thereby reduce perceived exertion and rescue self-selected training intensity.

Previous studies evaluating the acute effect of the "train low" overnight-fasted model have used mostly cycling exercise in male athletes (9,21,43). Cycling recruits a smaller active muscle mass for a given metabolic demand (or oxygen consumption) than running (7). For this reason, when cycling and running are matched for whole-body relative exercise intensity, cycling places a higher demand on carbohydrate utilization than running (6,24). Similarly, in women, the ovarian hormones alter substrate partitioning in favor of greater fat utilization at any given relative exercise intensity (8,24) and may suppress hepatic glucose production (10) compared with men. Thus, prescribed training or supplement strategies based on findings in male cyclists can possibly not be extrapolated to running or female athletes.

Therefore, the current study evaluated whether ingesting a typical dose of casein protein hydrolysate (15.8 $g \cdot h^{-1}$ in 10–15 minutes aliquots) during moderate to hard overnight-fasted exercise better supports euglycemia, reduces rating of perceived exertion (RPE), and increases self-selected training intensity compared with ingesting placebo-water in male cyclists, male runners, and female cyclists.

Methods

Experimental Approach to the Problem

This double-blind randomized crossover design study included 3 subject groups: male cyclists, male runners, and female cyclists. Each subject completed 2 trials, once with the experimental casein hydrolyzate beverage and once with the placebo-water beverage, in a randomized order and after a 10–12 hours overnight fast. For male athletes, trials were spaced 7 days apart to match typical research practice in male athletes. For female athletes, trials were spaced 2–4 days apart to limit large fluctuations in ovarian hormones between trials as previously applied (16) and corresponded with the most stable ovarian hormone phases, namely midfollicular phase (days 5–10) for non-oral contraceptive (non-OC) users and active pill phase for OC users. The exercise protocol matched the routine training loads of the respective athlete groups and thereby ensured that the time span between trials would be sufficient to achieve full recovery. Diet was replicated for 24 hours before, and exercise training replicated for 2–7 days before each trial. Each cycling trial consisted of a PPO test, 3 × 15-minute steady-state intervals at 60% PPO and 20-minute time trial (TT), performed in continuous succession. Each running trial consisted of a maximum running speed test (Vmax), 3 × 10-minute steady-state intervals at 70% Vmax, and 20-minute TT performed in continuous succession. The cycling and running protocols were designed to be matched for training load and intensity (Figure 1). Cycling at a given percent of PPO is linearly related to a percent of $\dot{V}O_2max$, such that 60% PPO typically equates to ~70% $\dot{V}O_2max$ (21,32), and running at a speed relative to a percent of Vmax, in this instance 70% Vmax, equates to the same speed when at 70% $\dot{V}O_2max$ (28). The running duration (~60 minutes) was modestly less than the cycling duration (~80 minutes) to approximate typical daily training durations in these athlete groups.

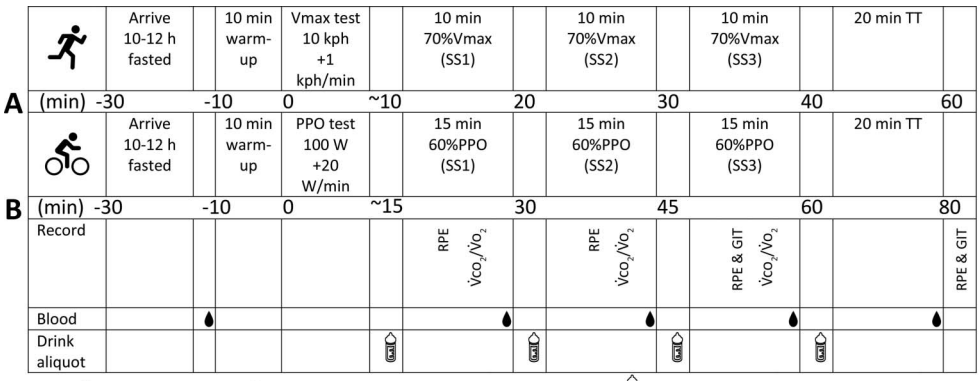
Subjects

Twenty-four young, healthy well-trained athletes aged between 18 and 49 years old completed all parts of the study (Table 1). The athletes were recruited from local sports clubs with the following minimum inclusion requirements: body mass index <26 $kg \cdot m^{-2}$, followed a mixed diet including all macronutrients, nonsmoker, not taking medication, and cycled ≥4 sessions/week (≥1.5 h/session) or ran ≥4 sessions/week (≥10 km/session). In addition, female cyclists were included if they reported experiencing regular menstrual cycles (between 25 and 35 days); both OC users and nonusers were included. The OC users (n = 4) used monophasic OCs comprising ethinyl estrogen (0.02–0.035 $mg \cdot d^{-1}$) and progestins with low androgenicity (drospirenone 3 $mg \cdot d^{-1}$ or cyproterone acetate 2 $mg \cdot d^{-1}$). One female athlete had an intrauterine device with slow-releasing progestin (levonorgestrel 0.02 $mg \cdot d^{-1}$). The remaining n = 3 female athletes experienced natural eumenorrheic cycles, 27–28 days long. The study protocol was conducted in accordance with the Declaration of Helsinki and was granted ethical clearance by the University of Cape Town human research ethics committee (medical) (547/2018). All athletes signed written informed consent to participate after the study protocol and risks were explained.

Procedures

Pretrial Controls. Athletes recorded their training for 7 days (men) or 2–4 days (women) before their first trial on a tabulated form and replicated this training routine before their second trial and submitted recorded evidence of their compliance. They were instructed to perform no exercise or only low-intensity exercise the day before their trials. Athletes recorded all their meals and beverages ingested the day before their first trial on a tabulated form that provided instructions on describing food and beverage type, quantity, and timing. They then replicated this diet record the day before their second trial and again submitted diet records as evidence of their compliance. Athletes were instructed to ingest their final meal before 20:00 hour and after that were only permitted to drink water as needed.

Experimental Trials. Athletes arrived at the laboratory at the same prescheduled time for both trials between 06:30 hours and 08:30 hours after a 10–12 hours overnight fast (Figure 1). Height, body mass, and sum of 4 skinfolds (bicep, tricep, subscapular, and



Key: Running timeline · Cycling timeline · Capillary blood sample · Drinking aliquot (runners-150 mL; cyclists-200 mL) · Vmax Maximum running speed · PPO Peak power output · SS Steady-state interval · TT Time trial · RPE Rating of perceived exertion · $\dot{V}co_2/\dot{V}o_2$ Indirect calorimetry recording · GIT Rating of gastrointestinal discomfort

Figure 1. Timeline for the running (A) and cycling (B) experimental trials that were completed twice by each runner and cyclist, respectively, in a randomized double-blind order, once ingesting placebo-water and once ingesting the casein protein hydrolysate drink.

suprailiac) were measured, with athletes wearing only cycling or running shorts and sports brassiere (for female cyclists). Before a finger was cleaned with an alcohol swab and capillary blood sample obtained using a softclix needle-pen, athletes rested while seated for 20 minutes. A free-flowing blood droplet was drawn up by capillary suction into glucose and ketone test strips and analyzed for blood glucose and β-hydroxybutyrate (β-OHB) concentrations, respectively (Freestyle Optium Neo; Abbott Diabetes Care, Inc., Oxon, United Kingdom). The accuracy of the glucose and β-OHB automated analyzer has been validated and has high intraclass correlations with laboratory reference methods (r = 0.98, slope = 0.99, intercept = 0.05 mmol·L^{-1} and r = 0.98, slope = 1.06, intercept = 0.07 mmol·L^{-1}) and interassay coefficient of variabilities of 3.4 and 3.8% for glucose and β-OHB, respectively. Athletes were fitted with a chest strap (TickrX, Wahoo Fitness, Atlanta, GA) that recorded heart rate (HR) continuously. Athletes warmed up at a self-selected low intensity for 10 minutes. Laboratory ambient conditions were maintained constant by air conditioning (21 ± 1 ° C, 66% ± 4% relative humidity), and athletes were cooled by a fan set at a constant speed and position.

Cycling Protocol. Cyclists cycled on their own bicycles fitted to a calibrated stationary ergometer (Kickr 4; Wahoo Fitness). The recorded data were analyzed using fitness training software (PerfPro Analyzer; Hartware Technologies, Rockford, IL). All cyclists were accustomed to training using this ergometer. Cadence was continuously displayed and recorded by a sensor (Cadence) secured to the cyclists' shoe. Cyclists were instructed to maintain a pedaling cadence between 70 and 120 rpm for the entire trial.

Peak Power Output Test. Immediately after the warm-up, the cyclists commenced a continuous step incremental PPO test that was included to impose an initial maximal effort to initiate rapid mobilization of hepatic glycogen stores. The PPO test began at 100 W

Table 1
Subject characteristics.*†

	Runners	Male cyclists	Female cyclists
Sample size (n)	8	8	8
Age (y)	30.5 ± 9.3	26.6 ± 8.2	29.8 ± 11.2
Body mass (kg)	69.9 ± 7.8	71.3 ± 6.3	60.8 ± 8.1‡
Height (cm)	174 ± 4	180 ± 6	169 ± 8§
BMI (kg·m^{-2})	23.1 ± 2.6	22.1 ± 1.1	21.2 ± 1.5
Sum of 4 skinfolds (mm)	33.7 ± 8.6	25.3 ± 4.3	31.5 ± 7.2
HRmax (b·min^{-1})	189 ± 11	183 ± 9	180 ± 11
Vmax (km·h^{-1})	17.9 ± 1.6	—	—
70%Vmax (km·h^{-1})	12.6 ± 1.2	—	—
PPO (W·kg^{-1})	—	4.5 ± 0.7	4.1 ± 0.6
60% PPO (W·kg^{-1})	—	2.7 ± 0.4	2.4 ± 0.3
60% PPO (W)	—	193 ± 36	147 ± 12§
SS intensity (%critical speed or power)	97 ± 10	96 ± 19	92 ± 6
Training history (y)	8.9 ± 5.2	11.9 ± 6.3	7.3 ± 6.1
Weekly training (h·wk^{-1})	5.7 ± 0.6§‖	8.9 ± 2.6	10.6 ± 2.1

*BMI = body mass index; HRmax = maximum heart rate; Vmax = maximum running speed; PPO = peak power output; SS = steady-state interval.
†Data are mean ± *SD*.
‡Significantly different to male cyclists, $p < 0.05$.
§Significantly different to male cyclists, $p < 0.01$.
‖Significantly different to female cyclists, $p < 0.001$.

Table 2
Amino acid profile of the casein hydrolysate (g/100 g protein).

Amino acid	g/100 g of protein‡
Alanine*	2.7
Arginine*	3.5
Aspartic acid/asparagine*	6.1
Cysteine*	0.6
Glutamic acid/glutamine*	20.5
Glycine*	2.1
Histidine*	3.0
Isoleucine*†	4.8
Leucine†	8.9
Lysine†	7.7
Methionine*	3.1
Phenylalanine*†	5.0
Proline*	9.6
Serine*	5.4
Threonine*	4.0
Tryptophan*†	1.3
Tyrosine*†	5.5
Valine*	6.2
Glucogenic only	66.8
Ketogenic only	16.7
Glucogenic and ketogenic	16.6

*Glucogenic amino acid.
†Ketogenic amino acid.
‡Values derived from technical information published by the casein protein hydrolyzate supplier (39).

increased in 20-W increments per minute until volitional exhaustion or until the cyclist was unable to maintain a pedaling cadence of >70 rpm. PPO was defined as the power output of the last completed workload plus the fraction of time completed in the final workload multiplied by the 20-W workload increment. In the second trial, the PPO test was terminated at the same time point as occurred in the first test. Immediately after the PPO test, the cyclists ingested a 200-ml bolus of the supplied test beverage and then commenced the next steady-state exercise stage of the trial.

Steady-State Cycling. Immediately after the PPO test, the cycling ergometer was set at 60% PPO for 45 minutes of steady-state exercise as 3 × 15-minute intervals (SS1, SS2, and SS3) for metabolic measurements. In the last 5 minutes of each interval, cyclists were asked to rank their RPE using the 6–20 Borg scale (3). Indirect calorimetry measurements were recorded in the last 3 minutes of each interval with athletes breathing through a one-way valve facemask (Hans Rudolph, Shawnee, Kansas) that allows inspiration of ambient air while expired air is passed through a metabolic analyzer (Quark CPET; COSMED, Rome, Italy) for breath-by-breath recording of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). The metabolic analyzer was calibrated before each trial using a 3-L syringe for volume and flow rate calibration and using room air plus a mixture of known gas composition (16% oxygen and 5% carbon dioxide) for gas sensor calibration. At the end of each 15-minute interval, blood glucose and β-OHB concentrations were immediately measured as previously described and then the workload was reduced by 100 W for a brief 30-second period while a further 200-ml bolus of the supplied test drink was ingested before commencing the next 15-minute interval at 60% PPO. The brief 30 seconds pause between intervals was used to match the steady-state running routine described below. After the final 15-minute interval, cyclists were asked to rank their perceived gastrointestinal discomfort for symptoms of thirst, bloating, stomach cramps, and nausea using a 0–10-point scale as previously used (32). The cyclists then immediately commenced a 20-minute TT as a measure of self-selected training intensity.

Cycling Time Trial. The cycling ergometer was set to linear mode, where the cyclists determined their own cycling intensity and instantaneous power output by gear ratio selection and altering their cadence and applied pedal force. Cyclists were instructed to perform the 20-minute TT to the best of their ability with the goal of completing as much total work and distance possible, without further verbal encouragement. Elapsed time and instantaneous power and cadence were visible, but speed, distance, HR, and average power were concealed. Immediately on completion of the TT, final blood glucose and β-OHB concentrations were measured and cyclists were asked to rank RPE and gastrointestinal discomfort that were perceived during the TT.

Running Protocol. Runners ran on a treadmill (h/p/cosmos sports & medical gmbh, Traunstein, Germany) and were all familiar with training and pacing on a treadmill. After a 10-minute warm-up at a self-selected running pace, the runners immediately commenced a maximum running speed (Vmax) test.

Maximum Running Speed Test. The Vmax test was a continuous step incremental test to volitional exhaustion. The treadmill was set at 10 $km \cdot h^{-1}$ (0% gradient) for the first minute and then the speed was increased in 1 $km \cdot h^{-1}$ increments every minute until volitional exhaustion. Vmax was described as the last completed running speed plus the fraction of time completed at the final speed multiplied by the step increment (1 $km \cdot h^{-1}$). In the second trial, the Vmax test was terminated at the same time point, as occurred in the first test. Immediately after the Vmax test, the runners ingested a 150-ml bolus of the supplied test beverage and then commenced the next steady-state exercise stage of the trial.

Steady-State Running. The treadmill speed was set at 70% Vmax for 30 minutes of steady-state exercise as 3 × 10-minute intervals (SS1, SS2, and SS3) for metabolic measurements. In the last 5 minutes of each interval, runners were asked to rank their RPE using the 6–20 Borg scale (3). Indirect calorimetry measurements were recorded in the last 3 minutes of each interval with athletes breathing through a one-way valve facemask, as described in the cycling protocol. At the end of each 10-minute interval, the runners jumped to the side of the treadmill belt for a short 30-second pause period during which blood glucose and β-OHB concentrations were immediately measured and a further 150-ml bolus of the supplied test drink was ingested before the runners resumed with the next interval. After the final 10-minute interval, the runners were asked to rank their perceived gastrointestinal discomfort, as described in the cycling protocol. The runners then immediately commenced a 20-minute TT as a measure of self-selected training intensity.

Running Time Trial. Runners were instructed to perform the 20-minute TT to the best of their ability with the goal of completing as much distance possible, without further verbal encouragement. The TT commenced with the treadmill speed set at 70% Vmax, and runners were instructed to alter the speed at will. Elapsed time and speed were visible, but distance and HR were concealed. Immediately on completion of the TT, final blood glucose and β-OHB concentrations were measured and runners were asked to

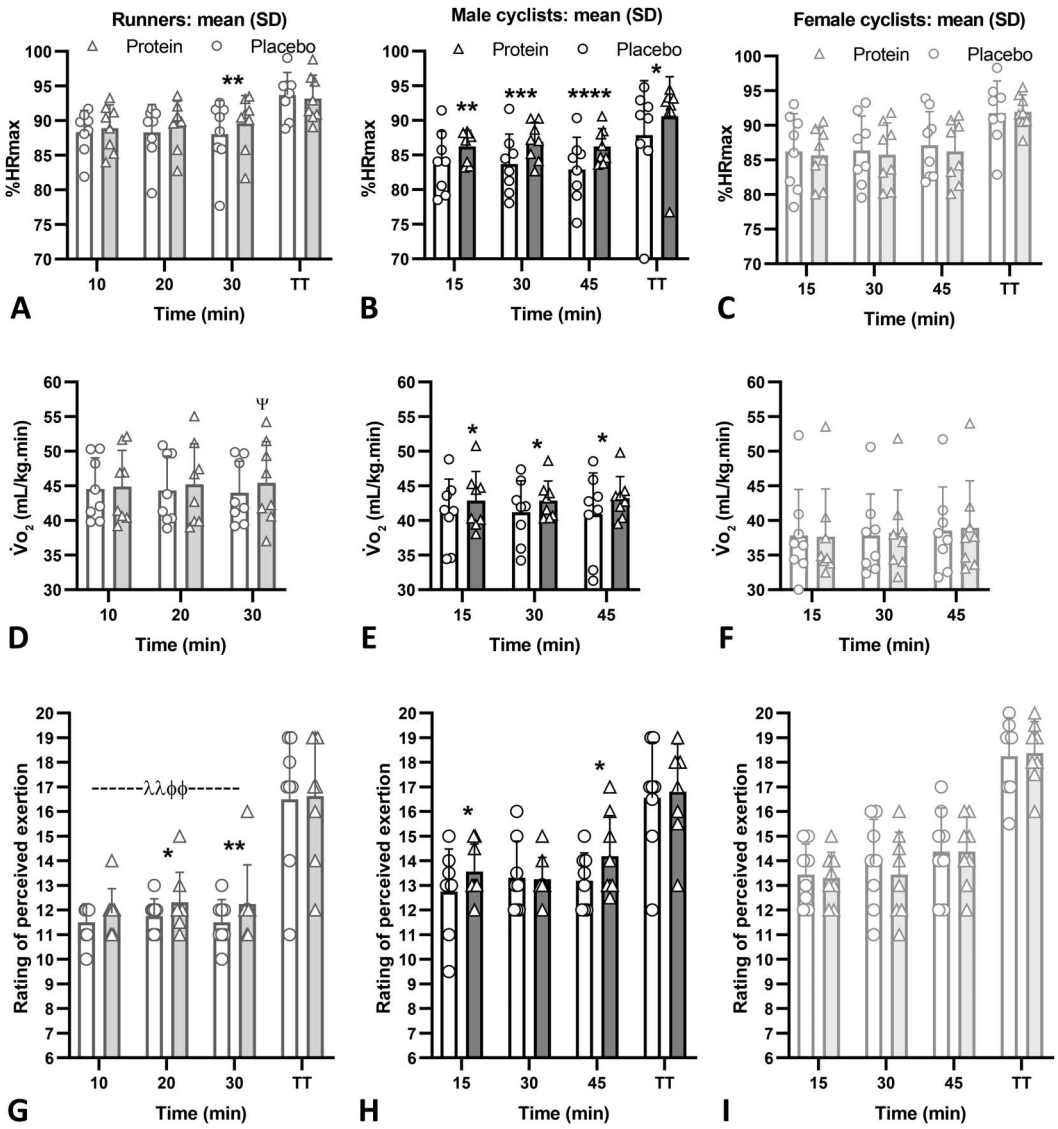


Figure 2. Exercise intensity as percent of maximum heart rate (%HRmax) (A–C), oxygen consumption ($\dot{V}O_2$) (D–F), and rating of perceived exertion (G–I), in male runners, male cyclists, and female cyclists over time during steady-state exercise and time trial (TT) when ingesting casein protein hydrolysate (protein) or placebo. * and **Significantly greater with protein than placebo within an athlete group, $p < 0.05$ and $p < 0.01$, respectively; Ψ denotes trend to be greater with protein than placebo in male runners, $p = 0.06$; λλ and ϕ denotes lower rating of perceived exertion during steady-state exercise in runners than both male and female cyclists during placebo ($p < 0.01$) and protein ($p < 0.05$) trials, respectively.

rank RPE and gastrointestinal discomfort that were perceived during the TT.

Experimental Beverages. Both placebo and protein beverages were composed of an orange-flavored electrolyte hydration effervescent tablet (Gu Hydration Tab, Berkeley, CA) in 600 ml of water. In addition, the protein beverage was then prepared as a 2.63% protein solution by adding 20 g of a commercially available protein supplement per 600 ml (PeptoPro Litely Fruity, DSM Nutritional Products, Heerlen, Netherlands) that provided 15.8 g of casein protein hydrolysate (Table 2) per 600 ml. Beverages were coded as X and Y, respectively, and prepared by the same investigator; in this way both the athletes and the investigators who supervised the trials were blind to the drink designation. The trial order was randomized using an alternating sequence and ensured that an equal number of athletes received the placebo and protein beverage in their first trial. The athletes ingested the beverages in 4 × bolus doses, where cyclists ingested a total of 800 ml during ~80 minutes of exercise and runners 600 ml during ~60 minutes of exercise resulting in an equal rate of protein ingestion of 15.8 $g \cdot h^{-1}$.

Metabolic Calculations. The rate of whole-body carbohydrate (CHO) and fat oxidation was calculated from indirect calorimetry measurements using equations for moderate-intensity to high-intensity exercise (22) as follows:

$$\text{CHO oxidation}\left(g \cdot \text{min}^{-1}\right) = 4.210\ \dot{V}_{CO_2} - 2.962\ \dot{V}_{O_2}$$

$$\text{Fat oxidation}\ \left(g \cdot \text{min}^{-1}\right) = 1.695\ \dot{V}_{O_2} - 1.701\ \dot{V}_{CO_2}$$

Energy equivalents applied for carbohydrate and fat oxidation were 4.07 and 9.75 $kcal \cdot g^{-1}$, respectively (22).

Critical power or speed defined as the intensity of cycling or running, respectively, that can be sustained for ~60 minutes is recommended as the best reflection of maximum metabolic steady state (23). Critical power/speed can be accurately derived from average power (or speed) in a 20-minute TT (FTP20) performed after a ~45 minutes exercise preload (4), where

$$\text{Critical power (or speed)} = 0.95 \times \text{FTP20}$$

Accordingly, critical power or speed was derived for each athlete using their average power or speed achieved in the 20-minute TT during the placebo trial, for the purpose of comparing steady-state interval intensity as a percent of maximum metabolic steady state between athlete groups.

Statistical Analyses

The Shapiro-Wilks test verified normality, and data were analyzed for mean drink effects using two-way repeated measures analysis of variance (drink × time), and Bonferroni test was used to evaluate differences between specified time points using GraphPad Prism 8.3 (GraphPad Software, San Diego, CA). Considering a priori pilot measured within-individual *SD* of 0.28 mmol·L^{-1} and between-individual *SD* of 0.42 mmol·L^{-1} for blood glucose concentration during 45 minutes of overnight-fasted exercise at 60% PPO preceded by an incremental test to exhaustion, a sample size of $n = 8$ per group provides 90 and 80% statistical power of identifying a meaningful change in a blood glucose concentration of 0.45 mmol·L^{-1} within an athlete group and 0.52 mmol·L^{-1} between athlete groups, respectively, at $\alpha = 0.05$. Between athlete group differences were analyzed by two-way analysis of variance and the false discovery rate with the 2-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli to control for multiple comparisons. Data are presented as mean ± *SD* or mean change and 95% confidence intervals (CIs). Data describing gastrointestinal discomfort were analyzed by the Wilcoxon signed-rank test or Kruskal-Wallis test with Dunn's post hoc test as appropriate and presented as median and interquartile range. $p \leq 0.05$ was accepted as significant. Magnitude of effect was described by Cohen's standardized change (*d*) and interpreted as follows: 0.0–0.2, trivial; 0.2–0.6, small; 0.6–1.2, moderate; and 1.2–2.0, large effect.

Results

Exercise Intensity and Metabolic Demand

Steady-state exercise intensity was similar between groups when described as percent of maximum metabolic steady state ($p = 0.7721$) (Table 1) or as %HRmax ($p = 0.1399$) (Figure 2A–C). Time trial intensity described as %HRmax was also similar between groups ($p = 0.2061$).

In male cyclists, a small to moderate effect for a higher HR occurred with protein compared with placebo at all time points during steady-state exercise (+3.0% HRmax, 95% CI [1.5%–4.4%], mean $p = 0.0008$, $d = 0.66$) and during TT in the first 15 minutes (+3.0% HRmax, 95% CI [0.9%–5.1%], mean $p = 0.0083$, $d = 0.40$) (Figure 2B). This corresponded with a small effect for oxygen consumption that was also higher with protein than placebo at all time points during steady-state exercise (+4.7%, 95% CI [0.6%–8.7%], mean $p = 0.0297$, $d = 0.37$) in male cyclists (Figure 2E).

Likewise, in male runners, a higher HR (+1.6% HRmax, 95% CI [0.4%–2.8%], $p = 0.007$, $d = 0.33$) and oxygen consumption (that increased in all but one runner) (+3.2%, 95% CI [−0.1% to 6.6%], $p = 0.061$, $d = 0.31$) were recorded with protein compared with placebo but only during SS3 (Figure 2A, D).

In female cyclists, HR and oxygen consumption were not different at any time points between the protein and placebo trials (Figure 2C, F).

Rating of Perceived Exertion

Male runners reported lower RPE by on average 2 units than both male ($p = 0.009$ and $p = 0.0079$) and female ($p = 0.0004$ and $p = 0.0079$) cyclists during the steady-state intervals with placebo and protein, respectively (Figure 2G–I). There was no difference in RPE between the male and female cyclists.

Male cyclists reported higher RPE with protein compared with placebo during SS1 ($p = 0.0416$, $d = 0.47$) and SS3 ($p = 0.0115$, $d = 0.88$) (Figure 2H). Likewise, male runners reported higher RPE with protein compared with placebo during SS2 ($p = 0.0287$, $d = 0.81$) and SS3 ($p = 0.0039$, $d = 0.80$) (Figure 2G). Conversely, female cyclists reported similar RPE during the placebo and protein trials (Figure 2I). Rating of perceived exertion was equally high during the TT in the protein and placebo trials in all athlete groups.

Blood Glucose Concentration

Resting blood glucose concentrations were not different between athlete groups. The number of athletes who incurred hypoglycemia (blood glucose concentration <4.0 mmol·L^{-1}) at any time point during exercise in the placebo trial were as follows: $n = 1$ runner, $n = 4$ male cyclists, and $n = 3$ female cyclists and during the protein trial were as follows: $n = 0$ runners, $n = 4$ male cyclists, and $n = 5$ female cyclists. In the placebo trial, runners had higher blood glucose concentrations after SS1 than male cyclists ($p = 0.0233$, $d = 1.13$) and after TT than both male ($p = 0.0002$, $d = 1.18$) and female cyclists ($p = 0.0061$, $d = 0.78$) (Figure 3A–C). In the protein trial, runners had higher blood glucose concentrations after SS1 ($p = 0.0054$, $d = 1.09$ and $p = 0.0054$, $d = 1.06$), SS2 ($p = 0.0201$, $d = 1.19$ and $p = 0.0678$, $d = 0.84$), and TT ($p = 0.0341$, $d = 0.76$ and $p = 0.0301$, $d = 0.87$) than both male and female cyclists, respectively (Figure 3A–C).

In male runners during the placebo trial, blood glucose did not change significantly from resting concentrations for the full duration of exercise (Figure 3D). In the protein trial, blood glucose increased above resting concentrations after SS1 ($p = 0.0098$, $d = 2.01$) and TT ($p = 0.0659$, $d = 1.54$) (Figure 3D). Blood glucose concentrations were higher with protein than placebo after SS2 (mean change [95% CI]: +0.63 mmol·L^{-1} [0.06–1.19 mmol·L^{-1}]; $p = 0.0285$, $d = 1.20$) (Figure 3A), and when expressed as the change-over, resting concentrations were higher with protein than placebo after SS2 (+0.71 mmol·L^{-1} [0.15–1.28 mmol·L^{-1}]; $p = 0.0124$, $d = 1.21$) and tended to be higher after SS3 (+0.54 mmol·L^{-1} [−0.03 to 1.10 mmol·L^{-1}]; $p = 0.0651$, $d = 0.76$) (Figure 3D).

Conversely, in male cyclists, blood glucose decreased below resting concentration after SS2 ($p = 0.013$, $d = -1.73$ and $p = 0.0018$, $d = -1.74$) and SS3 ($p = 0.003$, $d = -2.03$ and $p = 0.0018$, $d = -1.74$) with both placebo and protein, respectively (Figure 3E). Blood glucose remained below resting concentrations after TT ($p = 0.0035$, $d = -2.0$) with placebo but returned to resting concentration after TT with protein (Figure 3E). Blood glucose concentration was significantly higher after TT with protein compared with

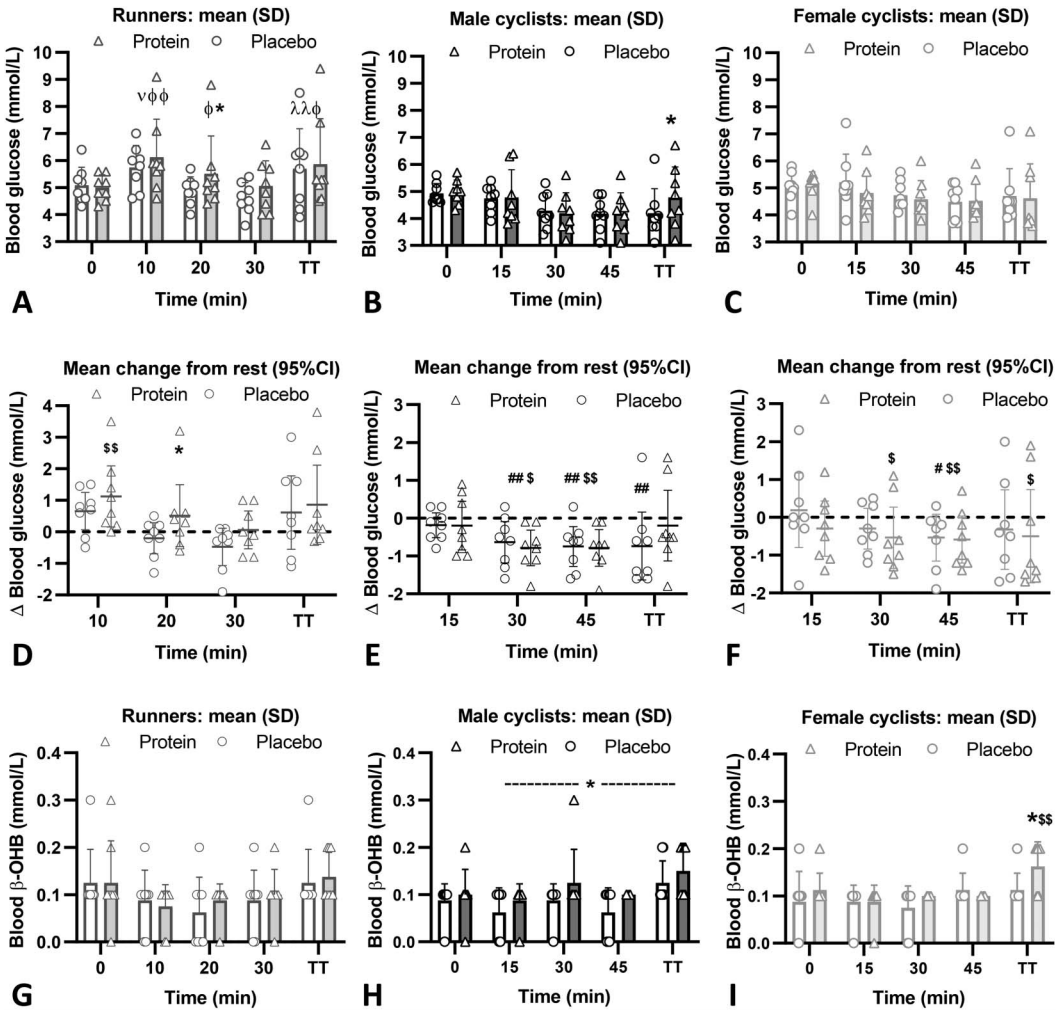


Figure 3. Blood glucose (A–C), change in blood glucose from resting (D–F), and β-hydroxybutyrate (β-OHB) (G–I) concentrations in male runners, male cyclists, and female cyclists over time during steady-state exercise and time trial (TT) when ingesting casein protein hydrolysate (protein) or placebo. *Significantly greater with protein than placebo within an athlete group, $p < 0.05$; $ and $$ significantly different to resting concentrations with protein, $p < 0.05$ and $p < 0.01$, respectively; # and ## significantly different to resting concentrations with placebo, $p < 0.05$ and $p < 0.01$, respectively; λλ, and ϕ or ϕϕ denotes higher blood glucose concentration in runners than both male and female cyclists during the placebo ($p < 0.01$) and protein ($p < 0.05$ or $p < 0.01$) trials, respectively; ν denotes higher blood glucose in runners than male cyclists in the placebo trial, $p < 0.05$.

placebo (mean change [95% CI]: +0.59 mmol·L^{-1} [0.04–1.14 mmol·L^{-1}]; $p = 0.0306$, $d = 0.64$) (Figure 3B).

In female cyclists during the placebo trial, blood glucose decreased below resting concentrations only after SS3 ($p = 0.0172$, $d = -0.46$) but was restored to resting concentrations after TT (Figure 3F). Conversely, in the protein trial, blood glucose decreased below resting concentrations after SS2 ($p = 0.0172$, $d = -1.11$) and remained below resting concentrations after SS3 ($p = 0.0082$, $d = -1.22$) and TT ($p = 0.0294$, $d = -1.04$) (Figure 3F). Despite this difference in the time effect between trials, there was no significant difference in absolute blood glucose concentrations at any time point between protein and placebo trials in female cyclists (Figure 3C).

Blood β-hydroxybutyrate Concentration

In male cyclists, mean β-OHB concentration during exercise was greater with protein than placebo (mean difference [95% CI]: +0.03 mmol·L^{-1} [0.01– 0.05 mmol·L^{-1}]; $p = 0.0112$, $d = 1.18$), but with no differences identified between specific time points (Figure 3H). In female cyclists, β-OHB concentration with protein was higher after TT than all other time points in that trial (mean $p = 0.0025$, $d = 1.89$) and was higher than placebo at this same time point (mean difference [95% CI]: +0.05 mmol·L^{-1} [0.01–0.09]; $p = 0.015$, $d = 1.41$) (Figure 3I). In runners, there were no differences in β-OHB concentrations between placebo and protein trials (Figure 3G). There were no differences in β-OHB concentrations between athlete groups.

Whole-Body Substrate Oxidation

In runners (Figure 4A, D, G) and male cyclists (Figure 4B, E, H), carbohydrate and fat oxidation expressed as both grams per minute and percent of energy expenditure were not different between placebo and protein trials at all time points during steady-state exercise; besides, in runners during SS2 there was a small effect for fat oxidation to be greater with protein than placebo (mean difference [95% CI]: +0.074 g·min^{-1} [0.01–0.14 g·min^{-1}], $p = 0.0218$, $d = 0.29$) (Figure 4D).

Conversely, in female cyclists, carbohydrate oxidation was notably greater and fat oxidation notably less with protein than placebo at all time points when expressed as both grams per minute (mean difference [95% CI]: CHO, +0.26 $g \cdot min^{-1}$ [0.12–0.39 $g \cdot min^{-1}$], mean $p = 0.0007$, $d = 0.67$ and fat, −0.11 $g \cdot min^{-1}$ [−0.16 to −0.05 $g \cdot min^{-1}$], mean $p = 0.0008$, $d = -0.64$) and percent of total energy expenditure (CHO, +8.6% [4.3–12.9%], mean $p = 0.0005$, $d = 0.65$ and fat, −8.8% [−12.9 to −4.6%], mean $p = 0.0003$, $d = -0.66$) (Figure 4C, F, I).

Carbohydrate and fat oxidation were not significantly different between athlete groups during steady-state exercise in both placebo and protein trials when expressed as percent of total energy expenditure (Figure 4G–I).

Time Trial Performance

There was no trial order effect for TT distance (runners, $p = 0.8312$; male cyclists, $p = 0.8384$; and female cyclists, $p = 0.9278$) or total work completed (male cyclists, $p = 0.9383$ and female cyclists, $p = 0.8382$) between the first and second trial, confirming that the athletes were well familiarized with high performance efforts on the treadmill or cycle ergometer and the time span between trials was adequate for achieving full recovery. Furthermore, a high %HRmax was achieved in all athlete groups (Figure 2A–C), confirming that the athletes applied their best effort.

Male runners completed a further total distance in TT with placebo than with protein, where the performance difference between trials is described as a trivial to small effect (mean ± *SD*, 4,609 ± 792 m; 4,471 ± 723 m, $p = 0.0018$, $d = -0.17$). The greater cumulative distance with placebo was already achieved by 15 minutes ($p = 0.0017$, $d = -0.24$) and that greater gain in distance was maintained to the end of TT at 20 minutes ($p = 0.0018$, $d = -0.17$) (Figure 5A). The modestly worse TT performance with protein may be a consequence of a slower starting pace in the protein trial where the distance covered in the first 5-minute split was less than the final 5-minute split in the protein trial (mean difference in first and fourth 5-minute split distance [95% CI], −99 m [−189 to −8 m], $p = 0.0268$) and coincided with a lower HR in the first 5-minute split in the protein compared with placebo trial (mean ± *SD*, 87.9 ± 5.9% HRmax; 89.5 ± 5.1% HRmax, $p = 0.0467$, $d = -0.32$). Conversely, runners maintained an even pace during the placebo trial covering a similar distance in all 5-minute splits.

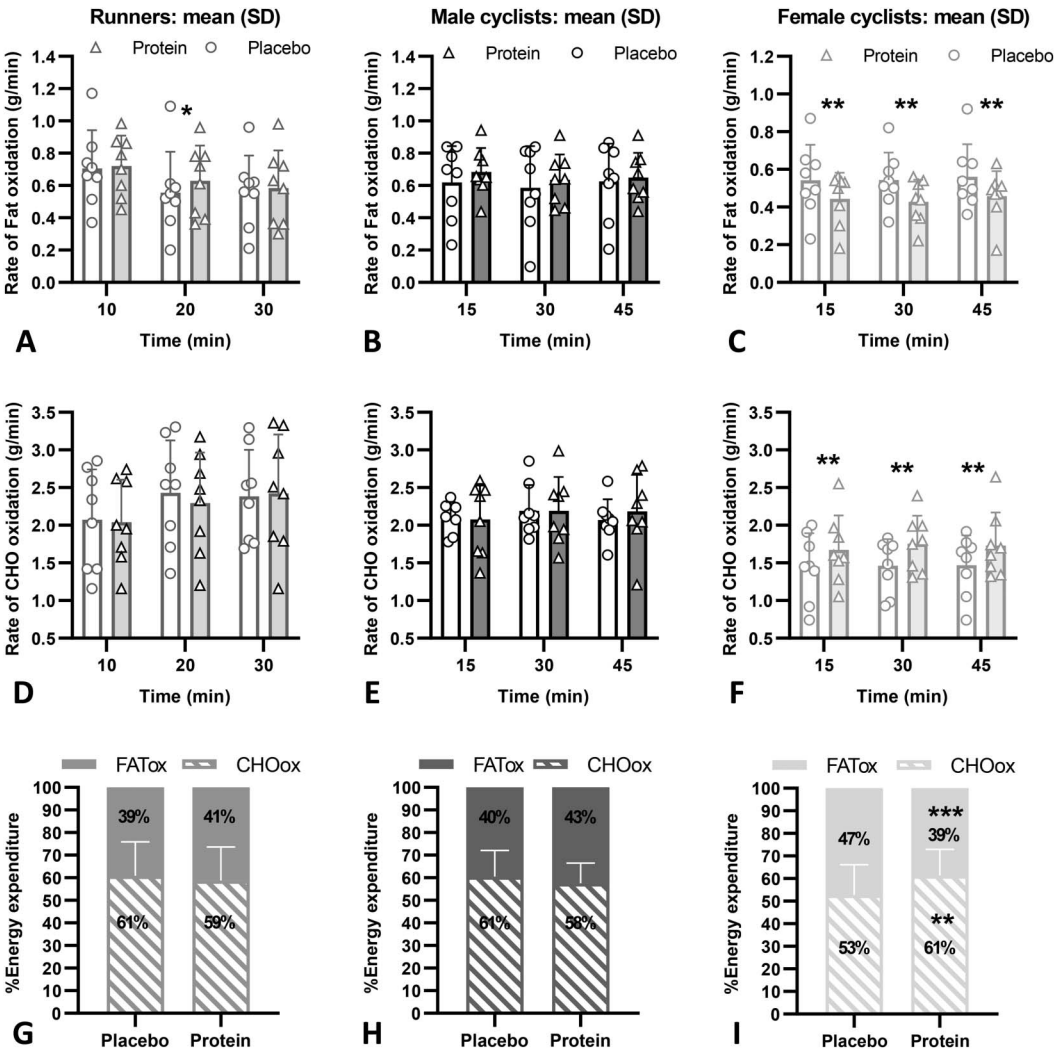


Figure 4. Rate of fat oxidation (A–C), carbohydrate (CHO) oxidation (D–F), and percent of energy expenditure derived from fat and CHO (G–I) in male runners, male cyclists, and female cyclists during steady-state exercise when ingesting casein protein hydrolysate (protein) or placebo. *, **, and ***Significantly different with protein than placebo within an athlete group, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Male cyclists completed a similar total amount of work (mean ± *SD*, 3.68 ± 0.94 and 3.73 ± 0.86 kJ·kg^{-1}, $p = 0.5794$, $d = 0.05$; %difference [95% CI]: +1.9% [−3.6% to 7.5%]) (Figure 5D) and similar distance (mean ± *SD*, 10,589 ± 1,050 m and 10,640 ± 1,092 m, $p > 0.9999$, $d = 0.05$) during TT with placebo and protein, respectively, with no differences in 5-minute split measurements (Figure 5B). The mean power output achieved during TT was 3.06 ± 0.78 and 3.10 ± 0.72 W·kg^{-1}, respectively.

Conversely, female cyclists completed a significant +2.5% (95% CI: 0.7%–4.3%) greater amount of total work (mean ± *SD*, placebo: 3.37 ± 0.52 kJ/kg; protein: 3.45 ± 0.54 kJ/kg, $p = 0.0164$, $d = 0.16$) (Figure 5E) and distance (mean ± *SD*, placebo: 9,492 ± 472 m; protein: 9,595 ± 491 m, $p = 0.0004$, $d = 0.22$) during TT with protein than placebo. The average distance recorded in the 5-minute splits was greater with protein than placebo ($p = 0.0232$) resulting in a significantly higher rolling cumulative distance by the 10-minute ($p = 0.0009$, $d = 0.32$), 15-minute ($p = 0.0008$, $d = 0.28$), and 20-minute ($p = 0.0004$, $d = 0.22$) time split in the protein trial (Figure 5C). The mean power output achieved during the TTs was 2.80 ± 0.43 and 2.86 ± 0.44 W·kg^{-1} with placebo and protein, respectively.

Gastrointestinal Discomfort

Both placebo and protein drinks were well tolerated by all 3 athlete groups, with very low rankings reported for gastrointestinal discomfort (see Figure S1, Supplemental Digital Content, http://links.lww.com/JSCR/A261). There were no significant differences in the rankings for gastrointestinal discomfort variables between placebo and protein trials in all groups.

Discussion

This study investigated the effect of ingesting a gluconeogenic precursor supplement, casein protein hydrolysate, during carbohydrate-restricted exercise on glycemia, RPE, and self-selected training intensity. Contrary to hypothesized, in male cyclists and runners, ingesting protein compared with placebo-water resulted in a small increase in RPE that coincided with a modest increase in the metabolic demand of exercise and an associated increase in HR. Conversely, this response was not observed in female cyclists where ingesting protein compared with placebo-water shifted whole-body substrate partitioning toward greater carbohydrate oxidation and less fat oxidation, occurring exclusively in the female cyclists with no net change in metabolic demand. Runners were better at maintaining euglycemia during fasted exercise than cyclists. Ingesting protein compared with placebo-water increased glycemia only in runners and restored blood glucose to pre-exercise concentrations after 80 minutes of exercise in male cyclists. The collective effect on self-selected training intensity was modestly negative in male runners, without clear effect in male cyclists and modestly improved in female cyclists with protein compared with placebo-water.

The modest increase in metabolic demand when ingesting the protein supplement noted particularly in male cyclists throughout steady-state fasted exercise and toward the end of steady-state exercise in male runners is a novel finding and is likely owing to the energy cost of upregulated hepatic gluconeogenesis. Hepatic gluconeogenesis

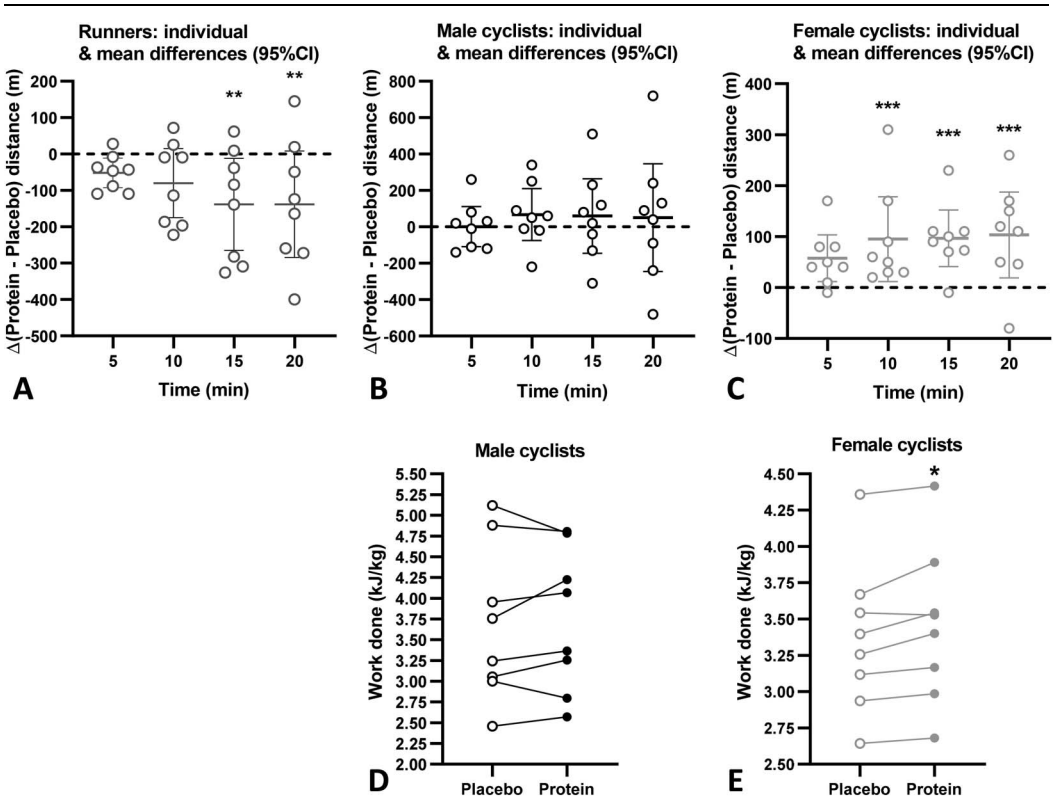


Figure 5. Difference in the distance covered by male runners, male cyclists, and female cyclists (A–C) and total work completed by male and female cyclists (D–E) during the 20-minute time trial in the protein compared with the placebo trial. *, **, and *** Significantly different with protein than placebo within an athlete group, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

is upregulated with the provision of exogenous amino acids, which stimulates glucagon secretion from pancreatic alpha cells (13). Glucagon then binds hepatic glucagon receptors promoting amino acid uptake and increased hepatic gluconeogenic flux (13,34). Gluconeogenesis is an energy-demanding pathway and previously resting metabolic rate increased by 4% when ingesting a high-protein low-carbohydrate diet in a glycogen-depleted state, where the energy cost of gluconeogenesis, measured by stable-tracers, equated to 33% of the energy derived from the gluconeogenic-produced glucose (or 1.32 $kcal \cdot g^{-1}$ of derived glucose) (44). Thus, it should not be surprising that ingesting the protein supplement during fasted exercise in the current study resulted in a modest increase in metabolic demand (+4.7% in male cyclists and +3.2% in male runners) and provides indirect evidence that the ingested protein likely was used in hepatic glucose production. An increase in metabolic demand, measured as $\dot{V}O_2$, has a strong linear correlation with increases in cardiac output, which is the product of stroke volume and HR (30). Heart rate will increase in response to even small increases in metabolic demand perceived through afferent signaling from metaboreceptors and chemoreceptors and hemodynamic shifts related to redirection of blood flow on a beat-by-beat basis (37). Therefore, it is also not surprising that the increase in metabolic demand noted in the male cyclists and runners also corresponded with an increase in HR in the protein trial compared with the placebo-water trial. Furthermore, the associated increase in RPE during the protein trial in the male athlete groups might therefore also be expected because RPE at a given workload is strongly related to HR, such that factors that govern HR may also influence RPE (15).

It might seem surprising then that the same outcome was not seen in female cyclists. Instead, in the female cyclists, metabolic demand, HR, and RPE were unchanged between protein and placebo-water trials. However, this does not necessarily imply that protein ingestion in female cyclists failed to upregulate gluconeogenic flux but may rather simply reflect no change in net energy balance between the 2 trials during steady-state exercise. The clear shift in whole-body substrate oxidation toward greater carbohydrate oxidation and lower fat oxidation with protein compared with placebo-water, which only occurred in female cyclists, is a novel finding and implies a more energy (or $\dot{V}O_2$) efficient state during exercise with protein ingestion. It is well known that less oxygen is consumed per kilojoule derived from carbohydrate compared with fat oxidation (22). Therefore, it is likely that unchanged $\dot{V}O_2$ (or metabolic demand) between protein and placebo-water trials in female cyclists may reflect a net balance where the energy saving from shifting to greater carbohydrate oxidation is set off against the probable modest additional energy demand from upregulated gluconeogenesis that is expected with the provision of exogenous amino acids.

In fact, it seems likely that the additional carbohydrate oxidized during the protein trial in female cyclists was fueled by plasma glucose derived from hepatic glucose production sourced from the ingested protein supplement. Considering that 83.3 g per 100 g of the casein hydrolysate supplement comprises glucogenic amino acids (Table 2) and that the cyclists ingested 15.8 g (3 × 200-ml aliquots) of the 2.63% protein supplement during 45 minutes of steady-state exercise, they therefore received exogenous glucogenic amino acids at a dose of 0.29 $g \cdot min^{-1}$. This approximates the observed increase in whole-body carbohydrate oxidation (+0.26 $g \cdot min^{-1}$) with protein ingestion compared with placebo in female cyclists. Likewise, although total energy expenditure during steady-state exercise (~11.3 $kcal \cdot min^{-1}$) was unchanged between protein and placebo trials in female cyclists, the proportion of energy derived from carbohydrate oxidation increased by +8.6% and is closely matched to the possible energy equivalent that could be derived from the exogenous glucogenic amino acids (1.18 kcal/min or 10.5% of total energy expenditure). Therefore, although blood glucose decreased below pre-exercise concentrations similarly in both trials in female cyclists, it seems likely that the protein supplement did increase hepatic glucose production, which was then possibly matched by an equal increase in glucose uptake. These findings should be supported by further isotopic tracer studies. An increased glucose uptake with casein hydrolysate might be expected considering that ingesting short-chain peptides containing isoleucine in rodents has previously been shown to increase skeletal muscle GLUT4 translocation and glucose uptake independent of insulin (31). Furthermore, including part OC users and nonusers is deemed best for assessing a composite effect outcome in premenopausal women (40). However, further studies are warranted to investigate for differences between OC users and nonusers and within variability between menstrual cycle phases.

Conversely, in the male athlete groups, the rate and ratio of carbohydrate to fat oxidation did not differ between protein and placebo trials. Furthermore, despite an increased metabolic demand that could suggest evidence for increased gluconeogenic flux during the protein trial, blood glucose decreased below pre-exercise concentrations similarly during steady-state exercise in both trials in male cyclists. However, it is probable that any additional plasma glucose derived from hepatic glucose production with the protein supplement was taken up by muscles at a matched rate and oxidized, sparing a proportional contribution from liver or muscle glycogenolysis. Muscle glycogenolysis has been shown to decrease during exercise in a glycogen-reduced state and is instead associated with increased plasma glucose uptake, (19) and the increase in energy derived from plasma glucose becomes increasingly notable during higher-intensity exercise (38,42), such as applied in the current study. Furthermore, under these exercise conditions, provision of an exogenous gluconeogenic source is expected to further increase reliance on plasma glucose oxidation with a proportional decrease in liver (11) and muscle glycogenolysis in male athletes (12), explaining our finding of no net change in glycemia and whole-body carbohydrate oxidation between protein and placebo trials in male cyclists.

However, unlike in cyclists, in runners ingesting the protein supplement tended to increase blood glucose above pre-exercise concentrations, compared with placebo. This may reflect a disproportionate increase in the rate of hepatic glucose production relative to whole-body/muscle glucose uptake in runners during the protein trial, which may be expected when exogenous amino acid availability induces a glucagon-stimulated hepatic response that can exceed whole-body plasma glucose demand (13). Higher blood glucose concentrations have previously been reported in runners compared with cyclists when exercising at moderate to high (70–80% $\dot{V}O_2max$) equal relative intensities (1), but not lower intensities (60% $\dot{V}O_2max$) (36). However, although exercise intensity of running and cycling may be matched relative to % HRmax or even percent of maximum metabolic steady state in the current or previous studies (1), running involves a greater active muscle mass than cycling and thus intensity per active muscle mass is greater in cycling than running at any given whole-body relative intensity index (6,7). In fact, this is evident in the current study based on the higher RPE of cyclists than runners during steady-state exercise. For this reason, the lower intensity per active muscle mass may impose less of a demand on plasma glucose utilization (38,42) during fasted running compared with cycling with or without the protein supplement and explain the higher blood glucose concentrations observed in runners. Following this same reasoning, the significantly greater blood glucose concentration during the second steady-state interval in runners

coincided with a small effect for a greater rate of fat oxidation at that same time point when ingesting protein compared with placebo, supporting an oversupply of hepatic glucose production with provision of exogenous gluconeogenic substrate in runners. The greater fat oxidation rate at the second steady-state time point in male runners, albeit not observed at other time points, agrees with previous reports for a shift to greater fat utilization when ingesting casein hydrolyzate during exercise in male athletes (32) that may be a glucagon effect (34) but possibly only evident at lower perceived intensities considering a lack of effect in male cyclists and the contrary response observed in female cyclists in the current study. Runners are more prone to gastrointestinal discomfort when ingesting supplements during exercise than cyclists (35). For that reason, in the current study, runners ingested modestly smaller volumes of the test beverages more frequently than cyclists to meet the fixed 15.8 $g \cdot h^{-1}$ dose with no adverse gastrointestinal symptoms experienced. Although unlikely, we cannot exclude possible minor effects of the modestly differing aliquot sequence between runners and cyclists.

Casein hydrolysate includes ketogenic amino acids but only produced modest increases in either mean β-OHB concentration in male cyclists or final β-OHB concentration in female cyclists compared with placebo, with no change in runners. This may not be surprising because the cyclists experienced bigger decreases in glycemia and higher incidences of hypoglycemia during exercise than runners.

The final 20-minute TT reflected self-selected maximal training intensity. Surprisingly, male runners performed modestly worse with the protein supplement compared with placebo owing to a poorer starting pace during the first 5 minutes of the TT. This poorer starting performance in runners may possibly be related to higher RPE and associated metabolic demand recorded at the end of the preceding steady-state exercise during the protein trial. Similarly, a greater metabolic demand (or oxygen cost) during elite race walking when following a low–carbohydrate high-fat diet compared with a high-carbohydrate diet coincided with higher RPE and worse race performances (5). Thus, in the current study, the increased perception of exertion may have carried over to the start of the TT. However, after the first 5 minutes, the runners pace corrected to match the pace during the placebo trial. Likewise, no clear differences in TT performance were observed in male cyclists between placebo and protein trials. However, higher blood glucose on TT completion that was restored to pre-exercise concentrations with protein compared with placebo in male cyclists may reflect possible sparing of hepatic glycogen stores with the ingestion of a gluconeogenic precursor (11). Future studies should investigate whether casein hydrolysate ingestion in male runners and cyclists during fasted exercise would support better performances in moderate-intensity to high-intensity exercise lasting longer than 60–80 minutes.

Conversely, ingesting casein hydrolysate modestly improved TT performance in female cyclists compared with placebo, owing to a consistent small increase in pace. The modest improvement in this final high-intensity TT effort in female cyclists is likely owing to the shift in substrate utilization in favor of greater carbohydrate utilization, which seems to be supported by plasma glucose derived from the ingested amino acids in the protein trial. The superior energy efficiency of carbohydrate utilization better supports high-intensity exercise performances (22).

Findings of the current study may be specific for casein hydrolysate and are not necessarily transferable to other whole-protein or protein hydrolysate supplements. Casein hydrolysate is composed of purely dipeptides and tripeptides that are rapidly absorbed even without further digestion, whereas other protein hydrolysates, such as whey hydrolysates, are often composed of a mix of some short and longer peptide chains that will require digestion before absorption (25,26). Previously, we have found more metabolic and performance effects with a casein hydrolysate-carbohydrate compared with whey hydrolysate-carbohydrate supplement during cycling (32). The current study determined whether ingesting a fixed absolute gram per hourly dose of casein hydrolysate during overnight-fasted exercise better maintained euglycemia than ingesting placebo-water rather than applying a body mass relative dose, for comparative purposes with previous similar studies (21,41). However, it would be insightful to use isotopic tracers in future studies to quantify rate of plasma glucose appearance and disappearance, gluconeogenesis, hepatic glycogenolysis, and oxidation of the ingested protein and to investigate for correlations between body mass and muscle mass to establish whether an absolute or relative protein dose is preferred. Nevertheless, the fixed absolute hourly dose in the current study was associated with distinct athlete group-specific directional changes in certain measured parameters and it is unlikely that a variable dose relative to body mass would alter the direction of observed changes. Furthermore, following a minimally invasive protocol, we did not quantify circulating glucoregulatory hormones. However, it is known that ingesting protein or amino acids increases plasma glucagon concentration (13) and such increases in glucagon will be associated with a counter reduced catecholamine response (11) even during hypoglycemia (33). Therefore, the metabolic outcome of ingesting the protein supplement in the current study is likely owing to both an increase in plasma glucagon and decrease in catecholamine concentrations. However, it is unlikely that the metabolic outcome can be attributed to differences in insulin concentration because previously casein hydrolysate or whey isolate ingestion during overnight-fasted exercise either resulted in a very modest increase (41) or even no change in insulin concentration (21), respectively. Furthermore, previously an increased rate of plasma glucose uptake during exercise occurred with lower or no change in insulin concentrations in a carbohydrate-depleted condition compared with a carbohydrate-loaded condition (19) or with provision of intravenous lactate as a gluconeogenic precursor compared with no exogenous substrate (11), respectively.

Practical Applications

Ingesting casein protein hydrolysate at a rate of 15.8 $g \cdot h^{-1}$ during carbohydrate-restricted, moderate-intensity to high-intensity exercise alters exercise metabolism and performance differently in male runners, male cyclists, and female cyclists. In male athletes alone, ingesting casein hydrolysate compared with placebo imposes a small increase in the metabolic demand of exercise that has a noticeable effect of increasing RPE and may have a modest to trivial negative effect on self-selected training intensity in runners. Conversely, in female cyclists alone, ingesting casein hydrolysate shifts substrate utilization in favor of more energy-efficient carbohydrate oxidation resulting in no net change in metabolic demand or RPE and promotes a modest increase in self-selected training intensity. Rating of perceived exertion is higher in cyclists than runners at the equal relative intensities used and may explain the increase in glycemia with casein hydrolysate ingestion only in male runners but not cyclists, in accordance with established principles of greater reliance on plasma glucose utilization as exercise effort increases.

Acknowledgments

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