Dietary protein digestion and absorption are impaired during acute postexercise recovery in young men

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The Gut is challenged with two major functions: digestion and absorption of nutrients and the maintenance of an intact barrier against potentially harmful luminal compounds. Exercise is known to cause gastrointestinal (GI) injury and gut barrier dysfunction, reflected by increased small intestinal permeability, bacterial translocation, and inflammation after exercise (6, 15). We demonstrated that 1 h of exercise induced small intestinal injury, leading to gut barrier dysfunction in healthy young athletes (30). These data gave rise to the intriguing question of whether exercise-induced intestinal injury compromises the digestive and absorptive capacities of the GI tract.

Dietary protein ingestion following exercise has been reported to increase postexercise muscle protein synthesis rates, thereby promoting early recovery after strenuous exercise (1). Impairments in dietary protein digestion and absorption may reduce the appearance rate of dietary protein-derived amino acids in the circulation (12), thereby lowering the postprandial availability of these amino acids for muscle damage repair and reconditioning. As muscle protein synthesis is maximally stimulated during acute postexercise recovery, a rapid supply of exogenous protein is required to allow muscle protein synthesis rates to increase (1, 16, 18, 19). The latter is important to athletes, as well as patients with chronic diseases, to allow proper adaptation to each successive exercise bout within exercise intervention programs designed to improve performance and health. Impairments in food digestion and nutrient absorption during and immediately after exercise may jeopardize the adaptive response to the combined effect of exercise and proper nutrition (1).

We hypothesized that exercise-induced intestinal injury impairs dietary protein digestion and absorption kinetics during acute postexercise recovery. The present study was designed to determine the impact of a single bout of resistance-type exercise on intestinal damage and on the subsequent digestive and absorptive function of the GI tract. Small intestinal injury and dietary protein digestion and absorption kinetics were assessed following ingestion of a single meal-like bolus of dairy protein at rest and after a single bout of resistance-type exercise in healthy young males. By combining the use of specifically produced intrinsically L-[1-13C]phenylalanine-labeled dairy protein with continuous intravenous L-[ring-2H3]phenylalanine infusion, we assessed in vivo dietary protein digestion and absorption kinetics (26, 29). This study shows novel evidence that resistance-type exercise induces small intestinal damage and impairs in vivo dietary protein digestion and absorption during acute postexercise recovery in young, healthy men.

MATERIALS AND METHODS

Ethical approval. The current study was approved by the Medical Ethical Committee of Maastricht University Medical Centre and was conducted in accordance with the Declaration of Helsinki (revised version, October 2008, Seoul). The study is part of a trial registered at clinicaltrials.gov as NCT00557388.

Participant characteristics. Twenty-four young, healthy male volunteers (21 ± 1 years of age) were included in the current study. The volunteers had no abdominal complaints during daily activities, had

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not taken any medication for at least 1 mo prior to participation, had no history of GI disease, and had had no abdominal surgery. All participants of the current study were recreationally active people, reporting to spend 5–10 h/wk on physical exercise. Participants were randomly assigned to the rest or exercise experiment. Participant characteristics, presented in Table 1, did not differ between groups. All participants were informed about the nature and risks of the experiments before written consent was obtained.

Pretest arrangements. Prior to the experiments, body composition analysis was performed using a dual-energy X-ray absorptiometry scan (DXA, Hologic, Bedford, MA), and leg volume was determined, as described previously (31). Electrocardiography was performed at rest and during exercise to exclude any heart failure in the selected volunteers. In addition, the subject’s maximal strength (one repetition maximum, 1RM) was estimated after a session of multiple repetitions of leg press and leg extension exercises (21). Subsequently, during a second session, the subject’s true 1RM was determined by setting the load to 90–95% of the estimated 1RM, and increased after each successful lift. An interval of 7 days or more was scheduled between the screening sessions and the experimental day.

Participants maintained normal activities of daily living, but they refrained from strenuous physical activity for 3 days prior to the experiments. The evening before the experimental day, participants received a standardized meal (33 ± 2 kJ/kg body wt, providing 55% of energy as carbohydrate, 15% as protein, and 30% as fat) at ~8:00 P.M., after which they remained fasted.

Study design and sampling. In the morning, a catheter was placed in the participant’s forearm vein for stable-isotope infusion. A second catheter was inserted in a heated dorsal hand vein of the contralateral arm and placed in hot box heated to 60°C for arterialized blood sampling. Blood was collected before (~30 min), immediately postexercise/rest (0 min), and 15, 30, 45, 60, 75, 90, 105, and 120 min postexercise/rest to enable thorough analysis of enterocyte integrity and protein digestion and absorption, especially in the immediate postexercise period. Blood was sampled in EDTA tubes, centrifuged at 1000 g for 5 min at 4°C, and stored until analysis at −80°C. After baseline sampling (t = −120 min), a single intravenous dose of the tracer, L-[ring-2H5]phenylalanine (2 μmol/kg) was infused to prime the plasma phenylalanine pool. Subsequently, the continuous tracer infusion was started at a rate of 0.044 ± 0.001 μmol·kg−1·min−1, after which the participants rested in upright position for 90 min. Subjects then either performed a 30-min standardized exercise protocol or remained seated for another 30 min of rest, according to random assignment. The exercise protocol comprised a 5-min self-paced cycling bout at 1.4 ± 0.1 W/kg body wt at a pace of 70–90 rotations/min, combined with 6 sets of 10 repetitions on a horizontal leg press machine (Technogym BV, Rotterdam, the Netherlands) and 6 sets of 10 repetitions on a leg extension machine (Technogym BV).

Each first two sets were performed at 40% of the subject’s 1 RM. Sets 3 and 4 and 5 and 6 were performed at 55% and 75% of 1RM, respectively, with a 2-min rest interval between sets.

After baseline sampling (t = 0 min), subjects received a single 250-ml bolus flavored drink containing 20 g intrinsically l-[1-13C]phenylalanine-labeled casein protein. The latter was obtained by infusing a Holstein cow with large amounts of l-[1-13C]phenylalanine and collecting the milk produced by this cow (29). The l-[1-13C]phenylalanine enrichment in the purified casein fraction of the cow milk averaged 29.2 mol percent excess. The drink met all specifications for safe human consumption.

Assessment of small intestinal injury. To determine whether resistance-type exercise leads to small intestinal injury, concentrations of human intestinal fatty acid binding protein (I-FABP) were analyzed by an ELISA in plasma samples collected before and after exercise. No samples were obtained during the short exercise bout. In short, 96-well plates (Greiner Microlon F, Greiner Bio-One, Frickenhausen, Germany) were coated overnight with anti-I-FABP immunoglobulin G (IgG) in a 2.5 μg/ml concentration in PBS at 4°C. Free sites were blocked with 1% BSA in PBS for 1 h at room temperature. Subsequently, plasma samples and human recombinant I-FABP, used to produce standard calibration curves, were allowed to incubate for 1 h at room temperature, after which 0.5 μg/ml of biotinylated anti-I-FABP IgG in 0.1% BSA-0.05% Tween 20-PBS was added to the plates and left to incubate for 1 h at room temperature. Horseradish peroxidase-streptavidin conjugate (Zymed Laboratories, San Francisco, CA) in 0.1% BSA-PBS and 3,3,5,5-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in substrate buffer were used to induce a color reaction, which was stopped after ~35 min by adding H2SO4. Color intensity was measured using an ELISA reader set at 450 nm. The detection window was 12.5 to 800 pg/ml.

Assessment of dietary protein-derived phenylalanine uptake. Plasma amino acid concentrations were determined by HPLC after deproteinization. The latter was done on ice with 10 mg of dry 5-sulfosalicylic acid, which was mixed with 100 μl of plasma and centrifuged, after which clear supernatant was collected. HPLC was used to determine plasma amino acid concentrations after precolumn derivatization with o-phthalaldehyde (28). Plasma phenylalanine enrichment was measured using its β-butyldimethylsilyl derivative, and its 13C and H enrichments were determined by electron ionization gas chromatography in combination with mass spectrometry (GC-MS; 6890 GC/5973N MSD; Agilent, Little Falls, DE). Molecular masses 336, 337, and 341 were assessed for unlabeled phenylalanine, l-13C phenylalanine, and ring-2H5 phenylalanine, respectively (32). Standard regression curves were used in all isotopic enrichment analyses. Enrichments were calculated according to Biolo et al. (2) to correct for the presence of the 13C and 2H isotopes.

Calculations. The total and exogenous (Exo) rate of phenylalanine appearance (Rexo) and plasma availability of phenylalanine (Pheplasma) were determined by modified Steele equations (5, 8). These parameters were calculated as

\[
\text{Total} \ Rexo = \frac{F - pV \cdot C(t) \cdot dE_jv/dt}{E_{jv}(t)}
\]

\[
\text{Exo} \ Rexo = \frac{\text{Total} \ Rexo \cdot E_{p\theta}(t) + pV \cdot dE_{p\theta}/dt}{E_{prot}}
\]

\[
\text{Pheplasma} = \frac{\text{AUC}_{\text{Exo} \ Rexo} \cdot R_{\text{ex}}}{\text{Phe}_{\text{prot}} \cdot 100}
\]

where F is the intravenous tracer infusion rate, pV is the distribution volume for phenylalanine (pV = 0.125) (14), C(t) is the mean plasma phenylalanine concentration between two consecutive time points, dE_jv/dt is the time-dependent variation of plasma phenylalanine derived from the intravenous tracer, and E_{jv}(t) is the mean...
plasma phenylalanine enrichment from the intravenous tracer between two consecutive time points. \( E_{\text{prot}} \) is the mean plasma phenylalanine enrichment form the oral tracer, \( dE_{\text{prot}}/dt \) represents the time-dependent variations in plasma enrichment from the oral tracer, \( E_{\text{int}} \) is the \( \ell \)-\([1-\text{13C}] \)phenylalanine enrichment in the dietary protein, \( \text{PhE}_{\text{prot}} \) is the amount of dietary phenylalanine ingested. AUC\(_{\text{Exo R}} \) is the area under the curve (AUC) of \( \text{Exo R} \), corresponding to the amount of dietary phenylalanine appearing in the circulation over the 6-h period after intake of the drink.

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism (version 5.00, GraphPad Software for Windows, San Diego CA). All data are presented as means ± SE. Normal distribution was tested using the D’Agostino and Pearson omnibus test. Paired data were analyzed by Friedman test/Wilcoxon signed rank test or repeated-measures analysis of variance/Student’s paired \( t \)-test. Unpaired variables were assessed by Mann-Whitney \( U \)-test or Student’s unpaired \( t \)-test. Correlation analysis was performed and expressed using Spearman correlation coefficient \((r_s)\). Linear regression was used to visualize the correlation. Correlations between plasma I-FABP levels and protein digestion and absorption rates were determined in samples collected immediately after rest/exercise and 45 min after rest/exercise, respectively, since we expected this to be the time interval in which the largest differences could be observed. A \( P \) value of <0.05 was considered statistically significant.

**RESULTS**

Small intestinal injury following resistance-type exercise. The 12 participants assigned to the exercise experiment all completed the 30-min standardized exercise protocol, consisting of 5-min cycling and resistance-type leg exercises. Intensity of cycling and leg exercises was based on the individual’s body weight and maximal strength, respectively. Plasma levels of I-FABP increased from 254 ± 31 pg/ml at baseline to 344 ± 53 pg/ml measured directly after the last leg exercise, i.e., an increase of 35.4% from baseline \((P < 0.05)\), while I-FABP levels did not change in resting conditions (Fig. 1). Plasma I-FABP levels after exercise, depicted as a percentage from baseline, were increased compared with levels observed in the resting control treatment \((P < 0.05)\; \text{Fig. 1}\).

Uptake of amino acids from ingested dietary protein. Digestion and absorption of dietary \( \ell \)-\([1-\text{13C}] \)phenylalanine-labeled protein were studied in 12 athletes after 30 min at rest and in 12 athletes after a single bout of exercise. In both resting and postexercise conditions, a trend toward increased plasma I-FABP levels was associated with protein intake (total increase of ~17% compared with preintake levels; \( n = 24, P = 0.06; \text{Fig. 1} \)). Oral intake of the labeled dietary protein increased plasma phenylalanine levels in both the resting and postexercise condition, reaching peak levels 30 min after protein intake (Fig. 2A). Plasma phenylalanine concentrations were higher in resting compared with postexercise conditions \((P < 0.0001; \text{Fig. 2A})\). Ingestion of the labeled dietary protein not only elevated the circulating levels of phenylalanine, but also increased plasma concentrations of other essential amino acids, both in resting and postexercise conditions (Fig. 2B). In line with the phenylalanine data, the postprandial rise in plasma essential amino acid levels was attenuated in the postexercise situation compared with the resting condition \((P < 0.0001; \text{Fig. 2B})\).

In the postexercise situation, plasma \( \ell \)-\([1-\text{13C}] \)phenylalanine enrichments were lower compared with resting conditions \((P < 0.05; \text{Fig. 3A})\). In line with this observation, a trend toward a lower AUC\(_{0-120 \text{ min}} \) of \( \text{Exo R} \) was observed after exercise when compared with resting conditions \((16 ± 2.0 \text{ vs. } 20 ± 1.5 \text{ mmol phenylalanine-kg body mass}^{-1} \text{-min}^{-1}, \text{respectively}; P = 0.09)\). Following protein consumption, plasma exogenous phenylalanine appearance rates increased in both
rS vivo dietary protein digestion and absorption rates (subset of exercising participants were analyzed (P<0.05). Plasma enrichment of L-[1-13C]phenylalanine (Phe) rate of appearance was decreased in the 0–120 min postexercise recovery period when compared with resting conditions (P<0.001). Data are expressed as means ± SE.

resting and postexercise conditions, reaching peak levels 30–45 min after protein ingestion (Fig. 3B). Interestingly, plasma phenylalanine appearance rates derived from the ingested dietary protein were lower during postexercise recovery when compared with resting conditions. Exogenous phenylalanine plasma appearance rates peaked at 0.18 ± 0.04 mmol phenylalanine·kg lean body mass⁻¹·min⁻¹ after exercise compared with 0.23 ± 0.03 mmol phenylalanine·kg lean body mass⁻¹·min⁻¹ at rest (Fig. 3B).

Exercise-induced intestinal injury attenuates postexercise dietary protein digestion and absorption kinetics. Changes in plasma I-FABP levels at rest or immediately after exercise correlated with in vivo dietary protein digestion and absorption rates (rS = −0.57; n = 24, P < 0.01 at 45 min). Specific analysis of the subset of exercising participants revealed a correlation between changes in plasma I-FABP levels with in vivo dietary protein digestion and absorption rates (rS = −0.62, n = 12, P < 0.05 at 45 min; Fig. 4). A correlation was observed between the total peak area of plasma I-FABP levels and total exogenous phenylalanine uptake (rS = −0.41, n = 24, P < 0.05). The latter correlation was also found if only the subset of exercising participants were analyzed (rS = −0.62, n = 12, P < 0.05 at 45 min).

DISCUSSION

The current study shows that resistance-type exercise increases plasma I-FABP levels, reflecting small intestinal cellular injury. In line with this observation, postprandial dietary protein digestion and absorption kinetics are shown to be attenuated during acute postexercise recovery. Plasma levels of I-FABP correlated with the appearance of the dietary protein-derived amino acids in the circulation, indicating that exercise-induced small intestinal injury impairs dietary protein digestion and absorption kinetics during acute postexercise recovery.

Previously, we demonstrated that exercise-induced small intestinal injury is caused by splanchnic hypoperfusion (30). Splanchnic hypoperfusion during exercise is a result of rapid redistribution of blood from the abdominal region toward the active muscle groups (7) and is known to predominantly affect the mature enterocytes at the upper half of the intestinal villi. The reason for the susceptibility of these cells to hypoperfusion is the countercurrent exchange mechanism in the villi, resulting in a low-flow and low-oxygen environment at the top of the villi (3, 4). The reduction of the splanchnic blood flow during exercise contributes to the physiological low-flow state of these enterocytes, further depriving the cells from nutrients and oxygen. I-FABP is a small protein present in the susceptible mature enterocytes and is rapidly released upon cellular injury due to hypoperfusion and ischemia (10, 13). The noted rise in I-FABP during exercise found in this study suggests the occurrence of ischemia-induced injury. These observations are in line with previous data showing a twofold increase in baseline I-FABP levels after a single 60-min session of endurance-type exercise (30). Similar plasma I-FABP levels have been reported in patients with splanchnic hypoperfusion during nonabdominal surgery (increase of ~60% from presurgical levels) (11) and in trauma patients brought into the emergency room (9). These data imply that the observations in the present study are not only relevant for athletes but may also be of important clinical relevance for more compromised patient groups.

Interestingly, a trend toward increasing plasma I-FABP levels was observed during the first 15 min after consuming a single 20-g bolus of dietary protein, both in rest and after exercise, suggesting that protein intake slightly disturbs enterocyte integrity. This observation might be explained by the fact that the presence of food in the GI tract rapidly increases the need for splanchnic blood flow (27). We speculate that in the short time period directly after food intake, splanchnic blood flow may not be adequate, leading to minor GI compromise. Nonetheless, this rapid postprandial increase in plasma I-FABP
levels is relatively small when compared with the substantial 40-100% increase in I-FABP levels observed after resistance-type or endurance-type exercise.

The production and use of intrinsically labeled milk protein in combination with continuous intravenous infusion of the tracer L-[ring-2H5]phenylalanine in this study provide a unique opportunity to assess dietary protein digestion and absorption in an in vivo human model (26, 29). Immediately after protein ingestion, protein-derived phenylalanine appeared rapidly in the circulation both at rest and after exercise. The postprandial rise in plasma 1-13C phenylalanine appearance rate was attenuated in the acute postexercise recovery period when compared with resting conditions. Uptake rates were lower after exercise than at rest, leading to reduced protein uptake during acute postexercise recovery. Furthermore, lower postprandial increases in plasma essential amino acid concentrations were observed after resistance-type exercise when compared with resting conditions. Altogether, these data show that dietary protein digestion and absorption are compromised during acute postexercise recovery. The latter is of considerable interest as dietary protein digestion and absorption kinetics are at least partly responsible for the postprandial muscle protein synthetic response to food intake (17, 25). However, despite diminished dietary protein digestion and absorption, postprandial muscle protein fractional synthesis rates were found to increase after exercise compared to rest in this population (25). In addition, another study showed that especially in the immediate postexercise period protein intake stimulates muscle protein synthesis compared with late protein intake (19). Hence, we do not believe that protein intake should be delayed based on the current findings; but, since even small changes can make the difference between winning and losing in athletic competition, it may be useful to determine whether such a difference in absorption found in this study has a meaningful effect on postexercise net muscle protein balance. Because a more rapid supply of amino acids following exercise has previously been shown to maximize postexercise muscle protein synthesis rates and improve the skeletal muscle adaptive response to more prolonged exercise training, new insights on gastrointestinal functioning may be used for fine-tuning the nutritional strategies to meet both gastrointestinal and muscle capacity. Our present findings indicate that dietary protein digestion and absorption are compromised in the postexercise recovery phase. In agreement, we previously demonstrated that exercise especially affects the proximal part of the GI tract (30), which is the primary location of dietary protein digestion and absorption (22). To further clarify the effects of exercise on postexercise dietary protein uptake, the relationship between exercise-induced intestinal injury and plasma appearance of the protein-derived amino acids was studied. Data revealed a strong, negative relation between plasma I-FABP levels and plasma appearance rates of exogenous phenylalanine. The presence of such a significant correlation between plasma I-FABP levels and plasma appearance rates of exogenous phenylalanine is needed to address the impact of exercise on gut function and the subsequent delivery of exogenous amino acids to allow postexercise muscle protein synthesis in an elderly population.

**Perspectives and Significance**

From this study, we conclude that exercise-induced loss of enterocyte integrity attenuates dietary protein digestion and absorption, impairing dietary protein-derived amino acid uptake during acute postexercise recovery in healthy, young recreationally active individuals. These data indicate that work is needed to address the impact of exercise on GI integrity and GI function. Dietary strategies need to consider compromised gut function, and food intake regimens should be modulated to allow proper nutrient delivery during exercise and subsequent acute postexercise recovery. The latter could be of considerable interest to optimize postexercise muscle reconditioning in both health and disease.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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