ABSTRACT
Background: Protein consumed after resistance exercise increases postexercise muscle protein synthesis rates. To date, dairy protein has been studied extensively, with little known about the capacity of other protein-dense foods to augment postexercise muscle protein synthesis rates.
Objective: We aimed to compare protein digestion and absorption kinetics, postprandial amino acid availability, anabolic signaling, and the subsequent myofibrillar protein synthetic response after the ingestion of milk compared with beef during recovery from resistance-type exercise.
Design: In crossover trials, 12 healthy young men performed a single bout of resistance exercise. Immediately after cessation of exercise, participants ingested 30 g protein by consuming isotopically labeled beef or milk. Blood and muscle biopsy samples were collected at rest and after exercise during primed continuous infusions of [ring-2H5]phenylalanine and [ring-3,5-2H2]tyrosine to assess protein digestion and absorption kinetics, plasma amino acid availability, anabolic signaling, and subsequent myofibrillar protein synthesis rates in vivo in young men.
Results: Beef protein–derived phenylalanine appeared more rapidly in circulation compared with milk ingestion (P < 0.001). The availability of phenylalanine during the 5-h postexercise period tended to be higher after beef (64% ± 3%) ingestion than after milk ingestion (57% ± 3%; P = 0.08). Both beef and milk ingestion were followed by an increase in the phosphorylation of mammalian target of rapamycin complex 1 and 70-kDa S6 protein kinase 1 during postexercise recovery. Milk ingestion increased myofibrillar protein synthesis rates to a greater extent than did beef ingestion during the 0- to 2-h postexercise phase (P = 0.013). However, the increase in myofibrillar protein synthesis rates did not differ between milk and beef ingestion during the entire 0- to 5-h postexercise phase (P = 0.114).
Conclusions: Both milk and beef ingestion augment the postexercise myofibrillar protein synthetic response in young men, with a stronger stimulation of myofibrillar protein synthesis during the early postprandial stage after milk ingestion. This trial was registered at www.clinicaltrials.gov as NCT01578590.

The American Journal of Clinical Nutrition

828

828

Nicholas A Burd,3 Stefan H Gorissen,3 Stephan van Vliet, Tim Snijders, and Luc JC van Loon*

NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, Netherlands

INTRODUCTION

The postprandial muscle protein synthetic response to protein ingestion has been shown to be modulated by dietary protein digestion and absorption kinetics (1, 2) and the amino acid composition (2, 3) of the ingested protein source. Both of these variables have been shown to affect the postprandial rise in circulating leucine concentrations and (essential) amino acid availability, thereby modulating the postprandial increase in muscle protein synthesis rates (2, 3). Studies that have investigated the postprandial or postexercise muscle protein synthetic response after the ingestion of various protein sources have mainly used isolated dairy (whey and casein) or soy proteins (2–5). However, the consumption of specific isolated protein sources (e.g., isolated protein powders) is not general practice because protein-dense foods, such as dairy milk or meat, are more commonly consumed. The consumption of whole foods implies that the protein is consumed within a specific food matrix with other macronutrients being available. Recent work has shown that carbohydrate coingestion with protein can modulate digestion and absorption kinetics compared with ingesting protein only (6). Such observations underline the necessity of studying digestion and amino acid absorption kinetics and the subsequent postprandial muscle protein synthetic response after the ingestion of protein-dense foods, as opposed to the ingestion of merely isolated protein fractions. Milk and beef are common protein-dense foods that are consumed within the Western diet and responsible for providing a large percentage of daily protein intake (7, 8). Interestingly, little research has been performed that assesses dietary protein digestion and absorption kinetics after the ingestion of dietary protein within its natural food matrix. Furthermore, no study has directly compared the muscle protein synthetic response with the ingestion of high-quality animal-derived proteins such as milk compared with beef.

This study compares protein digestion and absorption kinetics and the subsequent myofibrillar protein synthetic response after the ingestion of milk and beef during recovery.
from resistance-type exercise in young men. To provide a mechanistic basis for the regulation of myofibrillar protein synthesis after food ingestion during postexercise recovery, we also examined the phosphorylation status of key proteins involved in nutrient sensing and muscle anabolic signaling. To our knowledge, this is the first study to assess protein digestion and amino acid absorption kinetics and the subsequent postprandial myofibrillar protein synthetic response after the ingestion of 2 of the main protein-dense foods (milk and beef) commonly found in the Western diet in vivo in humans.

**METHODS**

**Participants and ethical approval**

Twelve healthy young men [mean ± SEM: age: 22 ± 1 y; body weight: 74 ± 3 kg; body fat: 13% ± 1%; BMI (in kg/m²): 22 ± 0.4] volunteered to participate in this study. Participants were recreationally active and engaged in exercise at least 2 times/wk for ≥1 y. All participants were deemed healthy based on their response to a routine medical screening questionnaire. Participants were informed of the purpose of the study, experimental procedures, and all its potential risks before providing written consent to participate. Volunteers had no history of participating in stable isotope amino acid tracer experiments. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre+, Maastricht, Netherlands, and conformed to standards for the use of human participants in research as outlined in the sixth version of the Declaration of Helsinki.

**Experimental design**

A within-subject crossover design was used for this study. Two weeks before the infusion trials, participants reported to the laboratory for familiarization with the exercise equipment and for maximum strength tests as determined by their one-repetition maximum for leg press (mean ± SEM: 217 ± 12 kg) and knee extension exercise (mean ± SEM: 132 ± 8 kg). In addition, body weight and height were measured as well as body composition by dual-energy X-ray absorptiometry (Discovery A; Hologic). Participants were instructed to refrain from vigorous physical activity for 3 d before each tracer infusion and to report their dietary intake in a food diary for 2 d before each infusion trial (Supplemental Table 1). All participants consumed a standardized meal of the same composition (mean ± SD): 32 ± 1 kJ/kg body weight, providing 33% of energy of carbohydrate, 51% of energy of fat, and 16% of energy of protein) the evening before each tracer infusion trial.

Participants were randomly assigned to consume skim milk or an isonitrogenous amount of minced beef during 2 subsequent infusions. Particpants were assigned to consume skim milk or an isonitrogenous amount of minced beef during 2 subsequent infusion trials. The time between crossover trials was 8–20 d (mean ± SD: 14 ± 1 d). On completion of the first infusion trial, a copy of the food diary was returned to the participants, and they were subsequently instructed to maintain their previously recorded dietary habits in the 2 d leading into the second infusion trial.

**Infusion protocol**

On the trial days, the participants reported to the laboratory at 0800 h after an overnight fast. A Teflon catheter was inserted into an antecubital vein in the hand and placed in a hot box (60°C) for arterialized blood sampling. After baseline blood sample collection (t = −240 min), the plasma phenylalanine and tyrosine pools were primed with a single intravenous dose of L-[ring-2H₅]phenylalanine (2 μmol/kg) and L-[ring-3,5-²H₂]tyrosine (0.615 μmol/kg). Subsequently, an intravenous infusion of L-[ring-2H₅]phenylalanine (infusion rate of 0.05 μmol·kg⁻¹·min⁻¹) and L-[ring-3,5-²H₂]tyrosine (0.15 μmol·kg⁻¹·min⁻¹) was initiated and maintained until the end of the trial. To minimize the number of biopsy samples collected and to provide a reference value for the assessment of postabsorptive myofibrillar protein synthesis rates (9–11), we collected a single resting muscle biopsy sample after 180 min of infusion. Afterward, the participants performed resistance-type exercise that consisted of 4 sets of 8–10 repetitions to volitional fatigue for both the leg press and knee extension exercise (t = −60 min). After the completion of the exercise bout, a muscle biopsy sample was collected, and subsequently the participants consumed 158 g (providing 30 g protein) minced beef or an isonitrogenous amount of nonfat skim milk (t = 0 h; Table 1). Additional muscle biopsy samples were obtained after 2 and 5 h of postexercise recovery. Arterialized blood samples were drawn every 30 or 60 min during the postabsorptive and postprandial states. The exercise external work (repetitions × load) was matched between the first (~9400 kg) and second (~9300 kg) trials. The biopsy samples were collected from the middle region of the vastus lateralis (15 cm above the patella) through separate incisions with a Bergstrom needle under local anesthesia. On each trial, the first resting biopsy sample was obtained from one leg (randomized) and the 3 postexercise biopsy samples were obtained from the contralateral leg. All biopsy samples were freed from any visible adipose tissue and blood, immediately frozen in liquid nitrogen, and stored at −80°C until subsequent analysis.

**TABLE 1**

Macronutrient and energy composition of the ingested meals

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Skim milk</th>
<th>Minced beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>EAAs, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.65</td>
<td>1.59</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.71</td>
<td>2.48</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.24</td>
<td>2.81</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.71</td>
<td>0.79</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.37</td>
<td>1.43</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.20</td>
<td>1.21</td>
</tr>
<tr>
<td>Valine</td>
<td>1.85</td>
<td>1.59</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.81</td>
<td>1.11</td>
</tr>
<tr>
<td>ΣEAAs, g</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Leucine by total amino acids, %</td>
<td>10.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>30.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Fat, g</td>
<td>0.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>247</td>
<td>164</td>
</tr>
</tbody>
</table>

4Abbreviations used: mTORC1, mammalian target of rapamycin complex 1; p70S6K1, 70-kDa S6 protein kinase; rpS6, ribosomal protein S6; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1.

1Amounts are shown per bolus (350 mL milk compared with 158 g beef). EAA, essential amino acid.

2ΣEAA includes Ile, Leu, Lys, Met, Phe, Thr, Val, and His. Trp was not measured.
Meal composition

The intrinsically labeled beef and milk was obtained by the infusion of L-[1,13C]phenylalanine into a lactating Holstein cow as described previously (12). The beef was minced before packaging and stored at −18°C. Before the infusion, the minced beef was thawed overnight in a refrigerator at 4°C. The minced beef was prepared into a patty (158 g) and grilled until the inner temperature reached 65°C. The 158-g minced beef patty provided 30 g protein intrinsically labeled with L-[1,13C]phenylalanine at an enrichment of 1.7 mole percent excess. The milk (skim milk powder; FrieslandCampina) was enriched to match the L-[1-13C]phenylalanine–labeled milk and beef combined with the intravenous infusion of L-[ring-2H5]phenylalanine and L-[ring-3,5-2H2]tyrosine. Total, exogenous, and endogenous phenylalanine rate of appearance, total rate of disappearance, and plasma availability of dietary protein–derived phenylalanine (the fraction of the dietary phenylalanine that appeared in systemic circulation) were calculated with modified Steele equations (19, 20). The fractional synthetic rates of myofibrillar protein were calculated with standard precursor-product methods by dividing the increment in tracer enrichment in the myofibrillar protein by the enrichment in the plasma precursor pool over time (10).

Plasma analyses

Plasma glucose and insulin concentrations were analyzed with commercially available kits [Glucose HK Gen.3, reference: 05168791190 (Roche) and Insulin, reference: 12017547122 (Roche)]. Plasma amino acid concentrations and enrichments were determined by gas chromatography–mass spectrometry analysis (Agilent 7890A GC/5975C; MSD), as previously described (6).

Western blotting

Phosphorylation status of mammalian target of rapamycin complex 1 (mTORC1) at Ser2448, 70 kDa S6 protein kinase 1 (p70S6K1) at Thr389, eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) at Thr37/46, and ribosomal protein S6 (rpS6) at Ser240/244 were quantified relative to total protein content as described in our previous work using antibodies from Cell Signaling (13).

Myofibrillar protein synthesis

Myofibrillar protein–enriched fractions were isolated as described in our previous work (14). Myofibrillar protein–bound enrichments were determined by gas chromatography–mass spectrometry analysis. To reduce the signal-to-noise ratio during gas chromatography–mass spectrometry analysis at low tracer enrichments, we enzymatically decarboxylated the phenylalanine from the myofibrillar protein hydrolysates to β-phenylethylamine (15) before tert-butyldimethylsilyl (tBDMS) derivatization (16, 17). Enrichments of the protein-bound samples were determined by selected ion monitoring for β-phenylethylamine–tBDMS mass-to-charge ratio at 183 (m+5) to 180 (m+2) and a single linear standard curve (to avoid slope influences on the measured enrichment) from mixtures of known m+5 to m+2 ratios. To avoid saturation of the mass spectrometer and eliminate bias due to any potential concentration dependencies (18), we adjusted the split ratio before the injection of each sample so that nearly equal amounts of phenylalanine were injected for all samples and standards.

Calculations

Whole-body amino acid kinetics were assessed in nonsteady conditions by the ingestion of intrinsically L-[1,13C]phenylalanine–labeled milk and beef combined with the intravenous infusion of L-[ring-2H5]phenylalanine and L-[ring-3,5-2H2]tyrosine. Total, exogenous, and endogenous phenylalanine rate of appearance, total rate of disappearance, and plasma availability of dietary protein–derived phenylalanine (the fraction of the dietary phenylalanine that appeared in systemic circulation) were calculated with modified Steele equations (19, 20). The fractional synthetic rates of myofibrillar protein were calculated with standard precursor-product methods by dividing the increment in tracer enrichment in the myofibrillar protein by the enrichment in the plasma precursor pool over time (10).

Statistics

A within-subject crossover design was used for the study. Differences in myofibrillar protein synthesis, whole-body amino acid kinetics, and muscle anabolic signaling were tested by 2-factor (treatment × time) repeated-measures ANOVA. When statistically significant interaction effects were observed, Bonferroni post hoc tests were performed to locate these differences. A power calculation based on previous research (2, 3) indicated that n = 10 was sufficient to detect differences in the postprandial muscle protein synthetic response to the ingestion of different protein sources when using a 2-sided statistical test (P < 0.05, 80% power). Considering a potential dropout rate of 20% during the protocol, the final number of participants recruited was 12. For all analyses, differences were considered statistically significant at P < 0.05. All calculations were performed with SPSS Statistics version 20 (SPSS Inc.). All data are expressed as means ± SEMs.

RESULTS

Plasma metabolites

Plasma glucose concentrations increased after milk ingestion (time × treatment, P < 0.001). Plasma glucose concentrations reached higher peak values (6.9 ± 0.3 compared with 5.9 ± 0.1 mmol/L) after milk than after beef ingestion, respectively (P = 0.003; Figure 1A). Plasma insulin concentrations increased after milk ingestion during recovery from exercise (P < 0.001). As such, plasma insulin concentrations reached higher peak values of 45 ± 6 compared with 17 ± 2 μU/L after milk than after beef ingestion, respectively (P < 0.001; Figure 1B). Plasma phenylalanine concentrations rapidly increased after milk and beef ingestion (P < 0.001), with plasma phenylalanine concentrations reaching higher peak values after beef than after milk ingestion (80 ± 2 compared with 73 ± 2 μmol/L, respectively; P < 0.001; Figure 2A). Plasma leucine concentrations rapidly increased after milk and beef ingestion (P < 0.001). Plasma leucine concentrations showed a more rapid increase during the first 30 min after milk than after beef ingestion. However, plasma leucine concentrations reached peak values of 277 ± 12 and 231 ± 11 μmol/L, (P = 0.002) at 115 ± 8 min and 135 ± 26 min after beef and milk ingestion, respectively (Figure 2B). Plasma tyrosine concentrations transiently increased after protein ingestion (P < 0.001). The postprandial rise in tyrosine concentrations was greater after beef than after milk ingestion (time × treatment: P < 0.001; Supplemen tal Figure 1).
Plasma tracers and whole-body amino acid kinetics

Basal plasma L-[ring-2H5]phenylalanine enrichments did not differ between treatments ($P = 0.89$; Figure 3A). L-[ring-2H5]phenylalanine enrichments decreased after milk and beef ingestion ($P < 0.001$), with a more rapid decline in L-[ring-2H5]phenylalanine enrichments after beef ingestion ($time \times treatment$ interaction, $P = 0.001$). Plasma L-[1-13C]phenylalanine enrichments increased to a greater extent after ingestion of beef than after ingestion of milk ($time \times treatment$ interaction, $P < 0.001$; Figure 3B).

Exogenous phenylalanine rates of appearance (representing the appearance of dietary protein–derived phenylalanine into the circulation) increased after beef or milk ingestion ($time$ effect, $P < 0.001$; $time \times treatment$, $P = 0.001$). Exogenous phenylalanine appearance rates were higher between 60 and 150 min after ingestion of beef than after ingestion of milk ($time \times treatment$ interaction, $P < 0.001$; Figure 4A). The amount of dietary protein–derived phenylalanine that appeared in the circulation over the 5-h postprandial period tended to be higher after ingestion of beef than after ingestion of milk ($64% \pm 3\%$ and $57% \pm 4\%$, respectively; $P = 0.08$). Endogenous phenylalanine rates of appearance (representing the appearance of phenylalanine derived from whole-body protein breakdown into the circulation) were lowered after beef or milk ingestion ($time$ effect, $P < 0.001$; $time \times treatment$, $P = 0.02$; Figure 4B). Total phenylalanine rates of appearance and total phenylalanine rates of disappearance increased to a greater extent after beef than after milk ingestion ($time \times treatment$ interaction, $P < 0.001$ and $P < 0.001$, respectively; Figure 4C and D). Phenylalanine can be converted (oxidized) to tyrosine. Intravenous infusions of L-[ring-2H6]phenylalanine and L-[ring-3,5-2H2]tyrosine were applied, and plasma L-[ring-2H6]phenylalanine, L-[ring-2H6]tyrosine, and L-[ring-3,5-2H2]tyrosine enrichments were measured to allow for the assessment of phenylalanine oxidation rates. Phenylalanine oxidation rates did not differ ($P = 0.51$) between the beef ($7.2 \pm 0.3 \mu mol \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and milk treatments ($7.4 \pm 0.3 \mu mol \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$).

Muscle anabolic signaling

Immediately after resistance exercise, mTORC1 phosphorylation did not differ from resting, baseline conditions. During postexercise recovery, mTORC1 phosphorylation increased at 2 h ($P = 0.006$) and returned to baseline values at 5 h ($P = 0.64$), with no differences between the milk and beef conditions ($P > 0.05$; Figure 5A). Phosphorylation of 4E-PB1 was...
Myofibrillar protein synthesis

Myofibrillar protein synthesis rates increased after milk and beef ingestion during both the early (0–2 h) and late (2–5 h) postprandial phase (128% ± 23% and 91% ± 15% above baseline values; P < 0.001; Figure 6). During the early postprandial phase (0–2 h), milk ingestion resulted in a greater myofibrillar protein synthetic response than did beef ingestion (P = 0.013). However, the cumulative myofibrillar protein synthetic response calculated over the entire 5-h postprandial phase did not differ (P = 0.114; 95% CI: −0.033, 0.004) between ingestion of beef (0.057 ± 0.006% · h⁻¹) and ingestion of milk (0.071 ± 0.005% · h⁻¹).

DISCUSSION

The present study demonstrated that the ingestion of beef resulted in a more rapid release of protein-derived phenylalanine into the circulation and greater postprandial plasma amino acid availability throughout 5 h of postexercise recovery compared with the ingestion of an isonitrogenous amount of milk. Despite the more rapid postprandial rise in plasma amino acid availability after beef as opposed to milk ingestion, milk ingestion resulted in a greater myofibrillar protein synthetic response during the early stages of the postprandial period (0–2 h). The overall postprandial myofibrillar protein synthetic response assessed over 5 h of postexercise recovery did not differ between milk and beef consumption.

Various studies have investigated the postprandial muscle protein synthetic response after the ingestion of isolated protein sources (such as soy, casein, or whey protein) to show that the rate of protein digestion and amino acid absorption and the subsequent rise in plasma amino acid availability (with leucine being of particular interest) form key factors that modulate the postprandial muscle protein synthetic response (1–4). In the present study, we assessed the impact of ingesting protein-dense foods (milk and beef) on dietary protein digestion and absorption kinetics and the subsequent postprandial myofibrillar protein synthetic response. The protein meals contained different amounts of carbohydrate and fat and thus varied in energy content (Table 1). However, previous work has shown that the energy content of a meal modulates dietary protein digestion and absorption kinetics (6) but does not affect postprandial or postexercise muscle protein synthesis rates (6, 21, 22).

Here, we observed a more rapid protein digestion and amino acid absorption after beef ingestion, which resulted in a greater rise in plasma amino acid availability (64% ± 3% compared with 57% ± 3%; P < 0.05) and higher peak plasma leucine concentrations (277% ± 12% μM compared with 231% ± 11% μM; P < 0.05; Figure 2B) in the beef compared with the milk treatment, respectively. Although this would predict a greater postprandial myofibrillar protein synthetic response after beef than after milk ingestion, we did not observe any statistically significant differences in the overall 5-h myofibrillar protein synthetic response between treatments (0.057 ± 0.006% · h⁻¹ and 0.071 ± 0.005% · h⁻¹, respectively; P > 0.05; Figure 6). Interestingly, we observed an even greater myofibrillar protein synthetic response after milk than after beef ingestion during the early (0–2 h) postprandial phase (Figure 6). This greater early myofibrillar protein synthetic response after milk than after beef ingestion may be attributed to the small but rapid rise in circulating plasma leucine (Figure 2B) and/or insulin (Figure 1B) concentrations during the first 30 min after milk ingestion. This rapid rise in circulating plasma leucine concentrations after milk ingestion may have initiated an accelerated rise in postprandial myofibrillar protein synthesis rates and allowed for a greater myofibrillar protein synthetic response during the early stages of the postexercise recovery in the milk treatment than in the beef treatment. It is generally assumed that the stimulation of postprandial muscle...
protein synthesis is attributed to the acute rise in plasma essential amino acid availability and is independent of the effects of increased insulinemia after food intake in healthy young or older adults (23). Hence, it is unlikely that the greater postprandial rise in circulating insulin after milk ingestion is responsible for the greater early postprandial myofibrillar protein synthetic response (0–2 h) compared with the ingestion of beef. However, the cumulative postprandial myofibrillar protein synthetic response (0–5 h) after milk and beef ingestion did not show a statistically significant difference between treatments (P = 0.12), despite greater postprandial amino acid availability and higher peak leucine concentrations after beef ingestion. The cumulative muscle protein synthetic response likely provides more insight into the efficacy of protein intake after exercise to augment gains in muscle mass (24). The fact that the greater postprandial plasma leucine availability after beef compared with milk ingestion did not lead to a greater postexercise muscle protein synthetic response may be, at least partly, explained by the increased sensitivity of skeletal muscle tissue to circulating leucine during recovery from exercise (25). In addition, the generous amount of protein provided via milk or beef ingestion (both 30 g protein) may have precluded our ability to detect differences in the entire 0- to 5-h postexercise myofibrillar protein synthetic response between beef and milk ingestion.

Alternatively, it has also been speculated that milk has various other anabolic properties that may explain the absence of a difference in the postprandial muscle protein synthetic response between groups. For example, milk is a potent insulin secretagogue (26) (Figure 1B). However, it is unlikely that the greater postprandial rise in circulating insulin further increased postprandial muscle protein synthesis rates because the postprandial insulin response has been reported to be permissive rather than stimulatory under conditions of increased plasma amino acid availability (22, 23, 27). Moreover, bioactive peptides in milk (28) or the endogenous release of microRNAs into circulation after milk ingestion (29, 30) may have served as an anabolic signal to peripheral tissues (muscle). It is worth noting that Wilkinson et al. (31) showed that milk consumption immediately after the cessation

---

**FIGURE 4** Whole-body phenylalanine kinetics. Mean ± SEM exogenous Rₐ (A), endogenous Rₐ (B), total Rₛ (C), and total Rₐ (D) in the fasting state and after the ingestion of beef (n = 12) or milk (n = 12) during postexercise recovery. The gray area corresponds to the exercise bout; the dashed line refers to protein ingestion. *Treatment conditions differ at these time points. Data were analyzed with a 2-factor repeated-measures ANOVA with time and treatment as within-subject factors. A Bonferroni post hoc test was used to locate differences between means for all statistically significant interactions. Exogenous Rₐ: time × treatment interaction, P < 0.001. Endogenous Rₐ: time × treatment interaction, P = 0.12; time effect, P < 0.001; treatment effect, P = 0.02. Total Rₛ: time × treatment interaction, P = 0.001. Total Rₐ: time × treatment interaction, P = 0.001. Beef, minced beef; Milk, skim milk; Rₐ, rate of appearance; Rₛ, rate of disappearance.
of resistance-type exercise resulted in higher postexercise muscle protein synthesis rates compared with the ingestion of equivalent bolus of soy protein. In short, the greater myofibrillar protein synthetic response after milk than after beef ingestion during the early postprandial phase may also be partly attributed to these and/or other nutritional factors present in protein-dense foods.

The initiation of mRNA translation has been reported to be primarily responsible for the regulation of muscle protein synthesis (32). Translation initiation is largely controlled by the activation of mTORC1-related signaling molecules (33). Exercise and protein ingestion have been shown to activate mRNA translation initiation through the mTORC1 pathway (34–36). As such, it is often suggested that maximizing mTORC1 activation throughout postexercise recovery is essential for facilitating the skeletal muscle adaptive response (37). Here, the examination of mTORC1, 4E-BP1, p70S6K1, or rpS6 phosphorylation provided

*Significantly different compared with rest.
†Significantly different compared with immediately after resistance exercise (t = 0 h).

Beef, minced beef; Milk, skim milk; mTOR, mammalian target of rapamycin; Phos, phosphorylated; p70S6K1, 70-kDa S6 protein kinase 1; rpS6, ribosomal protein S6; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1.
measures ANOVA with time and treatment as within-subject factors. A Bonferroni post hoc test was used to locate differences between means for all statistically significant interactions. Time × treatment interaction, $P = 0.006$; time effect, $P < 0.001$; treatment effect, $P = 0.055$. *Significantly different compared with rest. $^\ddagger$Significantly different between treatments. Beef, minced beef; FSR, fractional synthesis rate; Milk, skim milk.

Little insight into the mechanisms that govern changes in myofibrillar protein accretion after milk or beef ingestion (Figure 4). We have previously shown that the ingestion of carbohydrate and protein during recovery from exercise further elevated the phosphorylation of mTORC1 signaling-related proteins compared with the ingestion of carbohydrates alone (34). In the current study, we did not observe any differences in the protein phosphorylation status throughout postexercise recovery after milk compared with beef ingestion. It might be possible that the mTORC1 pathway was being activated via different mechanisms between the milk and the beef condition. For example, the higher leucinemia after beef ingestion may have been a principle driver, whereas in the milk condition, other factors (e.g., postprandial insulin response, microRNAs, or bioactive peptides) may have been responsible for activating mTORC1 signaling proteins. The reduced phosphorylation of 4E-BP1 immediately after exercise is consistent with previous observations (36) and likely represents a shift of energy provision to fuel muscle contraction rather than the synthesis of de novo myofibrillar protein (38).

In conclusion, ingestion of 30 g protein, provided in beef or milk, during recovery from exercise strongly increases myofibrillar protein synthesis rates in young men. Beef ingestion during recovery from resistance-type exercise is followed by more rapid protein digestion and amino acid absorption, resulting in greater postprandial plasma amino acid availability and higher peak plasma leucine concentrations compared with the ingestion of an isonitrogenous amount of milk. However, milk ingestion during recovery from exercise is followed by a greater early (but not overall) postprandial myofibrillar protein synthetic response than is beef ingestion.

We thank Joan Senden, Janneke van Krabbenburg, Annemie Gijsen, and Antoine Zorenc for their analytical assistance.

The authors’ responsibilities were as follows—NAB and LJvCL: contributed to the conception and the design of the experiment; NAB, SHG, SvV, TS, and LJvCL: contributed to collection, analysis, and interpretation of data; NAB, SHG, and LJvCL: contributed to drafting or revising intellectual content of the manuscript and had primary responsibility for the final content; all authors: read, edited, and approved the final version of the manuscript. The authors declared no conflicts of interest related to this study.

![FIGURE 6 Mean ± SEM myofibrillar protein synthesis rates expressed as FSR before (rest) and after 30 g milk ($n = 12$) or beef ($n = 12$) ingestion during recovery from exercise. Data were analyzed with a 2-factor repeated-measures ANOVA with time and treatment as within-subject factors. A Bonferroni post hoc test was used to locate differences between means for all statistically significant interactions. Time × treatment interaction, $P = 0.006$; time effect, $P < 0.001$; treatment effect, $P = 0.055$. *Significantly different compared with rest. $^\ddagger$Significantly different between treatments. Beef, minced beef; FSR, fractional synthesis rate; Milk, skim milk.](#)


