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Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans

B. Esmarck, J. L. Andersen*, S. Olsen, E. A. Richter†, M. Mizuno‡ and M. Kjær

Sports Medicine Research Unit, Bispebjerg Hospital, *Copenhagen Muscle Research Centre, CMRC, Rigshospitalet, †Department of Human Physiology, University of Copenhagen and ‡Department of Anaesthesia, Rigshospitalet, Denmark

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1. Age-associated loss of skeletal muscle mass and strength can partly be counteracted by resistance training, causing a net synthesis of muscular proteins. Protein synthesis is influenced synergistically by postexercise amino acid supplementation, but the importance of the timing of protein intake remains unresolved.

2. The study investigated the importance of immediate (P0) or delayed (P2) intake of an oral protein supplement upon muscle hypertrophy and strength over a period of resistance training in elderly males.

3. Thirteen men (age, 74 ± 1 years; body mass index (BMI), 25 ± 1 kg m⁻² (means ± S.E.M.)) completed a 12 week resistance training programme (3 times per week) receiving oral protein in liquid form (10 g protein, 7 g carbohydrate, 3 g fat) immediately after (P0) or 2 h after (P2) each training session. Muscle hypertrophy was evaluated by magnetic resonance imaging (MRI) and from muscle biopsies and muscle strength was determined using dynamic and isokinetic strength measurements. Body composition was determined from dual-energy X-ray absorptiometry (DEXA) and food records were obtained over 4 days. The plasma insulin response to protein supplementation was also determined.

4. In response to training, the cross-sectional area of m. quadriceps femoris (54.6 ± 0.5 to 58.3 ± 0.5 cm²) and mean fibre area (4047 ± 320 to 5019 ± 615 µm²) increased in the P0 group, whereas no significant increase was observed in P2. For P0 both dynamic and isokinetic strength increased, by 46 and 15%, respectively (P < 0.05), whereas P2 only improved in dynamic strength, by 36% (P < 0.05). No differences in glucose or insulin response were observed between protein intake at 0 and 2 h postexercise.

5. We conclude that early intake of an oral protein supplement after resistance training is important for the development of hypertrophy in skeletal muscle of elderly men in response to resistance training.

Ageing is associated with a progressive reduction of skeletal muscle volume (Lexell et al. 1988) and a concomitant reduction in strength (Grimby & Saltin, 1983; Young et al. 1985; Vandervoort & McComas, 1986; Frontera et al. 1991). This influences the physical performance and thereby the daily function of the elderly. However, resistance training has been shown to counteract the atrophy and loss of strength in this age group (Frontera et al. 1988; Fiatarone et al. 1990; Charette et al. 1991; Welle et al. 1996).

Hypertrophy following resistance training requires net protein synthesis of the myofibrillar proteins, and hence, a maximal stimulation of protein synthesis is favourable for the development of muscle hypertrophy in the elderly. Mixed muscle protein synthesis rate is increased in humans after bouts of resistance training provided the stimulus is of a sufficient magnitude (Chesley et al. 1992). This transient increase in protein synthesis is marked and surpasses the increase in degradation rate, and persists for up to 48 h following an acute exercise bout (Phillips et al. 1997). Evidence exists in favour of the balance between protein synthesis and degradation in skeletal muscle being tipped in favour of protein synthesis by protein intake and hyperaminoacidaemia during rest (Bennet et al. 1989; Biolo et al. 1997; Smith et al. 1998), and net protein balance remains negative after training if...
Anthropometrical measurements (DEXA scanning), food registration (weighed food records) and muscle cross-sectional area measurements (MRI scanning and muscle biopsy) were performed before (pre-training) and after (post-training) the training period. On two occasions during the period of training acute studies were conducted to measure the insulin response.

All subjects, P-tot (n = 13), were matched in pairs based on body composition and daily protein intake, and randomly assigned to either of two groups, P0 (n = 7) and P2 (n = 6), after the pre-training test round. The odd number is due to one subject's withdrawal from the study due to reasons not associated with the study.

Training
A supervised progressive bilateral resistance training programme was performed 3 times a week for 12 weeks (~36 training days) in the morning between 08.00 and 10.00 h. All subjects were individually supervised at all training sessions by one of the study investigators. Each bout of training lasted approximately 30 min starting with a 5–10 min warm up on a cycle ergometer (Monark, Sweden). The resistance training consisted of three different concentric strength exercises: leg press (Casall Leg Press), latissimus dorsi (lat) pulldown (Technogym lat machine, Italy) and knee extension (Technogym leg extension R.O.M. (range of movement)), always performed in the described order. The load for the leg exercises increased from 20 repetition maximum (RM) to 12 RM during the first 6 weeks (10–12 repetitions, 3–4 sets), and during the last 6 weeks it remained at 8 RM (8 repetitions, 3–5 sets) to ensure as high an exercise intensity as possible, taking into account the need for graded adaptation in these previously untrained individuals. The load for lat pulldown increased from 20 to 10 RM over the period of training (8–12 repetitions, one set). The load for all exercises was adjusted at every third training session to the exact RM according to the subject’s schedule.

Protein supplementation
The supplement consisted of a protein gel (JogMate Protein, Otsuka Pharmaceutical, Japan) containing 10 g protein (from skimmed milk and soybean), 7 g carbohydrate and 3.3 g lipid, corresponding to an energy intake of 420 kJ. The protein gel was dissolved in warm water (approximately 35°C) before oral ingestion. P0 ingested the protein supplement within 5 min after the termination of each training session and P2 ingested the protein supplement 2 h after each training session. Subjects were not allowed to ingest anything but the protein supplement during the 2 h following training, and after that free access to food was allowed. Further, subjects were instructed to have finished breakfast 1.5 h before the onset of training. The subjects were allowed water ad libitum.

Daily food intake
Self-reported food records (Team Danmark, Denmark) were kept for 4 consecutive days starting on a Sunday to determine daily food intake. Subjects were given verbal and written information and received electronic scales (Tefal, France) with ±1 g accuracy for use at home.
Strength measurements

**Isokinetic strength.** Unilateral maximal isokinetic strength was defined as the maximal voluntary torque produced in an isokinetic dynamometer (KinCom, Kinetic Communicator, Chattanooga Corp., Chattanooga, TN, USA). Knee joint angular velocity was 60 and 180 deg s\(^{-1}\), and the range of motion was 85 to 0 deg (0 deg = full knee extension). Only the concentric strength of knee extension of the right leg was measured as the two legs presumably had been trained equally. The number of subjects with the right leg as the dominant leg did not differ between the two groups. Subjects were familiarised with the apparatus 1 week before the pre-training test.

After a 10 min warm up on the cycle ergometer, subjects were tested seated in a rigid chair with a 90 deg hip flexion and arms crossed. The hip, thigh and lower leg were held in position by straps attached to the chair and lever arm of the dynamometer, respectively. The axis of rotation of the lever arm was aligned with the transversal axis of the knee joint. Gravity correction was measured at an angle of 30 deg in order to avoid passive hamstring strain to contribute to the reference gravity torque.

During testing, verbal encouragement was given, and the subjects were able to follow the force production during the trial. A multiple trial protocol was used, where the voluntary maximal isokinetic torque was defined as being achieved when the subject was unable to produce a higher torque in the two succeeding attempts. This was typically reached within six to eight attempts. All trials were saved for further analysis. The torque produced at each degree of movement was sampled, saved and automatically computed for gravity correction. The torque at 60 deg was chosen a priori for comparison of the voluntary isokinetic strength as peak force is typically close to this angle.

**Dynamic training strength.** A value of 5 RM (repetition maximum) was used as a measure of dynamic training strength. This was measured with the training apparatus (Technogym leg extension R.O.M.) used and was always carried out after the isokinetic measurements.

Muscle cross-sectional area (CSA)

MR scanning was conducted by a 1.5T Philips ACS-NT scanner within 1 week prior to the 12 week training period and 1 week after the termination of training. Subjects had not been engaged in any testing or training 3 days prior to the MR scanning. Subjects were placed within the stationary, external field in the supine position with the mid-point of the thigh (half-way between trochanter major and epicondylus lateralis) as the point of reference. For determination of CSA, five transaxial images were obtained at mid-thigh in a flip angle of 35 deg with the following parameters: repetition time = 500 ms and echo time = 14 ms. Each data set was obtained with field of view = 200 mm consisting of a 212 pixel matrix. Slice thickness was 6 mm and interslice gap equalled 0.3 mm. The transaxial images were transferred to a Unix Workstation (SPARCreation 20, Sun Microsystems, Mountain View, CA, USA) and analysed using the software program Easy Vision 4.2. All four heads of m. quadriceps femoris were outlined manually 3 times for analysis, and CSA was determined as the average of the three analyses.

Biopsies were obtained from m. vastus lateralis after local anaesthesia using the percutaneous needle biopsy technique (Bergström, 1962) with suction. A part of the biopsy was oriented, mounted and immediately frozen in isopentane cooled by liquid nitrogen and kept at −80°C till further analysis. The rest of the biopsy was frozen directly in liquid nitrogen.

Analysis

**ATPase histochemistry.** The embedded (Tissue-Tek) biopsy was serially cryosectioned (10 µm) at −25°C in a cryostat. For identification of fibre types the sections were mounted on glass slides and stained for ATPase activity after preincubation at pH 4.37, 4.60 and 10.3 (Brooke & Kaiser, 1970). The serial sections were visualised and analysed using an Olympus BX40 microscope (Olympus Optical, Tokyo, Japan), a Sanyo high-resolution colour charge-coupled device camera (Sanyo Electronic) and an 8-bit Matrox Meteor Framegrabber (Matrox Electronic Systems, Quebec, Canada), combined with an image-analysing computer program (Tema, Scanbeam, Hadsund, Denmark). Five fibre types were distinguished (1, 1/2a, 2a, 2a/b and 2b) from the staining pattern (Andersen & Aagaard, 2000). For determination of fibre-type distribution 214 ± 11 fibres (mean ± S.E.M.) were analysed per biopsy. For assessment of mean fibre area (MFA) 123 ± 10 fibres were analysed per biopsy.

**Electrophoretic separation of myosin heavy chain (MHC) isoforms.** SDS-PAGE was conducted to assess the relative expression of MHC isoforms in the biopsies. Twenty serial cryosections (10 µm) from the Tissue-Tek-embedded biopsy were collected in Eppendorf tubes. Denaturation buffer (Fry et al. 1994) was applied to the Eppendorf tubes, which were then heated for 3–5 min at 90°C. Gels were run at 70 V and at 4°C for 42–45 h (Andersen & Aagaard, 2000). Subsequently, the gels were Coomassie stained and the relative amount of each MHC isoform (I, IIA and IIX) was quantified using a scanner and densiometric software (Cream, Ke-Men-Tec Aps, Copenhagen, Denmark).

**The acute insulin response**

Six of the subjects were included in an additional acute study (3 from each training group, P0 and P2). The study was performed twice on two normal training days by use of a crossover design. Subjects arrived at 07.00 h after an overnight fast, and had a catheter inserted in the antecubital vein. The procedures of training and protein intake were identical to those described previously, except that strength exercise for the upper extremities in this case was excluded. Blood samples were collected before and every 30 min during the 4 hours following the training session. After sampling, plasma glucose concentration was analysed immediately (Yellow Springs Instruments, Yellow Springs, OH, USA) and the remaining blood was centrifuged and stored at −80°C until analysed for plasma adrenaline, noradrenaline and insulin concentration by immuno-radiometric (RIA) assays (Pharmacia, Uppsala, Sweden).

**Calculations**

The results for MFA were based on calculations where normalisation of the fibre-type distribution was performed in order to reduce the influence of the increased degree of heterogeneity observed in the ageing muscle (Lexell & Downham, 1991). Hence, the calculation of MFA of all fibres analysed (MFA-total) included the MFA and distribution (relative number) of all subtypes. Due to the low number of hybrid fibres (fibre-type 1/2a and 2a/b), these fibres were evenly divided into fibre-type 1, 2a and 2b for calculation of MFA-1, -2a and -2b, respectively.

Insulin availability during the 120 min after the intake of the supplement was assessed as the area under the curve calculated by the trapezoid method.

**Statistics**

The statistical analysis of the data was performed with SPSS standard version (7.5.3). As the data were non-normally distributed, the dependent variable was tested by non-parametric statistics. Wilcoxon signed-rank test (2-tailed) was applied for dependent
statistical comparison between two time points (intra-group). Mann-Whitney U test (2-tailed) was used for independent statistical comparison between two groups (inter-group). The relationship between variables was tested by the Spearman rank-difference correlation method (2-tailed). The significance level was set at $P < 0.05$.

**RESULTS**

**Anthropometrical measurements**

No significant difference was observed between P0 and P2 for any anthropometrical measurement before or after training. However, whole-body lean body mass increased $1.8 \pm 0.7\%$ ($P < 0.05$) in P0 and decreased $1.5 \pm 0.7\%$ ($P < 0.05$) in P2 (Table 1).

**Daily food intake**

No significant difference in any dietary parameter was observed between P0 and P2 either before or after training when assessed absolutely and relative to body weight. Further, training did not change dietary habits in either group (Table 1) or in the total of all subjects, P-tot. The protein supplementation was $0.13 \pm 0.01\text{ g (kg body weight)}^{-1}$ in both P0 and P2.

**Strength measurements**

In P-tot, dynamic training strength (5 RM), and isokinetic 60 and 180 deg $s^{-1}$ strength increased $42 \pm 6$, $13 \pm 7$ and $16 \pm 5\%$ ($P < 0.05$), respectively, with training. No difference was observed between values for P0 and P2 pre-, mid- or post-training for any of the strength measurements.

In P0, 5 RM, and isokinetic 60 and 180 deg $s^{-1}$ strength increased ($P < 0.05$) $47 \pm 4$, $24 \pm 9$ and $21 \pm 5\%$, respectively, with training (Fig. 1), whereas in P2, an increase in strength ($P < 0.05$) was only observed for 5 RM (36 $\pm$ 12%). Only three and four out of six subjects in P2 showed an increase in isokinetic strength at 60 and 180 deg $s^{-1}$, respectively. No significant difference in the relative increase in strength was observed between P0 and P2 for any measurement. However, isokinetic strength at 60 deg $s^{-1}$ tended ($P=0.07$) to increase more in P0 than in P2 (24 $\pm$ 9 vs. 0 $\pm$ 8%). At 60 deg $s^{-1}$ peak torque was obtained at 57 $\pm$ 1 deg in P-tot with no significant differences between the two groups (P0 55 $\pm$ 2 deg and P2 59 $\pm$ 2 deg). Changes in peak force were in agreement with torque changes at 60 deg (data not shown).

**CSA of m. quadriceps femoris**

The CSA of m. quadriceps femoris (CSA-q.f.) increased $4 \pm 1\%$ ($P < 0.05$) in P-tot from pre- to post-training (54.0 $\pm$ 2.9 to 56.0 $\pm$ 3.1 cm$^2$). CSA-q.f. increased $7 \pm 1\%$ ($P < 0.05$) in P0 from pre- to post-training (54.6 $\pm$ 0.5 to 58.3 $\pm$ 0.5 cm$^2$), whereas no significant change in CSA-q.f. was observed in P2 (53.2 $\pm$ 0.2 to 53.3 $\pm$ 0.3 cm$^2$; see Fig. 2). Hence, the relative increase in CSA-q.f. from pre- to post-training was larger ($P < 0.01$) in P0 than in P2.

**MFA**

In P-tot, no significant increase was found in MFA-tot with training (4164 $\pm$ 226 to 4589 $\pm$ 391 $\mu$m$^2$). In P0, MFA-tot increased ($P < 0.05$) from pre- to post-training (4047 $\pm$ 320 to 5019 $\pm$ 615 $\mu$m$^2$) whereas no significant change was observed in P2 (4300 $\pm$ 338 to
4088 ± 415 µm²; see Fig. 3). Hence, MFA-tot increased relatively more in P0 than in P2 (22 ± 6 vs. -5 ± 6%; P < 0.01).

In P-tot, MFA of type 1 fibres (MFA-1) was larger than MFA of type 2 fibres (MFA-2) before training (P < 0.05), but not after. This was due to the fact that MFA-2 increased (P < 0.05) from pre- to post-training (3552 ± 215 to 4079 ± 360 µm²), whereas MFA-1 did not change significantly (4668 ± 321 to 5012 ± 482 µm²) in response to training. In P0, MFA-1 and MFA-2 increased 18 ± 5 and 29 ± 7% (4556 ± 462 to 5460 ± 764 µm² and 3485 ± 316 to 4520 ± 567 µm²), respectively (P < 0.05), thus the relative increase in MFA-2 was larger than in MFA-1 (P < 0.05). In P2, no significant changes of MFA-1 or MFA-2 were observed (4798 ± 480 to 4489 ± 537 µm² and 3630 ± 314 to 3564 ± 351 µm², respectively).

In P-tot, neither MFA-2a nor MFA-2b changed significantly with training (3885 ± 228 to 4413 ± 389 µm² and 2982 ± 205 to 3320 ± 349 µm², respectively). In P0, MFA-2a increased (P < 0.05) from pre- to post-training (3917 ± 341 to 4910 ± 630 µm²), while no change was observed in P2 (3849 ± 326 to 3835 ± 331 µm²). MFA-2b did not change significantly in

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Table 1. Anthropometrics and dietary intake

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P2</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Age (years)</td>
<td>74 ± 1</td>
<td>74 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ± 6</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>WB fat (%)</td>
<td>26 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>WB-LBM (kg)†</td>
<td>54 ± 2</td>
<td>55 ± 3*</td>
</tr>
<tr>
<td>Energy intake (MJ)</td>
<td>8.6 ± 0.6</td>
<td>8.2 ± 0.4</td>
</tr>
<tr>
<td>Fat (E%)</td>
<td>38 ± 4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Carbohydrate (E%)</td>
<td>43 ± 4</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Protein per BW (g kg⁻¹)</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Protein per LBM (g kg⁻¹)</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± s.e.m. for P0 (n = 7) and P2 (n = 6) before (Pre) and after (Post) a 12 week training period. *Significantly different from Pre (P < 0.05). †Significantly larger increase in P0 than in P2. E%, percentage of total energy; WB, whole body; LBM, lean body mass; BW, body weight.
either group with training (2849 ± 266 to 3730 ± 516 µm² in P0, and 3115 ± 332 to 2910 ± 450 µm² in P2).

**MHC isoforms and fibre-type distribution**

In P-tot, no change in the distribution of MHC isoforms was observed with training (data not shown). In P0, MHC-I decreased \((P < 0.05)\) 8±3% from pre- to post-training (63±5 to 57±5%; see Table 2), and the sum of MHC-IIA and MHC-IIX (MHC-II) increased \((P < 0.05)\) 17 ± 5% (see Fig. 4). Whereas MHC-IIA increased \((P < 0.05)\) with training in P0 (32±4 to 38±5%), no significant change was observed in MHC-IIX (see Table 2). In P2, a significant change was only observed in the relative distribution of MHC-IIX, which decreased \((P < 0.05; \text{see Table 2})\).

In P-tot, no change in the fibre-type distribution (ATPase histochemistry) was observed with training, evaluated on the relative number and area of fibres. In P0 and P2, the effect of the training was equal to the changes of the MHC isoforms, except no significant decrease was observed in the relative number of type 1 fibres (see Table 2).

### Table 2. Distribution of MHC isoforms and fibre types

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC-I</td>
<td>63 ± 5</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>MHC-IIA</td>
<td>32 ± 4</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>MHC-IIX</td>
<td>6 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>56 ± 6</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>Type 2a</td>
<td>29 ± 3</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Type 2b</td>
<td>15 ± 4</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Area (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>62 ± 6</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>Type 2a</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Type 2b</td>
<td>10 ± 2</td>
<td>14 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± s.e.m. for P0 \((n = 7)\) and P2 \((n = 6)\) before (Pre) and after (Post) a 12 week training period. * Significantly different from Pre \((P < 0.05)\).

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**Figure 4. MHC isoforms**

The relative distribution of the MHC isoform MHC-II pre- (■) and post-resistance training (□) for 12 weeks in the group ingesting protein immediately postexercise (P0, \(n = 7\)) and in the group ingesting protein 2 h postexercise (P2, \(n = 6\)). * Significantly different from pre-training \((P < 0.05)\). Bars are means ± s.e.m.

**Figure 5. Acute response to resistance exercise and intake of protein supplementation**

The plasma concentrations of insulin (plasma [Ins]) and glucose (plasma [Glc]) before and during 4 h of recovery from one bout of resistance exercise.

- ●, group ingesting protein immediately postexercise (P0, \(n = 7\)); ○, group ingesting protein 2 h postexercise (P2, \(n = 6\)). * Significant difference between P0 and P2 at specific time point \((P < 0.05)\); # significantly different from preceding data point in P0; † significantly different from preceding data point in P2.
The results of the two methods applied (gel electrophoresis and histochemistry) correlated significantly for all three MHC isoforms analysed ($n = 26$ biopsies). The Spearman correlation coefficient of MHC isoform composition and fibre-type area was $r = 0.936 (P < 0.001)$ for MHC-I:type 1, $r = 0.890 (P < 0.001)$ for MHC-IIa:type 2a and $r = 0.804 (P < 0.001)$ for MHC-IIx:type 2b.

**Acute glucose, insulin and catecholamine response**

Plasma glucose concentration increased 30 min after intake of the supplement in P2 ($P < 0.05$) at 150 min, and tended to increase in P0 ($P = 0.75$) at 30 min (Fig. 5). Correspondingly, plasma insulin concentration increased to peak values 30 min after the respective intakes of the supplement in both P0 ($23.2 \pm 6.7 \mu U \, ml^{-1}$ at 30 min) and P2 ($18.9 \pm 3.0 \mu U \, ml^{-1}$ at 150 min) with no difference between the groups (Fig. 5). Likewise no difference was observed between P0 and P2 in insulin availability 2 h after the respective intakes of the supplement assessed as the area under the plasma insulin concentration curve (data not shown).

Plasma adrenaline and noradrenaline concentrations increased with exercise in P0 (to $0.27 \pm 0.05$ and $4.05 \pm 0.46 \, mmol \, l^{-1}$, respectively) and in P2 (to $0.25 \pm 0.04$ and $3.88 \pm 0.72 \, mmol \, l^{-1}$, respectively; $P < 0.05$). At 30 min postexercise plasma adrenaline and noradrenaline concentration had reached basal levels and no differences in plasma adrenaline and noradrenaline concentration were observed between groups at any time point during the 240 min of recovery (data not shown).

The increase in MFA was more pronounced (22%) than that in CSA of the whole muscle (7%). This is in accordance with the findings of other groups (Frontera et al. 1988) and indicates that a concomitant reduction in the relative amount of non-muscular tissue (fat and connective tissue) to CSA takes place in response to training. This is supported by findings in resistance-trained fragile elderly women, where a 10% increase in CSA of quadriceps was measured when corrected for fat and connective tissue versus only a 5% increase when not corrected for this (Harridge et al. 1999). Furthermore, since the angle of pennation has been shown to increase with resistance training in young individuals (Aagaard et al. 2001) it is likely that this contributes to the discrepancy between the two measurements of the relative increase of the muscle mass, and thus to the fact that the anatomical CSA of the muscle (determined by MRI) underestimated the true increase in physiological CSA (determined by muscle biopsy).

The MFA of fibre-type 1 (MFA-1) was found to be larger than MFA-2 in P-tot (as well as in P0) in agreement with previous observations in the elderly (Lexell et al. 1983; Aniansson et al. 1992). However, with training MFA-2 increased significantly more than MFA-1 in P0, and concomitantly the distribution of MHC-II increased in P0 (Fig. 4). These changes with resistance exercise are similar to findings in both young (Andersen & Aagaard, 2000) and elderly individuals (Charette et al. 1991) and could indicate that training induces a larger increment in stress of the type 2 fibres than type 1 fibres compared to normal daily living (Henneman et al. 1965). Further, it was only the area of fibre-type 2a that increased, as MFA-2b determined histochemically was unaffected by training. In addition, lean body mass increased more in P0 than in P2 with training, in agreement with a larger net muscle protein synthesis in P0 over the 12 week period of resistance training compared to P2.

In line with the observed disparity in the degree of hypertrophy between the two groups, the muscle strength was also affected differently by the 12 weeks of training in P0 compared to P2. Thus, isokinetic strength increased at both measured velocities (60 and 180 deg s$^{-1}$) in P0, whereas no significant increase was observed in P2 at any velocity (see Fig. 1B). However, both groups showed an increase in dynamic training strength assessed as 5 RM (see Fig. 1A), but this is more likely to reflect the neural factors of learning and coordination resulting from training (Rutherford & Jones, 1986). Thus, differences between P0 and P2 were observed only in the isolated non-trained isokinetic knee extensions, where the increase in strength in P0 was in agreement with other studies investigating the effect of 12 weeks of resistance training in the elderly using knee extension as the exercise mode (Frontera et al. 1988; Lexell et al. 1995).

No significant differences were observed between P0 and P2 for any anthropometrical, dietary, muscle or strength

**DISCUSSION**

The major finding of the present study is that the timing of protein intake after resistance training bouts in elderly males is of importance for the development of hypertrophy in skeletal muscle. Thus, over a 12 week resistance training period, the CSA of m. quadriceps femoris and MFA-tot increased by 7% and 22%, respectively, when protein was supplemented 2 h postexercise (P2) (Figs 2 and 3). The degree of hypertrophy found in P0 was similar to the findings of other studies investigating the effect of 12 weeks of resistance training in the elderly where no specific dietary restrictions were reported (Frontera et al. 1988; Brown et al. 1990; Fiatarone et al. 1990; Welle et al. 1996). In addition to the difference in hypertrophy development between the two groups, it is interesting to note the absence of any detectable hypertrophy in P2 despite 12 weeks of resistance exercise identical to the P0 group. This points to the importance of the early timing of protein intake in recovery from resistance exercise in terms of the amount of net protein synthesis in skeletal muscle. Such a hypothesis somewhat resembles the findings of the importance of early carbohydrate intake for the magnitude of glycogen resynthesis after exercise-induced depletion of muscle glycogen (Ivy et al. 1988).
In the present study muscle protein synthesis was not determined, but it is evident that the resulting hypertrophy after training is a product of an accumulation of net synthesis of structural muscle proteins after each resistance exercise bout. As the synthesis of mixed muscle, myofibrillar and MHC proteins has been shown to increase in response to resistance training in the elderly (Yarasheski et al. 1993; Welle et al. 1999; Hasten et al. 2000), resulting in net protein synthesis over a period of resistance training (Frontera et al. 1988; Brown et al. 1990; Fiatarone et al. 1990; Welle et al. 1996), it might be surprising that in the present study one of the groups, P2, did not show any significant increase in muscle CSA. However, two studies have failed to show an acute response in muscle protein synthesis to resistance exercise (Tipton et al. 1996; Roy et al. 1997), although in these studies, trained subjects were used suggesting that the training stimulus could have been insufficient (Rennie & Tipton, 2000). In the present study all individuals were untrained prior to the programme, and furthermore relative loads at 75% of RM were used during the last 6 weeks, making it unlikely that loading was insufficient to stimulate muscle protein synthesis. Nevertheless, no hypertrophy was observed in P2 despite the fact that comparable studies with very similar (Hakkinen & Hakkinen, 1995) or slightly heavier training protocols (Frontera et al. 1988; Fiatarone et al. 1990; Welle et al. 1996) have found an increase in muscle mass. However, we have no well-founded reason for believing that these studies were carried out in circumstances in which the subjects ate ‘early’ though the changes in muscle mass resemble our findings in P0. Yet, none of these studies report the dietary habits in association with the training. Alternatively, it could be speculated that the time of day when the training was carried out was important. The diurnal hormonal profile could influence the anabolic response to resistance exercise. Thus, in the present study the subjects always trained in the morning between 08.00 and 10.00 h with no differences in the specific time points between the groups P0 and P2. This was done in order to standardise the conditions, which were best controlled at this time of the day. Hence, the subjects in the present study may have had a disadvantage in training compared with other studies, and this could explain the lack of hypertrophy in P2. Unfortunately, no previous studies report when the training was carried out; further investigations are required to elucidate this point.

In spite of this, the difference in hypertrophy between the two groups suggests that the isolated act of contraction is counteracted by other factors, e.g. delayed food intake. In line with this, Tipton et al. (1999) have shown that postexercise net muscle protein balance is negative when individuals are maintained in the postabsorptive state during recovery, whereas if they ingest protein and achieve hyperaminoacidaemia the protein balance becomes positive. Finally, we are confident that both groups trained properly as the training sessions were always supervised and loads adjusted at every third training session. This is supported by the increased training strength (5 RM), which was observed for both groups.

Although not directly determined in this study we do believe that the protein intake highly stimulated muscle protein synthesis, since in a recent study by Rasmussen et al. (2000) protein synthesis was elevated 3.5 times above pre-intake values when a supplement of only 6 g amino acid with 35 g carbohydrate was ingested, whereas we gave 10 g protein together with 7 g carbohydrate. Furthermore, with ageing the stimulation of protein synthesis by resistance exercise has been shown to be preserved (Welle et al. 1994). However, as the dietary restrictions ended 2 h postexercise the possibility cannot be ruled out that if P2 regularly ingested a meal shortly after the supplement then the maximal effective dose of protein was exceeded, hence the stimulatory effect of the protein supplementation was blunted compared to P0. Yet, the dose–response relationship of ingested protein and protein synthesis remains to be elucidated. Consequently, we suggest that the contraction-induced stimulation of protein synthesis was used to a lesser
extent in the formation of muscle protein in P2 compared
to P0, provided that the stimulation of the protein
synthesis follows a time course with a rapid increase
within the first few hours following exercise (Phillips et al.
1997). Moreover, since the amino acid delivery is
dependent on blood flow (Biolo et al. 1995), the
intracellular amino acid availability in the exercised
muscle may have been larger in P0 than in P2, hence
favouring an increased anabolic response in P0 as it
correlates with intracellular amino acid concentration
(Biolo et al. 1995). Interestingly, Rasmussen and
co-workers have found that protein synthesis and
breakdown are stimulated similarly by protein intake in
recovery from resistance exercise whether the protein is
ingested 1 or 3 h after the termination of exercise, at least
in young individuals when protein synthesis in the hour
following intake is compared (Rasmussen et al. 2000).
However, a 1 h measurement period may be too short to
determine differences that affect muscle protein
synthesis for many hours.

Altogether, our findings suggest that the first 2 h of
recovery after resistance exercise are important for the
net protein synthesis during a strength-training
programme evaluated over a period of time, and to
optimise the protein synthesis the intramuscular
concentration of free amino acids is critical if it is not to
be a limiting factor.

In the present study muscle fibre hypertrophy was more
pronounced in P0 than in P2 despite identical rises in
plasma insulin after the intake of a supplement
containing protein and carbohydrate (Fig. 5). However, it
may be speculated whether the insulin sensitivity of
protein turnover is markedly higher immediately after
exercise than 2 h later. This has, however, to our
knowledge not been studied in humans. Yet, the
importance of hyperinsulinaemia with respect to the
present study can be questioned. First of all, the effect of
postexercise hyperinsulinaemia has been shown to
decrease mixed muscle protein degradation whereas
synthesis is unaffected (Biolo et al. 1999) and,
presumably, the effect is primarily on lysosomal
degradation and not myofibrillar breakdown, which
follows on the ubiquitin–proteasome pathway. Second, a
recent study on postabsorptive exercise in diabetic/non-
diabetic rats observed that insulin only played a
permissive role at low concentrations in stimulating
protein synthesis (Fedele et al. 2000). Thus, it was
concluded that the effect of insulin on protein synthesis
was only apparent in the low range of plasma insulin,
whereas a further increase in insulin did not enhance net
protein synthesis additionally (Fedele et al. 2000).

Finally, we cannot exclude the possibility that our
finding of a difference in hypertrophy between the
groups is a result of the relatively low number of subjects.
However, no subjects were systematic outliers and,

furthermore, all subjects in P0 had a larger increase in the
relative change of the CSA of the quadriceps than any
subject in P2. Additionally, theoretically it cannot be
ruled out that exercise-induced changes in tissue and
serum levels of anabolic hormones such as growth
hormone, cortisol or insulin-like growth factor 1, which
were not determined in the present study, could
contribute to the difference between P0 and P2.

In conclusion, this study investigated the importance of
the timing of protein intake after each exercise bout over
12 weeks of resistance training on morphological and
strength characteristics of skeletal muscle in elderly
individuals. Based on the findings in the present study it
appears that the timing of protein intake after strength
training bouts can be important for protein synthesis and
hypertrophy of skeletal muscle in elderly individuals,
and that this appears not to be related to the
hyperinsulinaemia in response to the intake of a
protein–carbohydrate supplement. The present findings
support the hypothesis that early intake of protein after
resistance exercise enhances total muscle mass as well as
hypertrophy of single muscle fibres in elderly humans.

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Corresponding author

B. Esmarck: Sports Medicine Research Unit, Bispebjerg Hospital, 23 Bispebjerg Bakke, Building 8, 2400 Copenhagen NV, Denmark.

Email: bep01@bbh.hosp.dk
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B. Esmarck, J. L. Andersen, S. Olsen, E. A. Richter, M. Mizuno and M. Kjær

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