The Effects of Nutritional Supplementation Throughout an Endurance Run on Leucine Kinetics During Recovery

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This study determined the effect of nutritional supplementation throughout endurance exercise on whole-body leucine kinetics (leucine rate of appearance [Ra], oxidation [Ox], and nonoxidative leucine disposal [NOLD]) during recovery. Five trained men underwent a 2-h run at 65% VO$_{2\text{max}}$ during which a carbohydrate (CHO), mixed protein-carbohydrate (milk), or placebo (PLA) drink was consumed. Leucine kinetics were assessed during recovery using a primed, continuous infusion of 1-$\text{I}^{13}$C leucine. Leucine Ra and NOLD were lower for milk than for PLA. Ox was higher after milk-supplemented exercise than after CHO or PLA. Although consuming milk during the run affected whole-body leucine kinetics, the benefits of such a practice for athletes remain unclear. Additional studies are needed to determine whether protein supplementation during exercise can optimize protein utilization during recovery.

**Key Words**: whole-body protein turnover, milk supplementation

Endurance exercise alters rates of whole-body leucine kinetics during exercise and in the postexercise recovery period. During moderate- to high-intensity exercise there is a decrease in protein synthesis (3, 26, 39) and increases in protein breakdown (7, 24, 39) and leucine oxidation (3, 19, 24, 32). Because most endurance athletes are able to maintain lean body mass and improve strength and performance, the catabolic environment during exercise must be balanced by an anabolic phase during the recovery period (6, 10). Studies of protein utilization after exercise have shown increases in whole-body protein synthesis (3, 10, 26), whereas rates of breakdown decrease or do not differ from those observed at rest (3, 10, 26). Losses in lean body mass and strength can occur during heavy training periods if the proteolysis occurring during exercise is not countered by a comparable increase in protein synthesis postexercise. Nutritional interventions might be a way to optimize protein utilization in athletes participating in vigorous endurance training (5, 16).
Carbohydrate supplementation during exercise is the classic example of a nutrient intervention aimed at improving performance in endurance athletes. Because of its ability to increase insulin levels (12, 30), maintain blood glucose levels (12, 36), and spare muscle glycogen stores (40), carbohydrate supplementation during exercise likely affects whole-body protein utilization, as well. Providing carbohydrate during exercise can reduce the reliance on endogenous protein stores for energy, thus attenuating leucine oxidation (3, 9, 14) and ammonia production (30). Similarly, there is a relationship between glycogen stores and protein utilization, with glycogen-depleted individuals exhibiting greater protein breakdown (15) and branched-chain keto-acid dehydrogenase activity (36) than carbohydrate-loaded or -supplemented subjects. Although some studies have documented the relationship between carbohydrate intake and protein utilization during endurance exercise, the effect of protein ingestion during exercise on protein utilization has not been extensively studied.

Providing protein or amino acids influences protein utilization at rest and the protein-related metabolic response to resistance exercise. At rest, amino acid administration improves protein utilization (1, 22). Paddon-Jones et al. (23) observed ~60% increases in muscle protein-synthetic rates in young and old subjects after they ingested 15 g of essential amino acids at rest. Protein administration (alone or with carbohydrate) before, during, or after resistance exercise enhances protein utilization in response to exercise (1, 2, 25, 33, 34). Biolo et al. (1) compared the effects of amino acid infusion at rest and after resistance exercise, and, despite similar increases in arterial amino acid concentrations, there were increased rates of amino acid transport and a 2-fold increase in muscle protein synthesis after exercise. Furthermore, the observed effects of amino acids in increasing muscle protein synthesis postexercise have been noted with as little as 6 g of essential amino acids (2, 25). Although it seems clear that providing amino acids at rest and in association with resistance exercise improves protein utilization, there are limited data regarding the interaction of protein intake and endurance exercise on whole-body protein utilization.

Theoretically, ingesting amino acids during endurance exercise should attenuate endogenous protein breakdown occurring in response to demands for gluconeogenic precursors or metabolic intermediates during prolonged exercise (12, 20). In addition, an increase in plasma amino acids during exercise could set the stage for a more anabolic recovery period, aiding in tissue repair after exercise (5, 29). A few studies have investigated the metabolic impact of consuming a protein-carbohydrate supplement during the recovery period and observed increases in whole-body and leg protein gains (17), improved nitrogen balance (28), and enhanced glycogen replenishment (41) after supplementation. The effects of protein ingestion during endurance exercise on protein utilization during the recovery phase, however, have not been delineated.

Koopman et al. (14), to the best of our knowledge, conducted the only study that examined the effect of protein ingestion during endurance exercise on protein utilization. They found that consumption of a protein-carbohydrate mixture during 6 h of endurance exercise at 50% VO$_{2\text{max}}$ in endurance athletes improved net protein balance at rest, during exercise, and in the recovery period (14). Although protein oxidation increased almost 2-fold, the ~60% decrease in protein breakdown contributed to noted improvements in protein utilization in response to the supplement.
Koopman et al. (14) used a protein-carbohydrate supplement that contained ~50 g of carbohydrate and 18 g of protein per 30 min of exercise. The high energy and solute content of this beverage would likely limit its routine use during endurance exercise. The question remains, however, whether similar improvements in protein utilization can be achieved with consumption of lesser amounts of carbohydrate and protein during an endurance bout.

The current study was designed to compare the effects of consuming a carbohydrate and a mixed protein-carbohydrate beverage throughout an endurance-exercise bout on whole-body leucine kinetics during the recovery period after exercise. We hypothesized that consuming the protein-carbohydrate mix would improve protein utilization during the recovery period compared with consumption of either carbohydrate alone or placebo.

**Materials and Methods**

**Subjects**

After project approval by the institutional review board at the University of Connecticut, 5 endurance-trained men (age 18–30 y) were recruited from the community to participate in the study. Each participant provided a complete medical history, a training schedule, and a record of dietary intake. Subjects were required to be running a minimum of 56 km/wk for inclusion in the study. Individuals reporting metabolic or cardiovascular abnormalities, gastrointestinal disorders (i.e., lactose intolerance), or use of nutritional or sports supplements or anabolic steroids were excluded from the study. All subjects provided informed, written consent. Subject characteristics are provided in Table 1.

**Experimental Design**

This study employed a randomized crossover design, with volunteers serving as their own controls. After initial baseline testing, subjects participated in 3 exercise trials during which a supplement containing carbohydrate (CHO), mixed carbohydrate-protein (milk), or a nonnutritive placebo (PLA) was administered during a 2-h endurance bout. The trials were separated by 7-d “wash-out” periods for isotope-decay recovery from one trial to the next. Vigorous exercise, as well as consumption of alcohol and caffeine, was restricted on the day preceding each trial.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject Characteristics (N = 5)</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Age (y)</td>
<td>22 ± 1.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 3.0</td>
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<tr>
<td>Weight (kg)</td>
<td>71 ± 3.0</td>
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<tr>
<td>Fat-free mass (kg)</td>
<td>62 ± 2.0</td>
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<tr>
<td>VO$_{2 \text{max}}$ (mL kg$^{-1}$ min$^{-1}$)</td>
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</tbody>
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Values are means ± standard error of the mean.
Baseline Measures
Before initiation of the trials, subjects reported to the laboratory for preliminary data collection. They underwent a test of maximal oxygen consumption (VO\textsubscript{2max}) and then returned at a later time to determine the treadmill speed corresponding to 65% of their predetermined VO\textsubscript{2max}. Body composition was determined by hydrostatic weighing and calculated using the equations of Brozek et al. (4).

Dietary Records
Three-day food-intake records were obtained from subjects at baseline and for the days leading up to each trial. Mean energy intake and macronutrient composition of the diets were analyzed by a registered dietitian using Nutritionist IV software (N2 Computing, Salem, OR).

Isotopes
Stocks of 1-\textsuperscript{13}C-leucine (99% isotopic enrichment, Cambridge Isotope Laboratories, Cambridge MA) were determined to be pyrogen free by a commercial laboratory (South Mountain Labs, Lackawanna, NJ), and infusate was prepared aseptically on the morning of each study.

Composition of Supplements
The CHO drink consisted of 45 g of dextrose (table sugar) mixed with 835 mL of bottled water and nonsweetened flavoring (Kool-Aid, Kraft, Inc., White Plains, NY). The milk supplement consisted of 480 mL of skim milk (17 g of protein and 27 g of carbohydrate) diluted with 355 mL of bottled water. Flavoring and aspartame (Equal, NutraSweet Co., Deerfield, IL) were added to the milk drink in an effort to mask the nature of its composition and to increase palatability. The CHO and protein drinks were isocaloric, providing ~170 kcal in total. The PLA drink was prepared using bottled water, nonsweetened flavoring, and aspartame.

Study Protocol
Figure 1 illustrates the study protocol. Subjects arrived at the human-performance laboratory at the University of Connecticut at ~7 AM after an overnight fast. Before exercise a 19 G Teflon catheter (Jelco, Critikon, Tampa, FL) was inserted into a superficial forearm vein for baseline blood sampling. An intravenous saline drip was maintained to keep the line patent. Subjects then began running for 120 min at a speed predetermined to elicit an effort equal to 65% of their individual VO\textsubscript{2max}. Water was available for subjects to drink ad libitum throughout the endurance trial, and a fan was positioned near the treadmill to maintain a comfortable environment. At 20, 40, 60, and 80 min, subjects drank 200 mL of supplement while straddling the treadmill.

Assessment of Leucine Kinetics
After the exercise had been completed an additional catheter was placed in a contralateral hand vein for arterialized blood sampling during the isotope infusion. An intravenous saline drip was maintained to keep the line patent. The participant’s
hand was placed in a Plexiglas box and was heated to ~70 °C so that arterialized blood samples could be obtained (8). After collection of blood and breath samples for background 1-13C-leucine enrichment, a primed continuous infusion of 1-13C-leucine (4 µmol/kg, 4.8 µmol·kg⁻¹·h⁻¹) was initiated (Razel Syringe Pump, Razel Scientific Instruments Inc., Stamford, CT) and continued for 180 min. At 120 min, blood and breath measurements were collected at 15-min intervals for 1 h. Plasma samples were spun in a refrigerated centrifuge (4 °C) at 2000 rpm for 10 min and stored at –80 °C for subsequent analyses. For 13CO₂ breath enrichments, subjects breathed into a Douglas bag for 2 min at specified time intervals. Breath samples were then transferred to 20-mL Venoject containers for isotope-ratio analysis. Plasma 13C-KIC and 13CO₂ enrichments were determined by gas chromatography and isotope-ratio mass spectroscopy, respectively, by a commercial laboratory (Metabolic Solutions, Nashua, NH). Blood 13C-KIC and breath 13CO₂ data were reviewed at 5 time points (120, 135, 150, 165, and 180 min) to confirm steady-state conditions. Steady-state conditions were assumed when the coefficient of variation of the atom-percentage-excess values at isotopic plateau was <10%. To account for possible contributions of naturally occurring 13C in milk to expired 13CO₂ enrichment, 3 subjects were studied without concurrent infusion of 1-13C-leucine, and their breath 13CO₂ enrichments were determined at baseline (before milk consumption) and at the same time periods after exercise in the current study. No differences were noted in expired 13CO₂ enrichments as a result of milk consumption.

**Calculation of Leucine Kinetics**

Data from the 5 time points were averaged for each subject, and group means were determined. Leucine rate of appearance (leucine Ra; indicator of protein breakdown), leucine oxidation (Ox), and nonoxidative leucine disposal (NOLD; indicator of protein synthesis) were then calculated using the reciprocal-pool model (11).
Statistical Analysis

Primary outcome measures were leucine Ra, Ox, and NOLD in response to the 3 supplements. Treatment means were calculated for all variables and subjected to a 3-way analysis of variance (ANOVA) with differences detected using Tukey’s post hoc analysis with an \( \alpha \) level set at 0.05. Data are expressed as mean ± standard error of the mean. Statistical analyses were conducted using SAS software (Cary, NC).

Results

Dietary Intake

Energy intakes for the 3-d period preceding each trial and the single day before each study did not differ for or between subjects. Likewise, macronutrient composition of the diets before all trials was similar for all subjects.

Leucine Kinetics

Steady-state conditions were achieved during recovery from exercise as indicated by plasma \(^{13}\) C-ketoisocaprate and breath \(^{13}\) CO\(_2\) enrichments (Figure 2). Leucine Ra and NOLD were lower during recovery from milk-supplemented exercise than with PLA (Ra 125.0 ± 20.3 vs. 154.2 ± 17.2 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\) and NOLD 89.4 ± 20.5 vs. 129.7 ± 17 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\), \(P < 0.05\); Figure 3). Leucine Ox was higher after the milk-supplemented run (35.6 ± 1.9 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\)) than with either CHO (23.9 ± 3.7 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\)) or PLA (24.5 ± 2.9 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\)), \(P < 0.05\). Leucine kinetics did not differ between CHO and PLA.

![Figure 2](image-url) — \(^{13}\)C-KIC plasma enrichments during 3 h recovery from a placebo- (PLA), carbohydrate- (CHO), or milk- (MILK) supplemented endurance run.
Protein metabolism changes in response to submaximal endurance exercise (31, 38). During exercise there is an increase in protein breakdown and a decrease in protein synthesis that are often countered by an increase in protein synthesis and attenuated rates of breakdown during recovery (10). The purpose of this study was to characterize how nutrient provision during exercise affects whole-body protein utilization postexercise. Unlike previous supplementation trials, in which fairly large portions of carbohydrate or nutrients were administered, a marginal caloric load was used, taking into consideration the amount athletes would be willing to ingest during training or a typical endurance event.

The major findings from this investigation were that consuming a carbohydrate-protein supplement that provided 0.2 g·kg\(^{-1}\)·h\(^{-1}\) CHO and 0.12 g·kg\(^{-1}\)·h\(^{-1}\) protein during exercise resulted in a decrease in leucine Ra (indicative of whole-body protein breakdown), a decrease in NOLD (indicative of whole-body protein synthesis), and an increase in Ox during the 3-h recovery period after a 120-min run at 65% \(\text{VO}_{2\text{max}}\). Ingestion of carbohydrate alone (CHO) during exercise did not affect whole-body leucine utilization during recovery.

Theoretically, maintaining blood glucose and glycogen stores in response to carbohydrate ingestion should reduce the reliance on endogenous-protein breakdown to provide substrates for working muscle during prolonged exercise (3, 9, 14, 30). Previous studies have found a decrease in protein breakdown (30) and attenuation in Ox (3, 9, 14) during exercise in response to carbohydrate intake. Leucine oxidation during exercise was not measured in the current study, but respiratory-exchange-ratio data collected during exercise in this study (21) showed that drink composition did affect the fuel mix, with respiratory-exchange-ratio values being higher during the CHO-supplemented run than during the PLA run.
Despite differences in fuel utilization during exercise and higher plasma glucose values postexercise (21), consumption of the CHO drink during exercise did not differ from the PLA drink with regard to protein utilization during recovery (Figure 4). It is important to note that the amount of carbohydrate we provided (0.3 g·kg\(^{-1}·h^{-1}\)) was much less than that consumed in these previous reports (0.7 – 1.1 g·kg\(^{-1}·h^{-1}\)) (3, 14, 30). This might have contributed to the absence of an effect of carbohydrate intake on protein utilization in the current study.

Few studies have evaluated the role of protein in sports drinks for endurance athletes. Results from these studies suggest that glycogen storage and muscle recovery are enhanced and that subsequent exercise performance is improved when protein is added to recovery supplements (12, 13, 37, 41). Gains in whole-body and leg-muscle protein are improved with protein consumption postexercise (17). The addition of 10 g of protein to a carbohydrate-fat supplement postexercise increased leg protein synthesis 6-fold and shifted whole-body protein net balance from negative to positive (17), which supports the notion that protein supplementation might provide some benefit to endurance athletes.

Timing of protein ingestion is an important factor regarding its impact on protein utilization (18, 28). Levenhagen et al. (18) found that consuming a protein-carbohydrate mixture immediately after exercise improved net protein balance in the recovery period. Similarly, Roy et al. observed improved nitrogen balance, weight maintenance, and subsequent performance when a protein-containing supplement was consumed immediately postexercise compared with the response noted when

![Figure 4](image_url) — Whole-body protein turnover in trained men after a placebo- (PLA), carbohydrate- (CHO), or milk- (MILK) supplemented endurance run. Leucine rate of appearance (Ra), nonoxidative leucine disposal (NOLD), and oxidation (Ox) during recovery from endurance exercise. Values are means ± standard error of the mean for 5 subjects. *Different from PLA. †Different from CHO. P < 0.05.
the supplement was consumed several hours before endurance exercise (28). The observed decrease in proteolysis during recovery from a milk-supplemented endurance-exercise session suggests that providing exogenous amino acids throughout the run might have minimized dependence on endogenous-protein stores during exercise. We previously reported (21) that branched-chain amino acid levels were maintained during the milk- but not CHO- or PLA-supplemented runs. Protein synthesis, as reflected by NOLD, was also attenuated during recovery after the milk-supplemented exercise. The increase noted in Ox might partially explain the latter observation. Because kinetic assessments were not made during the exercise session, we postulate that dietary amino acids consumed during the run might have been preferentially oxidized, thus decreasing their availability for whole-body protein synthesis during the recovery period (35).

Koopman et al. (14) compared the effects on whole-body protein utilization during recovery of consuming protein and carbohydrate or carbohydrate alone during prolonged endurance exercise. Those authors used a higher level of protein than that of the current study and found that the addition of protein led to decreased rates of protein breakdown during the recovery period without a decrease in synthesis. Despite an increase in Ox in response to protein ingestion, protein supplementation resulted in a more positive net leucine balance than did ingestion of carbohydrate alone. In contrast, we observed suppression in both breakdown and synthesis after protein intake compared with carbohydrate alone. Although discrepancies in study findings are likely a result of differences in the quantity of protein ingested, it is important to note that the exercise protocols also differed between this study (2 h at 65% VO_{2max}) and that of Koopman et al. (6 h at 50% VO_{2max}). Perhaps greater protein content in the milk supplement would have provided sufficient amino acids to not only support the increase in oxidation but also enable an increase in protein synthesis. Potentially, the amount of protein needed for this to occur would fall between the amount consumed in the present study (~8 g/h) and that consumed by subjects in the study by Koopman et al. (~36 g/h) (14). Practically speaking, determining the level of protein required to elicit this desired response is very important to endurance athletes looking to optimize protein utilization for the purpose of maintaining lean body mass throughout training and the competitive season.

In summary, when compared with placebo, we observed a decrease in whole-body protein breakdown and synthesis and an increase in Ox as evidenced by leucine kinetics during the recovery period after a milk-supplemented endurance run. Perhaps these data suggest a need for greater protein intake during exercise to observe favorable results with respect to whole-body protein utilization, given that the quantity provided in the current study was enough to elicit an increase in oxidation and suppress breakdown but not enough to support synthesis. Carbohydrate ingestion did not influence whole-body protein turnover in the current study. Conclusions from these observations are limited in that the 1-^{13}C-leucine tracer model provides an index of whole-body protein metabolism and is not reflective of specific metabolic events occurring in muscle (27). Future studies are needed to determine the role of protein supplementation during endurance exercise in modulating skeletal-muscle protein turnover. Practically speaking, maintaining lean body mass and strength throughout the course of a vigorous training program is critical to endurance athletes’ performance. Nutritional supplementation might
be a means by which this could be accomplished in this population. Benefits of nutritional, particularly protein, supplementation must be convincingly demonstrated to justify efforts to change an athlete’s training beliefs and behavior with regard to this particular macronutrient. For competitive athletes seeking ways to maintain lean body mass, nutritional supplementation during training might prove to be a successful strategy in optimizing protein utilization after prolonged endurance exercise.

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References


