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## Branched-chain amino acid and branched-chain ketoacid ingestion increases muscle protein synthesis rates in vivo in older adults: a double-blind, randomized trial

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### ABSTRACT

**Background:** Protein ingestion increases muscle protein synthesis rates. However, limited data are currently available on the effects of branched-chain amino acid (BCAA) and branched-chain ketoacid (BCKA) ingestion on postprandial muscle protein synthesis rates.

**Objective:** The aim of this study was to compare the impact of ingesting 6 g BCAA, 6 g BCKA, and 30 g milk protein (MILK) on the postprandial rise in circulating amino acid concentrations and subsequent myofibrillar protein synthesis rates in older males.

**Methods:** In a parallel design, 45 older males (age:  $71 \pm 1$  y; BMI:  $25.4 \pm 0.8$  kg/m<sup>2</sup>) were randomly assigned to ingest a drink containing 6 g BCAA, 6 g BCKA, or 30 g MILK. Basal and postprandial myofibrillar protein synthesis rates were assessed by primed continuous L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine infusions with the collection of blood samples and muscle biopsies.

**Results:** Plasma BCAA concentrations increased following test drink ingestion in all groups, with greater increases in the BCAA and MILK groups compared with the BCKA group ( $P < 0.05$ ). Plasma BCKA concentrations increased following test drink ingestion in all groups, with greater increases in the BCKA group compared with the BCAA and MILK groups ( $P < 0.05$ ). Ingestion of MILK, BCAA, and BCKA significantly increased early myofibrillar protein synthesis rates (0–2 h) above basal rates (from  $0.020 \pm 0.002\%/h$  to  $0.042 \pm 0.004\%/h$ ,  $0.022 \pm 0.002\%/h$  to  $0.044 \pm 0.004\%/h$ , and  $0.023 \pm 0.003\%/h$  to  $0.044 \pm 0.004\%/h$ , respectively;  $P < 0.001$ ), with no differences between groups ( $P > 0.05$ ). Myofibrillar protein synthesis rates during the late postprandial phase (2–5 h) remained elevated in the MILK group ( $0.039 \pm 0.004\%/h$ ;  $P < 0.001$ ), but returned to baseline values following BCAA and BCKA ingestion ( $0.024 \pm 0.005\%/h$  and  $0.024 \pm 0.005\%/h$ , respectively;  $P > 0.05$ ).

**Conclusions:** Ingestion of 6 g BCAA, 6 g BCKA, and 30 g MILK increases myofibrillar protein synthesis rates during the early postprandial phase (0–2 h) in vivo in healthy older males. The

postprandial increase following the ingestion of 6 g BCAA and BCKA is short-lived, with higher myofibrillar protein synthesis rates only being maintained following the ingestion of an equivalent amount of intact milk protein. This trial was registered at Netherlands Trial Register ([www.trialregister.nl](http://www.trialregister.nl)) as NTR6047. *Am J Clin Nutr* 2019;110:862–872.

**Keywords:** aging, sarcopenia, chronic kidney disease, anabolism, milk, dietary protein, leucine,  $\alpha$ -ketoisocaproic acid

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Data described in the manuscript (in de-identified form), code book, and analytic code will be made available upon request pending application and approval from the corresponding author.

Supplemental Figure 1 and Supplemental Methods are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: BCAA, branched-chain amino acid; BCKA, branched-chain ketoacid; CKD, chronic kidney disease; EAA, essential amino acid; FSR, fractional synthesis rate; HbA1c, glycated hemoglobin; iAUC, incremental AUC; KIC,  $\alpha$ -ketoisocaproic acid; KIV,  $\alpha$ -ketoisovalerate; KMV,  $\alpha$ -keto- $\beta$ -methylvalerate; MILK, milk protein; MPE, mole % excess; NEAA, nonessential amino acid; TAA, total amino acid.

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## Introduction

Protein ingestion strongly increases muscle protein synthesis rates (1, 2). The postprandial increase in muscle protein synthesis rate has been attributed to the rise in circulating amino acids (3, 4). Amino acids serve as precursors for de novo muscle protein synthesis and can act as strong signaling molecules activating translation initiation via the mechanistic/mammalian target of rapamycin complex-1 (mTORC1) pathway (5, 6). Several studies indicate that senescent muscle is less sensitive to these anabolic properties of amino acids (7, 8). Anabolic resistance to feeding has been reported in the elderly and in several patient populations suffering from chronic disease (9). As a consequence, older and/or more clinically compromised patient populations require greater amounts of protein to be consumed (7, 10–14) or may benefit from food fortification with branched-chain amino acids (BCAAs) (15–19) to augment postprandial muscle protein synthesis rates. Limited data are available regarding the muscle protein synthetic response to ingesting BCAAs (leucine, isoleucine, and valine) only in humans. Recently, it was shown that BCAA ingestion increases myofibrillar protein synthesis rates during recovery from exercise in young males (20). However, whether BCAA ingestion can increase myofibrillar protein synthesis rates to a similar extent when compared with the ingestion of intact protein in vivo in older males remains to be assessed.

In many clinically compromised populations, simply increasing protein intake is not realistic and has been suggested to be unfavorable in patients with chronic kidney disease (CKD) because of potential renal injury (21). Hence (very) low protein diets are often prescribed in certain disease stages of patients with CKD, further compromising their capacity to preserve muscle mass (22–24). Supplementation with branched-chain ketoacids (BCKAs) has been applied in these conditions as these keto-analogs do not provide nitrogen (N) and may help to lower nitrogen intake as BCKAs can be transaminated into BCAAs (25, 26). BCKAs are readily available, safe for human consumption (27), and efficiently absorbed in the small intestine (28). However, intestinal absorption rates of BCKAs appear to be moderately lower when compared with BCAAs (29). In addition, oral administration of BCKAs, but not BCAAs, appears to induce substantial first-pass oxidation in splanchnic organs (30). Therefore, the nutritional efficiency, and thus bioavailability of ingested BCKAs, may be considerably lower when compared with BCAAs (26, 31). A lower bioavailability may suggest that BCKAs do not stimulate muscle protein synthesis to a similar extent when compared with BCAAs or intact protein. Furthermore, it has been observed that intravenous infusion of BCKAs does not stimulate whole-body protein synthesis (32). However, it is important to note that whole-body protein synthesis is not necessarily reflective of muscle protein synthesis and evidence from animal work in fact supports a role for BCKAs as a nutrient regulator of muscle protein synthesis (33, 34). To date, no studies have investigated the effects of ingesting BCKAs on muscle protein synthesis in humans. We hypothesize that ingestion of intact protein, BCAAs, as well as BCKAs stimulates myofibrillar protein synthesis in vivo in older males.

## Methods

### Subjects

Forty-five healthy (tracer naïve) older men (age:  $71 \pm 1$  y; BMI:  $25.4 \pm 0.8$  kg/m<sup>2</sup>) participated in this double-blind, parallel-group, randomized trial. The trial was conducted between January 2017 and May 2017 at Maastricht University Medical Centre+, in Maastricht, The Netherlands (for the consort flow chart, please see **Supplemental Figure 1**). The characteristics of the subjects are detailed in **Table 1**. All subjects were informed of the purpose of the study, experimental procedures, and possible risks before providing written consent to participate. The procedures followed were in accordance with the ethical standards of the Medical Ethics Committee of the Maastricht University Medical Centre+ on human experimentation and in accordance with the Helsinki Declaration of 1975 as revised in October 2013. This trial was registered at [www.trialregister.nl](http://www.trialregister.nl) as NTR6047.

### Ethics approval and consent to participate

This study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre+, The Netherlands (METC 16-3-035). All participants provided written informed consent before participation.

### Pretesting

Volunteers between the age of 65–80 y and with a BMI between 18.5 and 30.0 underwent medical screening to assess glycated hemoglobin (HbA1c), whole-body glucose tolerance [using a 2-h oral-glucose-tolerance test (35)], blood pressure, weight, height, and body composition (by DXA; Discovery A; Hologic). Subjects were deemed healthy based on their responses to a medical questionnaire and their screening results.

### Study design

Subjects were randomly assigned to consume a drink containing 30 g milk protein (MILK) (ReFit MPI 85; Friesland Campina;  $n = 15$ ), 6 g BCAAs (Evonik Industries;  $n = 15$ ), or 6 g BCKAs (Myolution; Evonik Industries;  $n = 15$ ). The 30 g MILK provides ~6 g BCAAs with a total of 2.64 g leucine, which should theoretically induce a measurable increase in postprandial muscle protein synthesis rates (36). The ratio of (keto-)leucine, (keto-)isoleucine, and (keto-)valine in both BCAA and BCKA drinks was 2:1:1. Hence, the BCAA and BCKA drinks provided 3 g (keto-)leucine, 1.5 g (keto-)isoleucine, and 1.5 g (keto-)valine (total 6 g). Randomization was performed by using a computerized random-number generator. An independent person was responsible for random assignment and drink preparation.

### Diet and physical activity

All subjects were instructed to refrain from any sort of strenuous physical activity 3 d prior to the infusion trial and to keep their diet as consistent as possible for 2 d prior to the experiment. On the evening before the experimental trial, all

**TABLE 1** Subjects' characteristics<sup>1</sup>

	BCAA	BCKA	MILK	<i>P</i>
Age, y	70 ± 1	71 ± 1	72 ± 1	0.201
Body mass, kg	78.5 ± 2.2	79.0 ± 3.2	80.7 ± 2.2	0.779
BMI, kg/m <sup>2</sup>	25.9 ± 0.9	25.4 ± 0.6	24.9 ± 0.8	0.568
Systolic BP, mmHg	138 ± 3	134 ± 4	131 ± 3	0.345
Diastolic BP, mmHg	72 ± 1	68 ± 3	66 ± 2	0.101
Fat, %	24.4 ± 1.6	24.1 ± 0.9	21.9 ± 1.6	0.297
Appendicular lean mass, kg	25.3 ± 0.8	26.1 ± 0.9	26.5 ± 0.9	0.558
Lean body mass, kg	57.2 ± 1.5	57.6 ± 2.2	60.7 ± 1.5	0.246
Fasting glucose (OGTT), mmol/L	5.2 ± 0.2	4.9 ± 0.2	5.0 ± 0.2	0.346
2-h glucose (OGTT), mmol/L	7.2 ± 0.9	5.6 ± 0.4	5.8 ± 0.6	0.103
Fasting insulin (OGTT), mU/L	10.8 ± 1.8	10.1 ± 1.7	10.0 ± 1.1	0.915
2-h insulin (OGTT), mU/L	74.0 ± 20.9	59.1 ± 19.1	39.9 ± 10.0	0.307
HbA1c, %	5.5 ± 0.2	5.5 ± 0.1	5.5 ± 0.1	0.986
HOMA2-IR	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	0.913
OGIS, mL/min/m <sup>2</sup>	367 ± 21	385 ± 19	409 ± 19	0.252

<sup>1</sup>Values represent means ± SEM, *n* = 15 per group. Data were analyzed using a one-factor ANOVA. No differences were detected between groups. BCAA, 6 g branched-chain amino acids; BCKA, 6 g branched-chain ketoacids; BP, blood pressure; HbA1c, glycated hemoglobin; HOMA2-IR, homeostatic model assessment of insulin resistance; MILK, 30 g milk protein; OGIS, oral glucose insulin sensitivity; OGTT, oral-glucose-tolerance test.

subjects consumed the same standardized meal (2061 kJ/487 kcal) providing 31.8 g protein, 58.7 g carbohydrate, and 11.3 g fat at ~1800 h followed by an evening snack (985 kJ/234 kcal) composed of 34.7 g protein, 18.5 g carbohydrate, and 0.0 g fat at 2200 h.

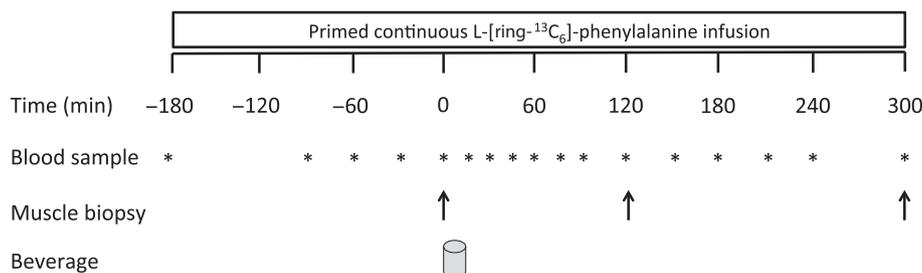
### Infusion protocol

At 0800 h, after an overnight fast, subjects arrived at the laboratory by car or public transport. A catheter was inserted into an antecubital vein for stable isotope labeled amino acid infusion. A second catheter was inserted into a dorsal hand vein of the contralateral arm and placed in a hot box (60°C) for arterialized blood sampling (37). After obtaining a baseline blood sample, the plasma phenylalanine pools were primed with a single dose of L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine (2.25 μmol·kg<sup>-1</sup>), after which a continuous L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine (0.05 μmol·kg<sup>-1</sup>·min<sup>-1</sup>) intravenous infusion was initiated (*t* = -180 min). Subsequently, the subjects rested in a supine position for 180 min during which 4 additional arterialized blood samples were drawn (*t* = -90, -60, -30, and 0 min). A muscle biopsy sample was then collected from the m. vastus lateralis of a randomly chosen leg (*t* = 0 min). After collection of the first muscle biopsy sample, subjects consumed a drink containing 30 g MILK (*n* = 15), 6 g BCAAs (*n* = 15), or 6 g BCKAs (*n* = 15)

at *t* = 0 min. A small amount of L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine (6%) was added to the MILK beverage to prevent precursor pool dilution. Additional arterialized blood samples were collected at *t* = 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, and 300 min. Second and third muscle biopsies were collected at *t* = 120 min and *t* = 300 min to determine postprandial myofibrillar protein synthesis rates. Blood samples were collected in EDTA-containing tubes and centrifuged at 1000 × *g* for 10 min at 4°C. Aliquots of plasma were snap frozen in liquid nitrogen and stored at -80°C. Biopsy samples were collected from the middle region of the M. vastus lateralis, ~15 cm above the patella and 3 cm below entry through the fascia, using the percutaneous needle biopsy technique (38). Muscle samples were dissected carefully, freed from any visible nonmuscle material, immediately frozen in liquid nitrogen, and stored at -80°C until further analysis. For a schematic representation of the infusion protocol, see **Figure 1**.

### Plasma and muscle tissue analysis

Details of analysis related to the determination of plasma (glucose, insulin, ammonia, ketoacids, amino acids, HbA<sub>1c</sub>, L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine, mixed plasma proteins) as well as muscle (myofibrillar protein L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine



**FIGURE 1** Schematic overview of the infusion protocol. Participants consumed either 30 g MILK, 6 g BCAAs, or 6 g BCKAs.

enrichments) data are presented in the **Supplemental Methods**.

### Calculations

Myofibrillar protein fractional synthetic rates (FSRs) were calculated using the standard precursor-product equation, as follows (39):

$$FSR = \frac{\Delta E_p}{E_{precursor} \cdot t} \cdot 100 \quad (1)$$

$\Delta E_p$  is the increment in myofibrillar protein-bound L-[ring- $^{13}\text{C}_6$ ]-phenylalanine enrichment after an incorporation period,  $E_{precursor}$  is the weighted mean plasma L-[ring- $^{13}\text{C}_6$ ]-phenylalanine enrichment during that incorporation period, and  $t$  is the incorporation period (h). Weighted mean plasma enrichments were calculated by taking the average enrichment between all consecutive time points and correcting for the time between these sampling time points. The weighted mean plasma precursor pool is preferred in this setting because the more frequent sampling time points allow a more accurate correction of the transient changes in precursor pool enrichments over time (40). For basal FSR, plasma protein samples at  $t = -180$  min and muscle biopsy samples at  $t = 0$  min were used; and for postprandial FSRs, muscle biopsy samples at  $t = 0, 120,$  and  $300$  min were used.

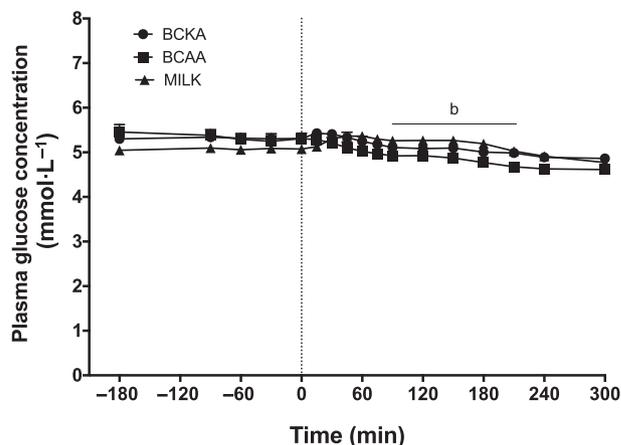
### Statistical analysis

All data are expressed as means  $\pm$  SEM. Baseline characteristics, incremental AUC (iAUC; for postprandial plasma insulin and ammonia concentrations), and plasma amino acid enrichments were compared between treatment groups using one-factor ANOVA. For time-dependent variables, repeated-measures ANOVA with treatment as a between-subjects factor and time as a within-subjects factor was used (i.e., all time points for plasma concentrations and basal compared with postprandial for muscle data). In case of significant interactions, separate analyses were performed within each treatment group, as well as between treatment groups for every time period separately (e.g., for FSR values, separate one-factor ANOVA for basal, 0–2 h, 2–5 h, and 0–5 h). In the case of significant treatment effects, Bonferroni post hoc analyses were performed to locate the effects. Significance was set at  $P < 0.05$ . Calculations were performed using SPSS (version 21.0, IBM Corp.).

## Results

### Plasma glucose, insulin, and ammonia

Plasma glucose concentrations (Figure 2) slightly declined over time in the BCKA and BCAA groups ( $P < 0.05$ ), whereas they slightly increased over time in the MILK group ( $P < 0.05$ ). Plasma glucose concentrations were significantly higher between  $t = 90$  and  $210$  min in the MILK group compared with the BCAA group ( $P < 0.05$ ). Plasma insulin concentrations (Figure 3A) showed a rapid and significant increase following beverage ingestion in the BCAA and MILK group ( $P < 0.05$ ), but not in the BCKA group. A greater increase in plasma insulin concentrations was observed in the MILK



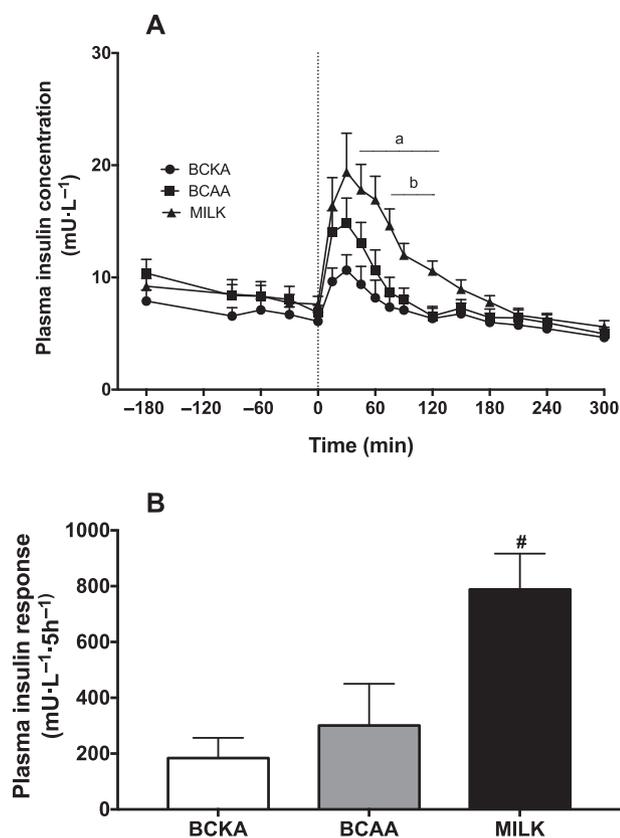
**FIGURE 2** Plasma glucose concentrations over time after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. The dotted line represents the ingestion of the drink. Values represent means  $\pm$  SEM. Data were analyzed with repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. Time  $\times$  treatment interaction,  $P < 0.001$ . b, MILK significantly different ( $P < 0.05$ ) from BCAA.

group compared with the BCAA group between  $t = 75$  and  $120$  min ( $P < 0.05$ ). In agreement, the iAUC above fasting plasma insulin concentrations was significantly greater in the MILK group compared with the BCKA and BCAA groups ( $P < 0.05$ ; Figure 3B). The iAUC of plasma ammonia (Figure 4) did not show significant differences between groups, despite the fact that plasma ammonia concentrations were substantially reduced during the postprandial period in the BCKA group.

### Plasma amino acids

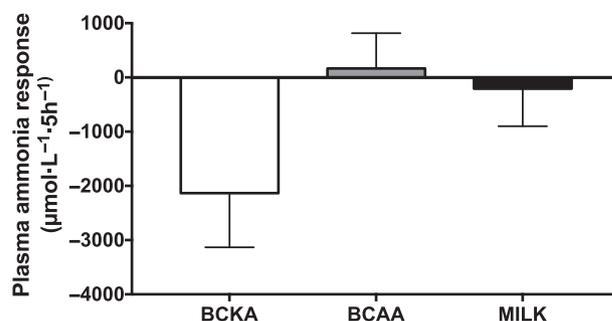
Plasma phenylalanine (Figure 5A), leucine (Figure 5B), isoleucine (Figure 5C), and valine (Figure 5D) concentrations over time are depicted in Figure 5. Significant time  $\times$  treatment interactions were observed for plasma phenylalanine, leucine, isoleucine, and valine concentrations ( $P < 0.001$ ). Following drink ingestion, a rapid increase in plasma phenylalanine concentrations was found in the MILK group ( $P < 0.05$ ), whereas plasma phenylalanine concentrations remained unchanged and decreased over time in the BCKA and BCAA groups, respectively ( $P < 0.05$ ). Plasma leucine concentrations increased significantly after drink ingestion in all groups ( $P < 0.01$ ), with the highest peak plasma leucine concentrations measured in the BCAA group ( $P < 0.05$ ). Plasma isoleucine and valine concentrations increased significantly after drink ingestion and remained elevated for the entire postprandial period in the BCAA and MILK groups ( $P < 0.01$ ), whereas no changes were observed in the BCKA group.

Plasma BCAA (Figure 6A), essential amino acid (EAA; Figure 6B), nonessential amino acid (NEAA-glutamine; Figure 6C), and total amino acid (TAA; Figure 6D) concentrations over time are depicted in Figure 6. For BCAA, EAA, NEAA, and TAA concentrations, significant time  $\times$  treatment interactions were observed ( $P < 0.001$ ). Plasma



**FIGURE 3** Plasma insulin concentrations over time (A) and total insulin responses, expressed as incremental AUC (B) after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. The dotted line represents the ingestion of the drink. Values represent means  $\pm$  SEM. Data in panel A were analyzed using repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. Time  $\times$  treatment interaction,  $P < 0.001$ . a, MILK significantly different ( $P < 0.05$ ) from BCKA; b, MILK significantly different ( $P < 0.05$ ) from BCAA. Data in panel B were analyzed using a one-factor ANOVA with Bonferroni correction. #Significantly different ( $P < 0.05$ ) from BCAA and BCKA.

total BCAA concentrations increased significantly after drink ingestion and remained elevated for the entire postprandial period in the BCAA and MILK groups ( $P < 0.001$ ). For the BCKA group, plasma total BCAA concentrations were only significantly higher than fasting values at  $t = 150$  and 300 min ( $P < 0.01$ ). Plasma EAA concentrations increased significantly after drink ingestion and remained elevated for the entire postprandial period in the BCAA (apart from  $t = 240$  min;  $P > 0.05$ ) and MILK groups ( $P < 0.05$ ), whereas no increase was found after BCKA ingestion. Plasma total NEAA concentrations following drink ingestion significantly increased and remained elevated until  $t = 240$  min after MILK ingestion ( $P < 0.001$ ), did not change after BCKA ingestion, and were significantly lower when compared to fasting values at  $t = 90$ , 180, 210, and 300 min in the BCAA group ( $P < 0.05$ ). Plasma TAA concentrations immediately increased after BCAA (until  $t = 120$  min;  $P < 0.001$ ) and MILK ingestion (remained elevated



**FIGURE 4** Plasma total ammonia concentrations, expressed as incremental AUC after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. Values represent means  $\pm$  SEM. Data were analyzed using a one-factor ANOVA. No significant differences between treatments ( $P > 0.05$ ).

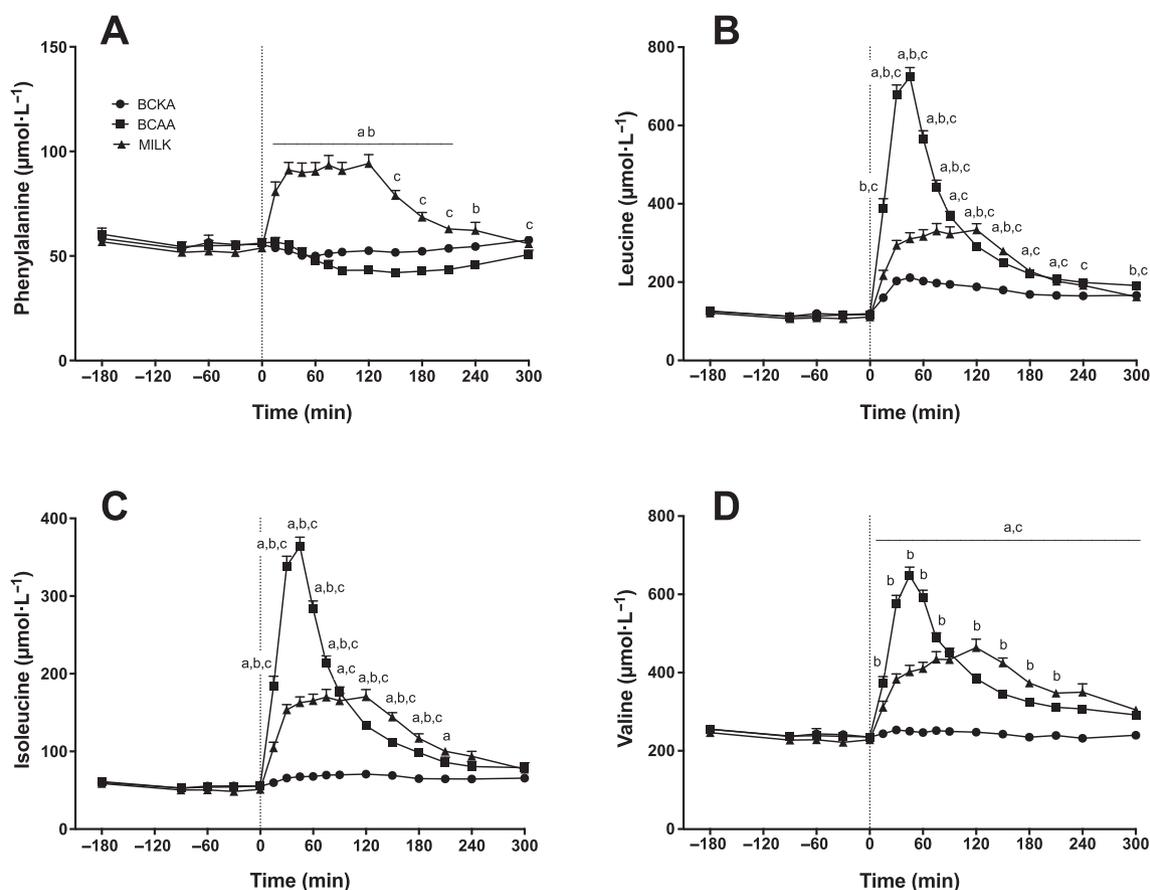
for the entire postprandial period;  $P < 0.01$ ). No significant changes were observed after BCKA ingestion.

### Plasma $\alpha$ -ketoacids

Plasma  $\alpha$ -ketoisocaproic acid (KIC; the  $\alpha$ -ketoacid of leucine; **Figure 7A**),  $\alpha$ -keto- $\beta$ -methylvalerate (KMV; the  $\alpha$ -ketoacid of isoleucine; **Figure 7B**),  $\alpha$ -ketoisovalerate (KIV; the  $\alpha$ -ketoacid of valine; **Figure 7C**), and total BCKA (**Figure 7D**) concentrations over time are depicted in **Figure 7**. Significant time  $\times$  treatment interactions were observed for plasma KIC, KMV, KIV, and total BCKA concentrations ( $P < 0.001$ ). Following drink ingestion, plasma KIC and KMV concentrations significantly increased and remained elevated for the entire postprandial period in the BCKA ( $P < 0.001$ , apart from  $t = 240$  min for KMV) and BCAA groups (from  $t = 30$  min;  $P < 0.05$ ). For the MILK group, KIC concentrations were only significantly higher than fasting values between  $t = 150$ –300 min and KMV concentrations were only significantly higher than fasting values between  $t = 75$ –210 min and at  $t = 300$  min ( $P < 0.05$ ). Following drink ingestion, plasma KIV concentrations significantly increased in the BCKA group (from  $t = 15$  until  $t = 150$  min;  $P < 0.01$ ), whereas no changes were observed in the BCAA and MILK groups. Plasma total BCKA concentrations increased significantly after drink ingestion for the entire postprandial period in the BCKA ( $P < 0.001$ ) and BCAA groups ( $P < 0.001$ ; apart from  $t = 15$  min), whereas they were only significantly increased between  $t = 90$ –300 min in the MILK group ( $P < 0.05$ ).

### Isotope tracer analysis

Prior to BCAA, BCKA, or MILK ingestion plasma L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine enrichments averaged  $7.5 \pm 0.3$ ,  $7.4 \pm 0.1$ , and  $7.4 \pm 0.2$  mole % excess (MPE), respectively, with no differences between treatments (data not shown). Plasma L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine enrichments during the postprandial period averaged  $8.7 \pm 0.3$ ,  $7.9 \pm 0.2$ , and  $7.3 \pm 0.2$  MPE for the BCAA, BCKA, and MILK groups respectively, with significant differences between the BCAA and BCKA groups ( $P < 0.01$ ) as well as the BCAA and MILK groups ( $P < 0.001$ ), but not between the BCKA and MILK groups ( $P = 0.082$ ).



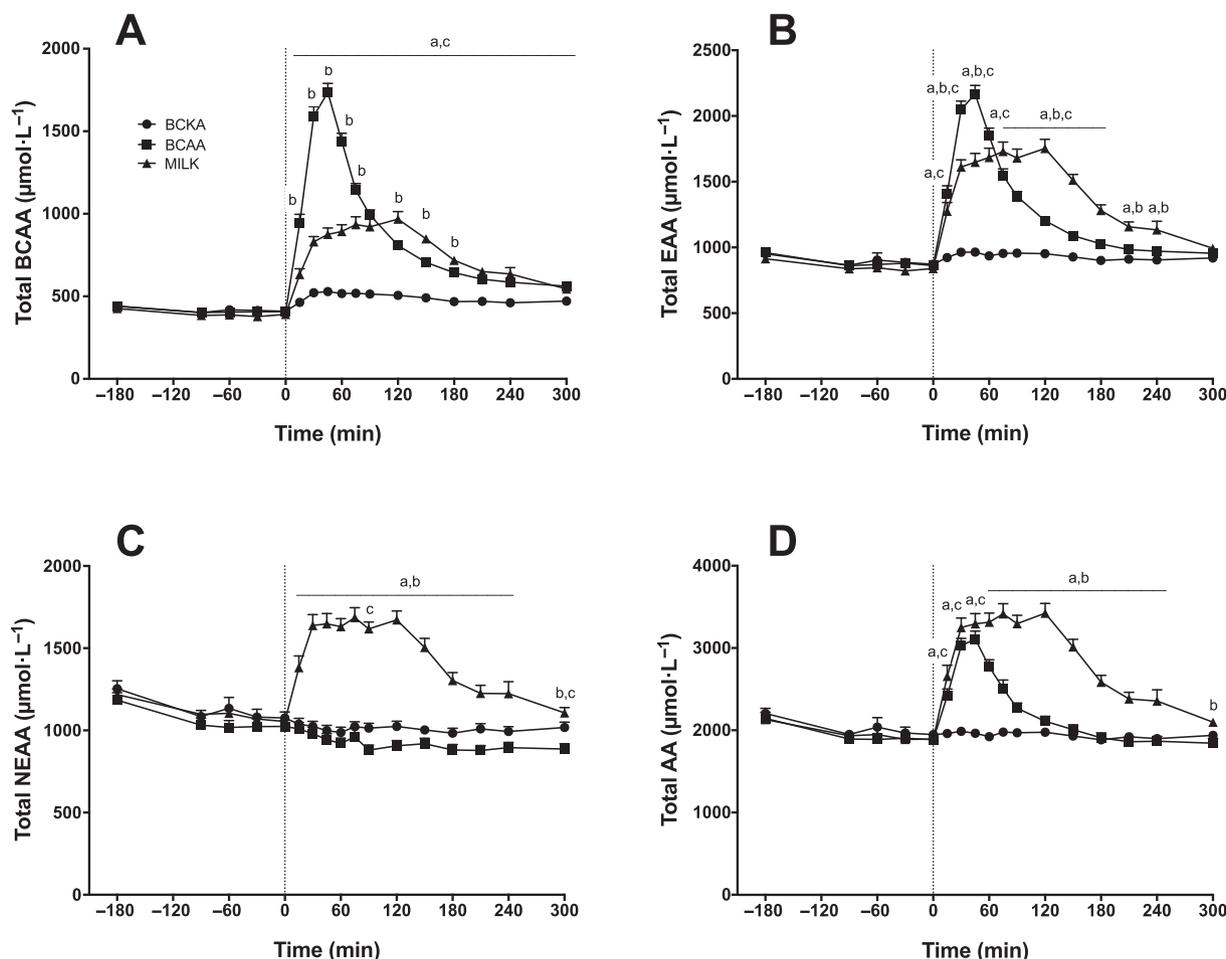
**FIGURE 5** Plasma phenylalanine (A), leucine (B), isoleucine (C), and valine (D) concentrations over time after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. The dotted line represents the ingestion of the drink. Values represent means  $\pm$  SEM. Data were analyzed with repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. Time  $\times$  treatment interaction,  $P < 0.001$ . a, MILK significantly different ( $P < 0.05$ ) from BCKA; b, MILK significantly different ( $P < 0.05$ ) from BCAA; c, BCAA significantly different ( $P < 0.05$ ) from BCKA.

Myofibrillar protein synthesis rates calculated based on the plasma precursor pool are depicted in **Figure 8**. No differences were observed in basal muscle protein synthesis rates between groups ( $P = 0.624$ ). Myofibrillar protein synthesis rates increased from basal to the early (0–2 h) postprandial period in all groups (from  $0.020 \pm 0.002\%/h$  to  $0.042 \pm 0.004\%/h$  in the MILK group,  $0.022 \pm 0.002\%/h$  to  $0.044 \pm 0.004\%/h$  in the BCAA group, and  $0.023 \pm 0.003\%/h$  to  $0.044 \pm 0.004\%/h$  in the BCKA group;  $P < 0.001$ ), with no differences between treatment groups (time  $\times$  treatment interaction:  $P = 0.969$ , main treatment effect:  $P = 0.732$ ). A significant time  $\times$  treatment interaction ( $P = 0.002$ ) showed that after ingestion of MILK, myofibrillar protein synthesis rates remained elevated over the 2–5 h postprandial period when compared with basal protein synthesis rates (from  $0.020 \pm 0.002\%/h$  to  $0.039 \pm 0.004\%/h$ ;  $P < 0.001$ ). In the BCKA and BCAA groups, myofibrillar protein synthesis rates decreased back to basal rates during the 2–5 h postprandial period ( $0.024 \pm 0.005\%/h$  and  $0.024 \pm 0.005\%/h$ , respectively;  $P > 0.05$ ). During the late (2–5 h) postprandial period, myofibrillar protein synthesis rates were higher in the MILK group when compared with the BCAA ( $P = 0.023$ ) and

BCKA groups ( $P = 0.023$ ). A significant time  $\times$  treatment interaction was also observed when comparing basal myofibrillar protein synthesis rates with the entire 5-h postprandial period ( $P = 0.003$ ). Over the entire 5-h postprandial period (0–5 h), myofibrillar protein synthesis rates were significantly elevated above basal for all treatments ( $P < 0.005$ ), but were significantly higher in the MILK group ( $0.040 \pm 0.002\%/h$ ) compared with the BCKA ( $0.032 \pm 0.003\%/h$ ;  $P = 0.023$ ) and BCAA groups ( $0.032 \pm 0.003\%/h$ ;  $P = 0.019$ ), with no significant differences between the BCKA and BCAA groups ( $P > 0.05$ ).

## Discussion

The present study demonstrated that ingestion of BCAAs, BCKAs, and MILK (providing an equivalent amount of BCAAs) significantly increased myofibrillar protein synthesis rates during the early (0–2 h) postprandial phase, with no differences observed between treatments. In the late (2–5 h) postprandial period myofibrillar protein synthesis rates remained elevated following MILK ingestion, with myofibrillar protein synthesis



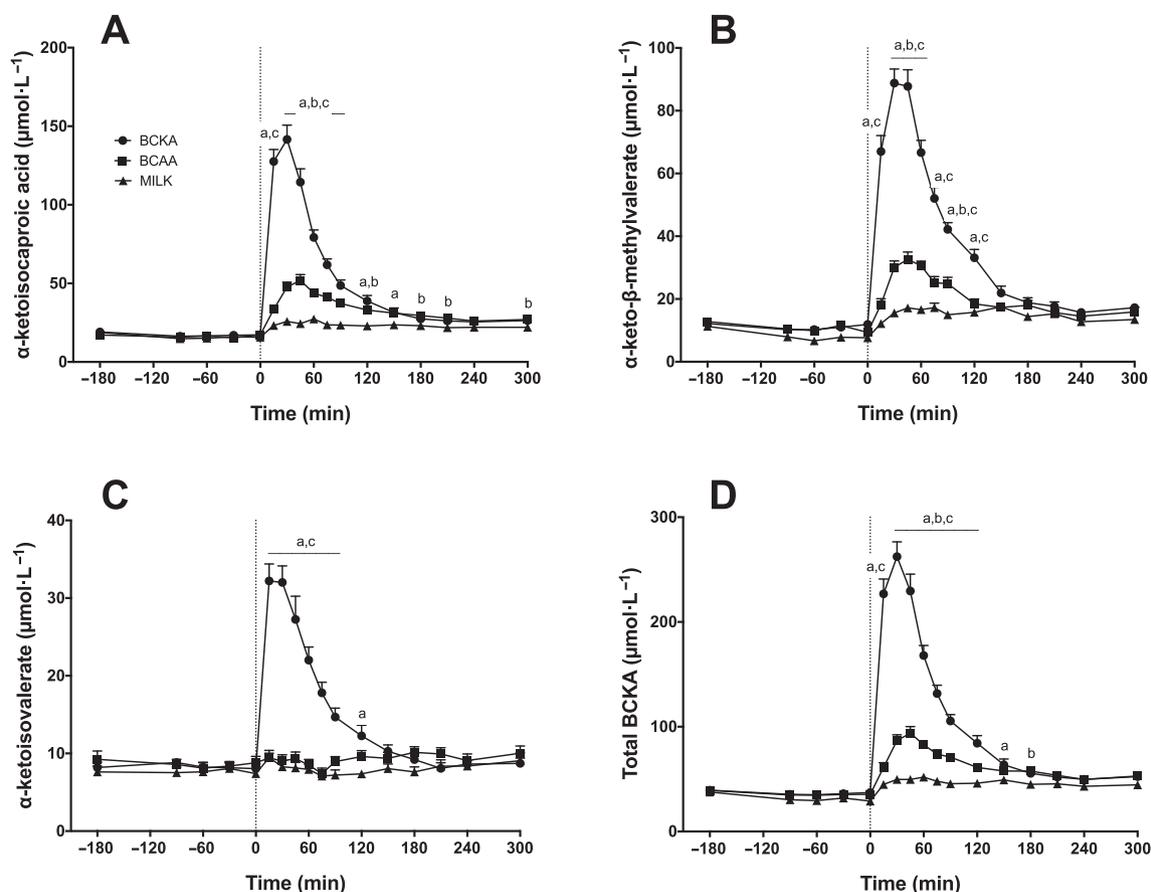
**FIGURE 6** Plasma branched-chain amino acids (BCAA; A), essential amino acid (EAA; B), nonessential amino acid (NEAA-glutamine; C), and total amino acid (TAA-glutamine; D) concentrations over time after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g BCAA ( $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. The dotted line represents the ingestion of the drink. Values represent means + SEM. Data were analyzed with repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. Time  $\times$  treatment interaction,  $P < 0.001$ . a, MILK significantly different ( $P < 0.05$ ) from BCKA; b, MILK significantly different ( $P < 0.05$ ) from BCAA; c, BCAA significantly different ( $P < 0.05$ ) from BCKA.

rates following BCAA and BCKA ingestion returning to baseline values.

It has been well established that ingestion of dietary protein strongly increases muscle protein synthesis rates (1, 2, 41). The postprandial increase in muscle protein synthesis rates has been attributed to the postprandial release of (essential) amino acids (3, 4, 42, 43), with the rise in circulating BCAAs and especially leucine being of particular relevance (16, 44–47). In line with previous work (10, 48, 49), we show a rapid postprandial increase in plasma (essential) amino acid concentrations following the ingestion of 30 g MILK, with plasma leucine concentrations reaching  $294 \pm 12 \mu\text{mol/L}$  within 30 min (Figure 5B). The postprandial rise in plasma amino acid availability resulted in a 111% increase in myofibrillar protein synthesis rates within the first 2 h after protein ingestion when compared with basal, postabsorptive myofibrillar protein synthesis rates (from  $0.020 \pm 0.002$  to  $0.042 \pm 0.004\%/h$ ; Figure 8). In addition, postprandial myofibrillar protein synthesis rates assessed during the entire 5-h postprandial period were also

higher following MILK ingestion when compared with baseline values ( $0.040 \pm 0.002$  compared with  $0.020 \pm 0.002\%/h$ , respectively).

As the postprandial increase in muscle protein synthesis has been attributed largely to the postprandial increase in plasma BCAAs, we also assessed the impact of ingesting BCAAs only. Following ingestion of 6 g BCAAs, which equals the amount of BCAAs present in 30 g MILK, we observed a rapid rise in circulating BCAAs (Figure 6A), with plasma leucine concentrations reaching 2–3 fold higher concentrations when compared with MILK ingestion (Figure 5B). We also observed a strong, significant increase in myofibrillar protein synthesis rates within the first 2 h after BCAA ingestion (from  $0.022 \pm 0.002$  to  $0.044 \pm 0.004\%/h$ ; Figure 8). This seems to be in line with previous work showing an increase in myofibrillar protein synthesis rate during a 2.5-h period after ingesting 3.4 g of free leucine (50). The rapid and marked increase in plasma BCAA availability after BCAA ingestion may have exceeded the maximal capacity to stimulate myofibrillar protein synthesis,

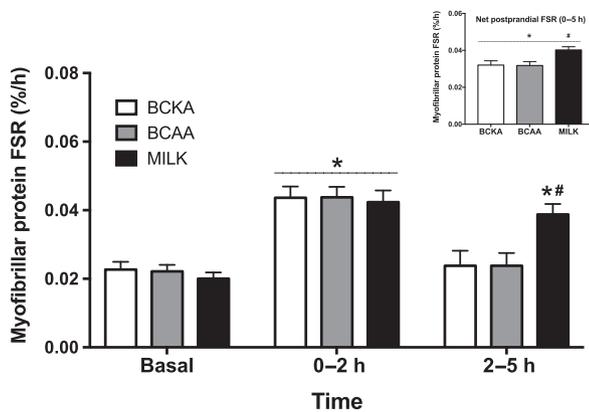


**FIGURE 7** Plasma  $\alpha$ -ketoisocaproic acid (KIC; the  $\alpha$ -ketoacid of leucine; A),  $\alpha$ -keto- $\beta$ -methylvalerate (KMV; the  $\alpha$ -ketoacid of isoleucine; B),  $\alpha$ -ketoisovalerate (KIV; the  $\alpha$ -ketoacid of valine; C), and branched-chain ketoacid (BCKA) (D) concentrations over time after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g BCKA ( $n = 15$ ) in healthy older males. The dotted line represents the ingestion of the drink. Values represent means  $\pm$  SEM. Data were analyzed with repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. Time  $\times$  treatment interaction,  $P < 0.001$ . a, MILK significantly different ( $P < 0.05$ ) from BCKA; b, MILK significantly different ( $P < 0.05$ ) from BCAA; c, BCAA significantly different ( $P < 0.05$ ) from BCKA.

so that during the 2-h postprandial period myofibrillar protein synthesis rates did not differ after ingestion of BCAA or MILK. Though this would be in line with the proposed muscle full effect (51, 52), these high myofibrillar protein synthesis rates could not be maintained following BCAA as opposed to MILK ingestion during the latter stages (2–5 h) of the postprandial period (Figure 8). These data suggest that besides the postprandial rise in plasma BCAA concentrations, other (essential) amino acids need to be provided to allow a more prolonged postprandial increase in muscle protein synthesis rate (53). Though the postprandial rise in muscle protein synthesis rate following protein ingestion can be initiated by the increased BCAA availability, it may be maintained only when sufficient other (essential) amino acids are provided to serve as precursors for de novo muscle protein synthesis. Alternatively, it could be speculated that ingestion of greater amounts ( $> 6$  g) and/or a more sustained provision of BCAA are needed to prolong the elevation in muscle protein synthesis rates.

BCKAs are ketoanalogs of the 3 BCAAs and play an active role in BCAA metabolism, due to their rapid in vivo inter-convertibility (by transamination) (26). As the transamination

capacity is high in skeletal muscle (54), when BCKAs are transaminated into BCAAs, they may directly stimulate muscle protein synthesis. It has previously been shown that infusion with the BCKA  $\alpha$ -ketoisocaproic acid stimulates muscle protein synthesis in neonatal pigs (33). However, the effect of BCKA ingestion on muscle protein synthesis rates in humans has never been assessed. Ingestion of 6 g BCKA, which was tolerated well in all subjects with no reported side effects, resulted in a strong postprandial increase in plasma BCKA concentrations (Figure 7). Concomitantly, we also observed a small but significant increase in plasma leucine concentrations (Figure 5B), suggesting that there is some, albeit limited, conversion of KIC to leucine in vivo in healthy humans. Despite only minimal changes in plasma BCAA or other amino acid concentrations following BCKA ingestion (Figure 6), we observed a rapid increase in myofibrillar protein synthesis rates during the early postprandial phase that did not differ from the early postprandial response following BCAA or MILK ingestion (Figure 8). However, similar to the postprandial response observed following BCAA ingestion, these elevated myofibrillar protein synthesis rates were not maintained during the latter postprandial phase



**FIGURE 8** Myofibrillar protein fractional synthetic rates (FSR; in %/h) during the fasting state (basal) and over the early (0–2 h), late (2–5 h), and entire (0–5 h) postprandial period after the ingestion of 30 g milk protein (MILK;  $n = 15$ ), 6 g branched-chain amino acids (BCAA;  $n = 15$ ), or 6 g branched-chain ketoacids (BCKA;  $n = 15$ ) in healthy older males. Values represent means + SEM. Data were analyzed with repeated measures (time  $\times$  treatment group) ANOVA and separate analyses were performed when a significant interaction was detected. Bonferroni post hoc testing was used to detect differences between groups. \*Significantly different ( $P < 0.01$ ) from basal; #significantly different ( $P < 0.05$ ) from BCAA and BCKA.

(2–5 h). The observation that BCKA ingestion strongly increases (early) postprandial myofibrillar protein synthesis rates, despite a minimal rise in circulating plasma leucine concentrations, implies that either BCKA-derived BCAA (by transamination) are directly used within muscle to stimulate muscle protein synthesis or that alternative metabolites may be involved in the early postprandial stimulation of muscle protein synthesis. It has previously been shown that leucine metabolites, such as  $\beta$ -hydroxy- $\beta$ -methylbutyrate, can stimulate myofibrillar protein synthesis (50, 55). In contrast to the ketoanalog of leucine,  $\beta$ -hydroxy- $\beta$ -methylbutyrate cannot be converted back into leucine. In line with the minimal increase in plasma leucine concentrations following BCKA ingestion, our findings suggest that the stimulatory effect of BCKA ingestion on myofibrillar protein synthesis may work directly (KIC) and/or indirectly via conversion to  $\beta$ -hydroxy- $\beta$ -methylbutyrate. Clearly, research is warranted to elucidate alternative metabolites and pathways that could be (at least partially) responsible for the stimulatory effect of BCKA ingestion on muscle protein synthesis. This will also yield important information on whether the anabolic properties of BCKA and BCAA involve separate pathways and, as such, may be combined to further increase postprandial muscle protein synthesis rates. Ultimately, net muscle protein accretion is determined by the balance between muscle protein synthesis and breakdown rates and it has been suggested that BCKA, with KIC in particular, has a profound impact on muscle protein breakdown. It appears that KIC, and not leucine per se, is primarily responsible for the inhibitory effect on muscle protein breakdown (56–59). This further supports the potential of BCKAs as an anabolic agent to stimulate muscle protein accretion.

BCKAs lack an amino group bound to the  $\alpha$ -carbon in their molecular structure and, therefore, do not provide nitrogen. In support, transamination of the BCKAs into their respective BCAAs was likely responsible for the observed decline in plasma

ammonia concentrations (Figure 4). The potential therapeutic value of BCKAs has been studied in several diseases and disorders, but appears particularly relevant for patients with CKD (25, 26). Patients with CKD are advised to adhere to low protein diets during certain stages of their disease (60), restricting them in their capacity to maintain muscle mass. The observed stimulatory effect of BCKA ingestion on early postprandial myofibrillar protein synthesis rates can be of particular relevance for this patient group as it may support them in preventing or attenuating the progressive loss of muscle mass without increasing nitrogen intake (34, 61). Furthermore, there are suggestions that a low protein diet improves the nutritional efficiency of BCKAs (26, 62). Therefore, future studies should look into the benefits of BCKA co-ingestion to stimulate postprandial muscle protein synthesis rates in patients with CKD ingesting a diet relatively low in dietary protein content.

In conclusion, ingestion of 6 g BCAAs, 6 g BCKAs, and 30 g MILK increases myofibrillar protein synthesis rates during the early postprandial phase (0–2 h) in vivo in healthy older males. The postprandial increase following the ingestion of 6 g BCAAs and BCKAs is short-lived, with higher myofibrillar protein synthesis rates only being maintained following the ingestion of an equivalent amount of intact milk protein.

The authors' contributions were as follows—CJF and LJCvL: designed the research; CJF, WJHH, AMH, and JSJS: conducted the research; CJF, JMS, JK, APG, WKHWW, HS, and LJCvL: analyzed the data; CJF and LBV: performed the statistical analysis; CJF and LJCvL: wrote the manuscript and hold primary responsibility for the final content; and all authors read and approved the final manuscript. LJCvL and LBV have received research grants, consulting fees, speaking honoraria, or a combination of these, from Evonik Industries, Friesland Campina, and Nutricia. All other authors report no conflicts of interest related to research presented in this article.

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