Stimulation of muscle protein synthesis by whey and caseinate ingestion after resistance exercise in elderly individuals

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Sarcopenia is a well-known phenomenon in elderly individuals and resistance exercise together with sufficient amino acid (AA) availability has proved to be a counteractive implement. However, the source of AA and supplement timing require further investigation. The objective was to compare muscle protein synthesis (MPS) to intakes of whey and caseinate after heavy resistance exercise in healthy elderly individuals, and, furthermore, to compare the timing effect of caseinate intake. Twenty-four elderly men and women (mean ± SEM; 68 ± 1 years) were randomized to one of four groups: caseinate intake before exercise (CasPre), caseinate intake immediately after exercise (CasPost), whey intake immediately after exercise (Whey), or intake of a non-caloric control drink (Control). Muscle myofibrillar and collagen fractional synthesis rates (FSR) were measured by a primed continuous infusion of L-[1-13C]leucine using labeled proteins during a 6-h recovery period. No differences were observed in muscle myofibrillar and collagen FSR with Whey (0.09 ± 0.01%/h) compared with CasPost (0.09 ± 0.003%/h), and it did not differ between CasPre (0.10 ± 0.01%/h) and CasPost. MPS does not differ with whey and caseinate feeding immediately after heavy resistance exercise in elderly individuals, and MPS is similar with caseinate ingestion before and after exercise.

In elderly persons, reductions of muscle mass and strength contribute significantly to a decline in physical performance and may ultimately result in a dependent lifestyle. This phenomenon, termed sarcopenia, has been reported to be responsible for approximately 1.5% of the total healthcare expenditures in Western countries (Janssen et al., 2004), and the burdens related to sarcopenia may increase concomitantly with increasing number of elderly in future decades. Therefore, it is important to optimize regimens that can counteract the development of sarcopenia.

Ingestion of protein is crucial for the maintenance of skeletal muscle mass (Campbell et al., 2008) primarily by increasing the protein synthesis rate (Biolo et al., 1997). The intrinsic characteristics of proteins determine their digestion and absorption rates following ingestion and have been shown to affect the protein turnover processes differently (Boirie et al., 1997; Dangin et al., 2001). The milk-derived proteins, whey and casein, are good models to study the significance of absorption rate, because while whey protein is digested and absorbed rapidly, casein protein clots in the stomach and is released more slowly (Boirie et al., 1997; Dangin et al., 2001; Calbet & Holst, 2004). When ingested, the “fast” whey protein induces superior stimulation of whole-body protein synthesis compared with the “slow” casein protein in both young (Boirie et al., 1997; Dangin et al., 2001) and elderly individuals (Dangin et al., 2003) in the rested state. Moreover, Tang et al. (2009) found a diminished effect on mixed muscle protein synthesis (MPS) of micellar casein compared with whey hydrolysate feeding following resistance exercise in young persons (Tang et al., 2009). However, others demonstrated similar improved muscle anabolism with whey and casein protein feeding following resistance exercise in young persons (Tipton et al., 2004; Reitelseder et al., 2011). In combination with the resistance exercise, the potential significance of protein digestibility has never been studied in detail in elderly.

Longitudinal training studies have shown that the combination of resistance exercise and immediate protein ingestion are more effective stimulators of skeletal muscle anabolism than exercise resistance without an acute supply of proteins or combined with carbohydrate ingestion alone (Esmarck et al., 2001; Andersen et al., 2005; Holm et al., 2006; Hartman et al., 2007). Hence, increased amino acid (AA) availability in the time period around the resistance exercise bout seems to be beneficial for
Milk proteins and resistance exercise

The aims of the present work were to determine the MPS rate of specific functional proteins in skeletal muscle during 6 h after one resistance exercise session combined with ingestion of caseinate, whey, or a non-caloric control drink immediately after the completion of exercise in elderly untrained individuals. We further sought to investigate how ingestion of caseinate 30 min before or immediately after exercise affected the outcome parameters. The milk proteins were intrinsically labeled, which allowed an applied design using one large bolus of protein without disturbing the tracer enrichment noticeably. We hypothesized that ingestion of whey would result in a superior MPS rate compared with caseinate in elderly subjects and that the muscle protein synthetic response would be equal with ingestion of caseinate before and after resistance exercise.

Subjects and methods

Subjects

Through newspaper and web advertisements, 15 elderly men and nine elderly women (age 68 ± 1 years, range 61–80 years) were recruited (Table 1). All subjects underwent a verbal and physical examination before inclusion, which included an evaluation of the history of medical intake, metabolic/cardiovascular diseases, locomotive limitations of the hips and knees, physical activity level, and dietary/smoking habits. Subjects were excluded if they were obese (BMI > 30); smokers; or suffering from myocardial infarction, severe hypertension, or metabolic diseases. Subjects enrolled in the project were moderately active and had not taken part in any form of strenuous endurance training or resistance exercise at least 1 year before the trial. Keeping an even distribution of sex and age in each group, all subjects were randomly allocated to one of four groups: Caseinate ingestion 30 min before exercise (CasPre; n = 6), caseinate ingestion immediately after exercise (CasPost; n = 6), whey ingestion immediately after exercise (Whey; n = 6), or non-caloric control drink ingestion immediately after exercise (Control; n = 6). All subjects were carefully informed in accordance with the Declaration of Helsinki before they gave their written consent to participate in the study. The study was approved by the local ethics committee of Copenhagen and Frederiksberg under journal H-D-2008-054.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CasPre</th>
<th>CasPost</th>
<th>Whey</th>
<th>Placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>4/2</td>
<td>3/3</td>
<td>4/2</td>
<td>4/2</td>
<td>–</td>
</tr>
<tr>
<td>Sex distribution (M/F)</td>
<td>4/2</td>
<td>3/3</td>
<td>4/2</td>
<td>4/2</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71 ± 3</td>
<td>70 ± 2</td>
<td>64 ± 1</td>
<td>68 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.04</td>
<td>1.74 ± 0.04</td>
<td>1.75 ± 0.02</td>
<td>1.73 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.5 ± 6.5</td>
<td>75.8 ± 6.1</td>
<td>76.1 ± 4.4</td>
<td>71.9 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 1.4</td>
<td>24.9 ± 1.6</td>
<td>24.7 ± 1.0</td>
<td>23.8 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Tissue mass (kg)</td>
<td>77.0 ± 6.3</td>
<td>72.3 ± 5.9</td>
<td>73.8 ± 4.2</td>
<td>68.0 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>53.4 ± 5.3</td>
<td>52.3 ± 5.1</td>
<td>54.5 ± 3.3</td>
<td>53.0 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>23.7 ± 2.4</td>
<td>20.0 ± 3.1</td>
<td>19.3 ± 1.0</td>
<td>15.0 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.1 ± 3.0</td>
<td>27.6 ± 4.0</td>
<td>26.2 ± 0.6</td>
<td>23.0 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>1 RM, leg press (kg)</td>
<td>193 ± 29</td>
<td>206 ± 21</td>
<td>201 ± 21</td>
<td>204 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td>1 RM, knee extension (kg)</td>
<td>46 ± 6</td>
<td>39 ± 7</td>
<td>49 ± 5</td>
<td>47 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Energy intake 3-day mean (MJ)</td>
<td>8.2 ± 0.1</td>
<td>8.0 ± 0.0</td>
<td>8.9 ± 0.0</td>
<td>8.8 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Protein intake 3-day mean (g/kg)</td>
<td>1.03 ± 0.08</td>
<td>1.03 ± 0.05</td>
<td>1.13 ± 0.05</td>
<td>1.14 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Physical activity index</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean (± SEM) subject characteristics (n = 24). Mass measures are by DEXA scan. A one-way ANOVA was conducted to determine inter-group differences. NS, not significant; CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise.

MPS, not significant; CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise. In contrast, Rasmussen and colleagues showed that an AA intake 1 h after resistance exercise (Dreyer et al., 2008) resulted in greater mixed muscle fractional synthesis rates (FSR) after the resistance exercise bout than an AA intake 1 h before exercise (Fujita et al., 2009). This discrepancy may be due to the different exercise timing of the AA intakes or due to methodological aspects, i.e. the MPS was measured indirectly in Tipton et al. (2001) and directly in Dreyer et al. (2008) and Fujita et al. (2009). Moreover, it was reported recently that ingestion of whey protein immediately before resistance exercise compared with 1 h after exercise stimulated muscle net balance to a similar degree during a 5-h post-exercise period in young subjects (Tipton et al., 2007).

The aims of the present work were to determine the synthesis rate of specific functional proteins in skeletal muscle during 6 h after one resistance exercise session combined with ingestion of caseinate, whey, or a non-caloric control drink immediately after the completion of exercise in elderly untrained individuals. We further sought to investigate how ingestion of caseinate 30 min before or immediately after exercise affected the outcome parameters. The milk proteins were intrinsically labeled, which allowed an applied design using one large bolus of protein without disturbing the tracer enrichment noticeably. We hypothesized that ingestion of whey would result in a superior MPS rate compared with caseinate in elderly subjects and that the muscle protein synthetic response would be equal with ingestion of caseinate before and after resistance exercise.
Pre-tests and food registration

At least 2 weeks before carrying out the acute study, the one repetition maximum (1RM) was defined for each subject as the heaviest load that could be lifted just once throughout the complete range of motion in the knee-extension and leg-press machines (Technogym, Gambettola, Italy). After a 5–10 min warm-up on a cycle ergometer (Monark, Sweden) at a light intensity, the subjects were familiarized with the exercise machines. Only one leg was investigated and this “exercising leg” was randomly chosen. In the knee-extension exercise, the 1RM strength was determined in the “exercising leg” only, whereas the leg-press exercise was completed bilaterally to maintain a correct lifting technique. Subsequently, the subjects completed a dual-energy x-ray absorptiometry (DEXA) scan. Approximately 20–30 min before performing the DEXA scan, the subjects were offered a cup of water as the hydration state affects the measurements of fat-free mineral-free tissue mass with the DEXA scan (Prior et al., 1997; Williams et al., 2006). All scans were performed while the subjects were wearing light clothing and no removable metal objects. The subjects were placed in a supine position and scanned (Lunar DPX-IQ, GE Healthcare, Chalfont St. Giles, UK) at a medium speed (~ 25 min). During the 3 days before the study, the subjects were instructed to follow their normal eating pattern and refrain from strenuous physical activity. Further, no intake of alcohol (1 week) and caffeine (1 day) was allowed before the experiment. Weighed food and physical activity registrations were performed during the 3 days before the study to determine each subject’s compliance to the prescribed instructions and to detect possible differences between groups in protein and energy intakes. The registrations indicated acceptable compliance and showed that the subjects had refrained from strenuous physical activity in the 3 days before the experiment. The food recordings were analyzed using Dankost 3000 software (Dansk Catering Center A/S, Herlev, Denmark). The 3-day mean energy and protein intakes were sufficient and similar among groups (Table 1). To validate the food registrations, the recorded energy intake was divided by the basal metabolic rate (BMR), which was estimated by the Cunningham equation (Cunningham, 1980): \[ \text{BMR(cal/day)} = 500 + 22 \times \text{LBM} \]. In this way, we obtained an indirect estimate of the physical activity index (Table 1).

Experimental protocol

The overall design of the acute studies was identical, and only the type and timing of the protein intake differed, as illustrated in Fig. 1. Each trial included heavy resistance exercise, ingestion of a L-[1-13C]leucine-labeled protein or a non-caloric control supplement, and an infusion of a stable isotope tracer. For every trial, the subjects arrived by car at 07:00 hours to the Institute of Sports Medicine (Bispebjerg Hospital, Copenhagen, Denmark). The subjects were placed in a supine position and catheters were retrogradly inserted into antecubital veins on both forearms. One catheter was used for an infusion of L-[1-13C]leucine (99% enriched, Cambridge Isotopes Laboratories, Andover, Massachusetts) and the other for repeated blood sampling. After a background blood sample was drawn for the measurement of tracer enrichment, a primed [1.97 mg/kg body weight (BW)], continuous (1.97 mg/kg BW/h) infusion of L-[1-13C]leucine was initiated and maintained for 8 h. The primed, continuous infusion of the leucine tracer was designed to obtain a tracer-to-tracee ratio (TTR) of 10% at an isotopic steady state in plasma-free leucine to mimic the leucine enrichment in the whey and caseinate drinks. The subjects then performed five sets of eight repetitions at 80% of 1 RM in both unilateral knee-extension and bilateral leg-press with 3 min of rest between sets.

Fig. 1. Experimental protocol for the acute studies. L-[1-13C]leucine was infused for 8 h while a heavy resistance exercise was performed and a drink was ingested either before (30 min) or immediately after exercise. In each study, 11 venous blood samples and two muscle biopsies were collected throughout the study period. CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise.
The “exercising leg”, carrying out the knee-extension exercise, was randomly chosen. Except for the time spent during the exercise session, the subjects rested in a bed throughout the entire study period.

At 30 and 390 min after the completion of the resistance exercise bout, muscle biopsies were taken from the lateral portion of the vastus lateralis muscle. The biopsies were taken from two incisions made in the mid-thigh region of the “exercising leg.” All biopsies were obtained under local anesthesia (lidocaine, 1%) using the percutaneous needle (Stille, Stockholm, Sweden) biopsy technique (Bergström, 1962) with manual suction (Evans et al., 1982). As illustrated in Fig. 1, the FSR was measured during a period from 30 to 390 min after exercise. The FSR period was started 30 min after the completion of the resistance exercise to optimize conditions of an isotopic steady state after the strenuous exercise bout. Additionally, 11 venous blood samples were drawn throughout the study period as illustrated in Fig. 1, of which some were used to evaluate the changes in insulin and AA concentrations, as well as 13C-ketoisocaproic acid (KIC) TTR-enrichment.

Protein supplementation

Milk proteins labeled with 13C-leucine were produced at Research Centre Foulim, Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, Aarhus, Denmark, as described elsewhere (Reitelseder et al., 2011). The 13C-leucine isotopic enrichment was measured using gas chromatography mass spectrometry (GC-MS) and the TTR of the milk proteins were 10.0% (Reitelseder et al., 2011). The subjects were fasting from 10.00 hours on the day before the study, and were offered a meal after the completion of the infusion trial. During this period, no nutritional intake was allowed, except for water and the protein supplement. Each subject consumed caseinate protein 30 min before (CasPre), or caseinate protein (CasPost), whey protein (Whey), or water (Control) immediately after the completion of the resistance exercise bout. The amount of labeled protein dissolved in ~300 mL water was 0.45 g/kg LBM, resulting in individualized drinks with a total content of 15.6–30.4 g protein, 7.8–14.6 g essential AAs (EAAs), and 1.5–3.4 g leucine (Table 3).

Blood analyses

Plasma insulin concentrations were determined using enzyme-linked immunosorbent assay kits (ELISA, DakoCytoamtion, Insulin K6219, Cambridgeshire, UK) with intra-assay CV < 7.5% and inter-assay CV < 10%.

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Plasma AA concentrations were determined with prior phenylisothiocyanate (PITC) derivatization and HPLC coupled with a UV-detector (Heinrikson & Meredith, 1984) (HPLC: SpectraSystem P4000, Thermo Separation Products, FinniganMat, Paris, France; Column: Nova-Pak C 18 60A 4 μm, 3.9×300 mm, Waters Corp., Milford, Massachusetts; UV-detector: UV6000LP PDA FINN 50 mm Cell UV photo array detector, Thermo Separation Products). Norleucine was used as internal standard, and 0.05 mol/L ammonium acetate was used as mobile phase A and 0.1 mol/L ammonium acetate in acetonitrile:methanol:water (44:10:46) as mobile phase B, both adjusted to pH 6.8 with acetic acid and filtrated before use.

Stable isotope analyses

Determinations of plasma 13C-KIC enrichments were performed on GC-MS with prior derivatization. One milliliter of ethanol (99.8%) was added to 140 μL of venous blood, and the mixture was spun down to precipitate the proteins. The supernatant was evaporated with N2 at 50 °C. 200 μL Millipore H2O and 200 μL acidified 2% w/v O-phenylenediamine solution were added. The sample was extracted twice by adding 1 mL ethylacetate and centrifuging. The acetate phase was collected and evaporated with N2. Fifty microliters of pyridine and 50 μL BSTFA+1% TMCS were added and the mixture was shaken. 50 μL of the sample was left for 30 min at room temperature to allow derivatization. Subsequent analyses were carried out on the GC-MS analyser (GC: Trace GC 2000 series, MS: Automass Multi, Thermo Quest Finnigan, Paris, France) as described elsewhere (Holm et al., 2010).

To determine leucine enrichment in myofibrillar and total intramuscular collagen protein pools, weighed pieces of muscle tissue (15–25 mg) were ground in liquid nitrogen and homogenized in a high-salt buffer (0.15 M NaCl, 0.1% Triton X-100, 0.02 M Tris, 5 mM EDTA). The resulting homogenate was subjected to repeated centrifugation and salt extraction procedures in order to separate the myofibrillar, collagen, and sarcoplasmic fractions. The sarcoplasmic fraction remained in the supernatant, and the myofibrillar and collagen fractions precipitated. The myofibrillar and collagen pellet was added to 1 mL 0.7 M KCl, vortexed, left for 30 min at 4 °C, and centrifuged at 1600 g at 4 °C for 20 min. The supernatant containing the myofibrillar fraction was collected, leaving the collagen protein fraction in the pellet. Ethanol was added to the myofibrillar supernatant and the mixture was centrifuged to precipitate the myofibrillar proteins. The washed myofibrillar pellet and the total salt-precipitated collagen fraction were hydrolyzed and derivatized...
separately but in a similar way, as described in the following. The myofibrillar and collagen pellets were hydrolyzed in 6 M HCl (110 °C for 18 h), and the constituent AA were purified by cation exchange resin columns (Dowex AG-50W, Bio-Rad, Copenhagen, Denmark). The samples were then n-propyl n-propyl acetate (NAP) derivatized through the addition of n-propyl chloroform. Fifty microliters of acetonitrile, 26 µL 1.4-dioxan, 38 µL triethylamine, and 24 µL acetic anhydride were added to the samples. The solvent was added to 50 µL chloroform and 3 × 50 µL 0.001 M NaHCO3 and the water was removed. The samples were analyzed on the gas chromatography combustion isotope ratio mass spectrometry analyzer (GC-C-IRMS) (DeltaPlus XL, Thermo Finnigan, Bremen, Germany) as described elsewhere (Holm et al., 2010).

FSR calculation
The myofibrillar and muscle collagen FSR were calculated from the 13C-leucine incorporation into muscle protein using the standard precursor–product method:

\[
FSR (\%/h) = \frac{\Delta E_{\text{product}}(E_{\text{precursor}} \times \Delta \text{time})^{-1} \times 100\%}{\text{time period between the two biopsies}},
\]

where \(\Delta E_{\text{product}}\) represents the change in protein-bound tracer enrichment between two biopsies, \(\Delta \text{time}\) is the time period between the two biopsies, and \(E_{\text{precursor}}\) represents the mean precursor pool enrichment in that time period (Wolfe & Chinkes, 2005). A weighted mean of the venous plasma 13C-KIC enrichments shown in Table 2 was used as an estimate of the precursor pool enrichment to calculate the myofibrillar and collagen FSR. Venous plasma 13C-KIC enrichment was chosen as an acceptable alternative of the real intracellular precursor pool (i.e., leucyl-transfer RNA) enrichment (Watt et al., 1991; Toffolo et al., 2003; Chow et al., 2006).

Statistical analysis
All values are presented as means ± standard error of the mean (SEM). Statistical analyses for all comparisons of subject characteristics and dietary intakes between groups were performed using one-way analysis of variance (ANOVA). Analyses of differences in insulin concentrations, AA concentrations, and KIC enrichments with time and between groups were carried out using two-way ANOVA with groups as unreplicated and time as repeated measures. When the main effects were obtained, Student–Newman–Keuls post hoc tests were performed to determine the specific differences between groups and time points. The areas under the insulin and AA curves were analyzed using one-way ANOVA and Student–Newman–Keuls post hoc tests. The FSR values were compared among groups using one-way ANOVA, with Student–Newman–Keuls post hoc tests. The level of significance was set at \(P<0.05\), and analyses were performed using SigmaPlot 11.0 (Systat Software Inc., San Jose, California).

Results
Subject characteristics
Subject characteristics are presented in Table 1. There were no differences in any of the variables between groups.

Systemic insulin concentrations
Plasma insulin concentrations are shown in Fig. 2. A significant effect of interaction (\(P<0.001\)) was observed. The insulin concentrations were increased in CasPost (15–30 min) and Whey (15–90 min) compared with the basal level. The insulin concentrations reached a peak of 57 ± 11, 72 ± 15, and 104 ± 8 pmol/L after exercise for CasPre, CasPost, and Whey, respectively. In the control group, the plasma insulin concentration did not change throughout the study period, and the average insulin concentration was 18 ± 2 pmol/L.

The total insulin response (i.e., the area under the curve [AUC]) in the time period 15–390 min after the resistance exercise bout was significantly greater in CasPost and Whey compared with Control (\(P<0.01\)).

Systemic AA concentrations
Plasma concentrations for leucine; the sum of EAA, except Phe, Thr, and Trp; and total AA (TAA),
except Asn, Asp, Cys, Phe, Thr, and Trp, are reported in Fig. 3. For leucine, EAA, and TAA, significant effects of interaction ($P<0.001$) were observed. The plasma TAA concentrations were increased in CasPre (15–150 min), CasPost (30–60 min), and Whey (30–60 min) compared with the basal levels. The plasma EAA concentrations were increased in CasPre (30 min), CasPost (30–270 min), and Whey (30–60 min) compared with the basal levels. The plasma leucine concentrations were increased during the entire post-exercise periods in CasPre, CasPost, and Whey compared with the basal levels. The leucine concentrations reached a peak of $227 \pm 11$, $282 \pm 17$, and $490 \pm 32 \mu$mol/L in CasPre, CasPost, and Whey, respectively. In Control, the plasma leucine, EAA, and TAA concentrations did not change throughout the study period and the average leucine concentration was $152 \pm 6 \mu$mol/L.

The total leucine response expressed as the AUC in the time period 15–390 min after the resistance exercise bout was significantly higher in Whey compared with all the other groups ($P<0.05$), higher in CasPost compared with CasPre ($P<0.05$), and lower in Control than in the other groups ($P<0.01$). The EAA AUC (measured 15–390 min after exercise) was significantly lower in Control than in all the other groups ($P<0.001$). The TAA AUC (measured 15–390 min after exercise) was lower in Control than in all the other groups ($P<0.01$).

Fig. 2. Mean (± SEM) plasma insulin concentrations (pmol/L) before (time = 120) and after resistance exercise. Data were analyzed using two-way ANOVA, with time being the repeated measure. A significant effect of interaction ($P<0.001$) was observed. *CasPre and CasPost were significantly higher compared with Control. ¤CasPost was significantly higher compared with CasPre. §CasPost was significantly higher compared with Control. Whey was significantly higher compared with all the other groups. — Significantly higher compared with basal in CasPost. - - - Significantly higher compared with basal in Whey (Student–Newman–Keuls post hoc test, $P<0.05$). CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise.

Fig. 3. Mean (± SEM) plasma leucine (a), essential amino acids (EAA; b), and total amino acid (TAA; c) concentrations (μmol/L) before (time = 120) and after resistance exercise. Data were analyzed using two-way ANOVA, with time being the repeated measure. For leucine, EAA, and TAA, a significant effect of interaction ($P<0.001$) was observed. *CasPre was significantly higher compared with all the other groups. CasPre and CasPost were significantly higher compared with Control. ¤CasPost was significantly higher compared with CasPre. §§CasPost was significantly higher compared with Control. Whey was significantly higher compared with Control. §Whey was significantly higher compared with all the other groups (Student–Newman–Keuls post hoc test, $P<0.05$). CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise. The TAA AUC (measured 15–390 min after exercise) was lower in Control than in all the other groups ($P<0.01$).
Venous plasma 13C-KIC enrichment

The mean venous plasma 13C-KIC enrichments are shown in Table 2. A significant effect of interaction \((P<0.05)\) was observed. In the CasPost group, a difference in enrichment was observed between 30 and 210 min, and between 30 and 390 min after resistance exercise \((P<0.01)\). In Whey, a difference was found between 30 and 210 min, and between 30 and 390 min after resistance exercise \((P<0.01)\). In the CasPre and Control groups, the enrichment did not change with time. Additionally, there were no differences between groups.

Myofibrillar and collagen protein synthesis

The myofibrillar and total muscle collagen FSR are presented in Fig. 4. As a precursor, we used a mean of each individual’s venous 13C-KIC enrichment determined at three time points throughout the incorporation period (Table 2). For the myofibrillar proteins, a significant difference between groups was observed overall \((P<0.05)\). The myofibrillar FSR was higher in CasPre \((0.10\pm0.007\%/h)\) compared with Control \((0.07\pm0.006\%/h)\) \((P=0.025)\). Furthermore, the myofibrillar FSR tended to be higher in Cas Post \((0.09\pm0.003\%/h)\) compared with Control \((P=0.052)\), and in Whey \((0.09\pm0.005\%/h)\) compared with Control \((P=0.025)\). The four mean FSR values were sorted in order, and no significant difference was found between CasPost and Control. As the difference of means was smaller in Whey compared with Control than in CasPost compared with Control, the difference between Whey and Control was not considered significant due to the procedural rule of the post hoc test, although \(P=0.025\). All other post hoc test comparisons had \(P>0.5\).

For the muscle collagen proteins, a strong tendency toward a significant difference between groups was observed \((P=0.07)\). The muscle collagen FSR were \(0.06\pm0.005\%/h\), \(0.06\pm0.006\%/h\), \(0.06\pm0.006\%/h\), and \(0.04\pm0.002\%/h\) in CasPre, CasPost, Whey, and Control, respectively.

Discussion

The most novel findings in the present study were that the consumption of whey and caseinate protein after resistance exercise resulted in a similar stimulation of both the myofibrillar and the collagen protein synthesis rate during the initial 6-h recovery period in elderly subjects. Moreover, there was no difference between caseinate ingestion 30 min before and immediately after exercise in either the myofibrillar or the collagen protein synthesis rate. Furthermore, the collagen synthesis rates tended to be higher after protein ingestion compared with the control group.

The majority of studies comparing whey and casein protein have found that whey is superior to casein in stimulating whole-body protein synthesis in young (Boirie et al., 1997; Dangin et al., 2001) as well as elderly (Dangin et al., 2003) resting individuals. In skeletal muscle, though, studies have shown that whey may be superior to casein protein to stimulate MPS over the very first hours in the recovery from a resistance exercise bout (Tipton et al., 2004; Tang et al., 2009; Reitelseder et al., 2011). However, it was

Fig. 4. Distribution of the fractional synthetic rate (FSR; %/h) of myofibrillar (a) and muscle collagen (b) proteins during a 6-h recovery period \((1/2–6\text{ h})\) after resistance exercise. As a precursor, we used a mean of each individual’s venous plasma 13C-KIC enrichment throughout the incorporation period. To illustrate female and male values, different symbols are used, "" females and ○ males. Data were analyzed using a one-way ANOVA. For the myofibrillar proteins, a significant difference between groups was observed overall \((P<0.05)\). *Significantly higher myofibrillar FSR in CasPre compared with Control \((P<0.05)\). **Tendency toward higher myofibrillar FSR in CasPost compared with Control \((P=0.052)\), and in Whey compared with Control \((P=0.025)\) (Student–Newman–Keuls post hoc test). The four mean FSR values were sorted in order, and no significant difference was found between CasPost and Control. As the difference of means was smaller in Whey compared with Control than in CasPost compared with Control, the difference between Whey and Control was not considered significant due to the procedural rule of the post hoc test, although \(P=0.025\). All other post hoc test comparisons had \(P>0.5\). For the muscle collagen proteins, a strong tendency toward a significant difference between groups was observed overall with the one-way ANOVA \((P=0.07)\). CasPre, caseinate intake 30 min before exercise; CasPost, caseinate intake immediately after exercise.
shown recently that the slower absorption rate of caseinate in the later acute recovery (3–6 h after exercise) continues to stimulate MPS while the whey protein is fully absorbed and its effect is markedly diminished (Reitelseder et al., 2011). Moreover, different types of casein protein may induce slightly different anabolic responses, with the micellar casein seemingly being less potent (Tang et al., 2009) than caseinate, which was used in the present as well as another study (Reitelseder et al., 2011).

The higher leucine and EAA concentrations observed after ingestion of whey than of caseinate (Fig. 3) are presumably caused by a faster digestibility and perhaps also a higher leucine content in whey compared with caseinate (11.8% vs 8.8%; Table 3). Hence, as elderly individuals possibly need a high leucine and EAA availability to maximize MPS (Paddon-Jones et al., 2004; Katsanos et al., 2006; Rieu et al., 2006), it was surprising that the MPS was not higher after ingestion of whey compared with caseinate in the present study. The explanation may be that the amount of protein ingested (0.45 g/kg LBM) even in the case of the slow digestible caseinate supplement was sufficient to allow a sufficient AA absorption from the gastric region to maximally stimulate the MPS in the elderly individuals (Cuthbertson et al., 2005). Thus, the higher delivery of leucine to the muscles following the ingestion of whey compared with caseinate probably resulted in excess amounts of leucine (AA in general), which was presumably oxidized as the muscle protein synthetic processes were fully loaded and maximally activated. It could be speculated, though, that if elderly individuals ingest smaller amounts of protein, a beneficial effect of whey compared with caseinate may be obtained.

The larger peak in circulating AA concentration from the whey compared with the caseinate protein intake presumably caused the higher insulin response (Fig. 2), which has been observed previously in young subjects (Dangin et al., 2001; Tipton et al., 2004). However, insulin seems to have minimal effect on MPS after resistance exercise and primarily blunts the exercise-mediated acceleration of muscle protein breakdown (Biolo et al., 1999; Miller et al., 2003; Borsheim et al., 2004; Koopman et al., 2007). Thus, with the indications of insulin resistance of basal muscle protein metabolism in non-diabetic aging persons in mind (Volpi et al., 2000; Rasmussen et al., 2006; Wilkes et al., 2009), it seems unlikely that the insulin response should have had any impact on the elevations in myofibrillar and collagen protein synthetic rates.

The present results indicate that it is of less importance whether caseinate protein is ingested 30 min before or immediately after resistance exercise (Fig. 4). This is in agreement with the study by Tipton et al. (2007) providing whey protein immediately before and 1 h after resistance exercise. Our results indicate that caseinate ingestion 30 min before exercise did induce a solid anabolic stimulus, which persisted beyond the time point where the EAA availability had returned to basal concentrations. Hence, it is likely that some long-lasting anabolic conditions can be achieved in the exercised muscles when AAs are available in excess amounts close to the exercise session. Additionally, it is possible that the overall anabolic effect is more in CasPre compared with CasPost, because the acute anabolic effect of caseinate ingestion in CasPre may not be fully included in the post-exercise FSR measurement. A concomitant increase in the blood flow and plasma AA concentration may have increased the delivery of nutrients to the working muscles, which may have prevented a decrease in Fujita et al. (2009) or actually stimulated (Beelen et al., 2008) MPS during exercise in the CasPre group. Owing to the relatively slow skeletal muscle protein turnover, the energy requirement for MPS is largely negligible compared with the massive energy requirements in contracting muscles. On the other hand, there is reason to believe that the muscle AA metabolism during heavy resistance exercise is mainly altered toward energy production (i.e. AA transamination and oxidation) to support muscle contraction rather than exercise-mediated adaptations (Rennie et al., 2004; Kumar et al., 2009). Hence, as long as the AA availability is increased close to the exercise session, it most likely

| Table 3. Amino acid distributions of Whey and caseinate protein |
|-----------------|-----------------|-----------------|
| 100 g protein   | Whey            | Caseinate       |
| Amino acid      |                 |                 |
| Alanine (g)     | 4.66            | 2.84            |
| Arginine (g)    | 2.65            | 3.31            |
| Asparagine (g)  | 11.25           | 6.73            |
| Cysteine (g)    | 2.65            | 0.35            |
| Glutamine (g)   | 16.58           | 20.61           |
| Glycine (g)     | 1.78            | 1.73            |
| Histidine (g)   | 2.25            | 2.87            |
| Isoleucine (g)  | 5.72            | 5.03            |
| Leucine (g)     | 11.77           | 8.77            |
| Lysine (g)      | 9.68            | 7.44            |
| Methionine (g)  | 2.10            | 2.71            |
| Phenylalanine (g)| 3.54          | 4.80            |
| Proline (g)     | 4.82            | 10.10           |
| Serine (g)      | 4.78            | 5.73            |
| Threonine (g)   | 5.05            | 3.97            |
| Tryptophan (g)  | 2.04            | 1.16            |
| Tyrosine (g)    | 3.59            | 5.38            |
| Valine (g)      | 5.29            | 6.46            |
| EAA (g)         | 50.09           | 46.53           |
| Total (g)       | 100             | 100             |

Amino acid composition of the whey and caseinate protein. 0.45 g protein/kg LBM was dissolved in ~ 300 mL water. Thereby, individualized drinks with total content of 15.6–30.4 g protein, 7.8–14.6 g essential amino acids (EAA), and 1.5–3.4 g leucine was ingested.
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makes no difference in protein synthetic stimulation whether protein is ingested before or after resistance exercise.

We found that the muscle collagen FSR was unaffected by the type and the timing of the protein intake, but that protein feeding strongly tended to increase the collagen FSR during the recovery period compared with the control group (Fig. 4). The present study is the first to demonstrate that a significant increase in circulating AA favors muscle collagen growth. It has been shown previously that the increases in muscle collagen and myofibrillar FSR follow an almost similar time course during 72 h of recovery from a non-damaging strenuous aerobic exercise bout (Miller et al., 2005) and during the 8.5 h following maximal shortening and lengthening contractions (Moore et al., 2005) in fed, young individuals. Additionally, recent studies have reported that feeding does not increase the muscle collagen synthesis rate either at rest (Mittendorfer et al., 2005; Holm et al., 2010) or in combination with resistance exercise (Holm et al., 2010) in young persons. The reason for these contrasting findings could very likely be the differences in study designs such as intermittent feeding (Holm et al., 2010) vs bolus feeding in the present study. This presumably led to different levels of AAs availability during the recovery period, resulting in different stimulatory effects. It may be that a high availability of AA is needed to obtain a favorable effect of feeding on muscle connective tissue protein synthesis following resistance exercise in elderly individuals.

In the present study, we wanted to make the study outcome relevant for an elderly population in general and, therefore, both men and women were included. Although the different groups were equal with regard to age, body composition, and strength, it was not possible to obtain a completely similar distribution of sex in each group. Although conflicting data exist on whether sex has an impact on the basal MPS rate (Balagopal et al., 1997; Smith et al., 2008), a study with over 200 inclusions recently reported that the basal mixed muscle FSR and whole-body protein turnover rates are higher in women than in men, irrespective of age (Henderson et al., 2009). In contrast, elderly obese women have been shown to respond less to feeding than elderly, obese men (Smith et al., 2008). Therefore, the lack of difference between men and women in the post-exercise and fed state could be due to a smaller stimulation of protein synthesis rate on top of a higher basal rate in the women compared with males. This phenomenon cannot be verified by the present study. However, the net balance outcome will only be influenced if also the responsiveness of the protein breakdown rate is affected by sex, which has not been investigated. Longitudinal training intervention studies though, have shown that elderly men respond better to resistance training compared with elderly women (Welle et al., 1995; Kosek et al., 2006). Therefore, it could be hypothesized that the improvements in net balance to exercise plus feeding in men are larger than those in women, despite the similar levels of synthesis rate as shown in Fig. 4.

To achieve and maintain an isotopic steady-state enrichment in the free AA pools in blood and muscle while AA from the ingested protein entered these pools, the subjects had received a relatively high amount (roughly 0.3–0.6 g) of leucine via a primed, continuous infusion at the initiation of the measuring period (30 min). However, as leucine is important for improving MPS in elderly humans (Katsanos et al., 2006; Rieu et al., 2006), we cannot exclude that the high amount of leucine tracer may have stimulated the protein synthetic machinery to some extent. However, the amount of leucine given during the primed, continuous infusion is equal among all groups. Thus, a possible anabolic effect should intuitively not have influenced the difference between groups. On the other hand, if the maximally stimulating level of leucine/EAA availability was achieved by the protein intake in the protein-supplemented groups, the leucine tracer could have biased the results in favor of the Control group, which did not seem to be the case.

The primed continuous tracer infusion was designed to ensure an isotopic steady state within 90 min (before finishing the resistance exercise). As the venous plasma $^{13}$C-KIC enrichment increased significantly in CasPost and Whey during the FSR measuring period, the alterations in muscle protein metabolism caused by the protein ingestion may have elongated the time course for enrichment equilibrium between the intracellular compartments and blood plasma. That is, the altered muscle protein metabolism may have resulted in an inconsistency in the intramuscular leucyl-tRNA:venous KIC enrichment ratio during the measuring periods, which may have ultimately affected the FSR calculations. However, it should be emphasized that the magnitude of the inconsistency in the intramuscular leucyl-tRNA:venous KIC enrichment ratio in the different groups is difficult to assess, as the intracellular trafficking of AA from different sources is unknown. Moreover, as we aimed for an isotopic steady-state enrichment of 10% and achieved a mean venous plasma KIC enrichment of 13%, the priming dose and the continuous infusion were too high. Thus, the labeled proteins may have diluted the isotopic enrichment obtained by the primed continuous infusion, which is supported by the fairly lower initial venous plasma KIC enrichments in the CasPost and Whey groups (Table 2). In fact, the lower initial enrichments at
30 min may, to a large extent, be responsible for the observed increases in the enrichments in CasPost and Whey at 210 and 390 min.

In summary, the present study displays a similar muscle protein synthetic response with caseinate and whey protein ingestion following heavy resistance exercise in elderly men and women. Contrary to our hypothesis, whey was not superior to caseinate in stimulating myofibrillar and collagen protein synthesis. Importantly, the amounts of protein provided may have possibly induced a maximal muscle protein synthetic response in the elderly individuals, and, thus, the type of caseinate and amount of protein ingested may explain the observed similarity between whey and caseinate protein. In addition, a similar muscle protein synthetic response was observed when caseinate was ingested 30 min before or immediately after resistance exercise. Furthermore, the collagen synthesis rates tended to be higher in the groups ingesting protein compared with the control group.

**Perspectives**

Because the protein distribution in milk is ~80% and 20% of casein and whey, respectively, it is reasonable to recommend milk consumption before and/or after each resistance exercise bout. Furthermore, both acute and longitudinal studies have shown that milk ingestion is superior to soy protein ingestion in enhancing muscle anabolism after resistance exercise (Hartman et al., 2007; Wilkinson et al., 2007). Obviously, it is of high importance to optimize the anabolic response to resistance exercise in elderly individuals. As elderly persons are considered to prefer well-known nutrient products that are accessible, inexpensive, and palatable, the recommendation of exercise-timed milk consumption appears to be especially applicable to elderly individuals involved in resistance training.

**Key words:** myofibrillar protein synthesis, collagen protein synthesis, insulin, amino acids.

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