

# High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women

STEPHEN WELLE<sup>1</sup> AND CHARLES A. THORNTON<sup>2</sup>

<sup>1</sup>Departments of Medicine, Pharmacology, and Physiology, and

<sup>2</sup>Department of Neurology, University of Rochester, Rochester, New York 14620

**Welle, Stephen, and Charles A. Thornton.** High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E677–E683, 1998.—This study tested the hypothesis that increasing the protein content of isocaloric meals increases the rate of myofibrillar synthesis in muscle of healthy subjects over 60 yr old and enhances the stimulation of myofibrillar synthesis induced by resistance exercise. Myofibrillar synthesis of sedentary and exercised quadriceps muscle was determined by incorporation of L-[1-<sup>13</sup>C]leucine. During the tracer infusion, subjects consumed meals with a low (7% of energy,  $n = 6$ ), normal (14%,  $n = 6$ ), or high (28%,  $n = 6$ )-protein content. In sedentary muscle, the mean ( $\pm$  SE) myofibrillar synthesis was  $1.56 \pm 0.13\%$ /day in the low-protein group,  $1.73 \pm 0.11\%$ /day in the normal-protein group, and  $1.76 \pm 0.10\%$ /day in the high-protein group ( $P = 0.42$ ). Myofibrillar synthesis was faster in exercised muscle (mean 27%,  $P < 10^{-6}$ ) in all groups ( $2.10 \pm 0.14\%$ /day in low protein;  $2.18 \pm 0.10\%$ /day in normal protein;  $2.11 \pm 0.09\%$ /day in high protein;  $P = 0.84$ ). The stimulation of myofibrillar synthesis by exercise was not significantly different among low-protein [ $0.54 \pm 0.12\%$ /day ( $37 \pm 9\%$ )], normal-protein [ $0.46 \pm 0.08\%$ /day ( $28 \pm 5\%$ )], and high-protein groups [ $0.34 \pm 0.04\%$ /day ( $20 \pm 3\%$ );  $P = 0.31$ ]. We conclude that high-protein meals do not enhance the stimulation of myofibrillar protein synthesis induced by resistance exercise.

muscle protein synthesis; amino acid concentrations; nutrition

MYOFIBRILLAR SYNTHESIS is ~30% slower in human muscle over 60 yr old than in young adult muscle (37–39). Because protein synthesis is necessary to maintain protein mass and protein quality, this slowing of myofibrillar protein synthesis could contribute to the diminished muscle mass and function in old age. The most effective strategy for stimulating myofibrillar protein synthesis, muscle bulk, and muscle strength is resistance training. This type of training can stimulate protein synthesis in both young and old human muscle (3, 4, 7, 11, 29, 40, 41). Anecdotally, many athletes involved in strength training claim that ingestion of large amounts of protein enhances the hypertrophic response. There is some experimental evidence to support this claim (14), but the effect of protein intake on myofibrillar protein synthesis has not been examined. Although it is known that feeding stimulates myofibrillar protein synthesis (39), the relative importance of energy and protein intake in determining the response to feeding is uncertain. In the present study, we tested the hypothesis that myofibrillar synthesis in untrained muscle and the stimulation of myofibrillar synthesis by

resistance exercise are proportional to protein intake when energy intake is constant.

## METHODS

### Subjects

Healthy volunteers 62–75 yr old were recruited by newspaper advertisements. Eighteen subjects (9 men and 9 women) were medically eligible and completed the study. None of the subjects had any medical conditions or disabilities that interfered with their ability to perform the exercise or that would be expected to influence protein synthesis, according to medical history, physical examination, electrocardiogram, and laboratory tests [thyroid-stimulating hormone (TSH), fasting glucose and electrolytes, liver enzymes, creatinine, urea nitrogen, albumin and total protein, and complete blood count]. Two women (one in high-protein group, one in low-protein group) were on estrogen replacement therapy. None was involved in any form of resistance training. Written consent was obtained from all participants after procedures and risks were explained verbally and in a written consent form. The project was approved by the University of Rochester Research Subjects Review Board.

### Procedures

**Exercise protocol.** The subjects exercised the quadriceps muscles of one leg (usually the right leg) on a Universal (Cedar Rapids, IA) knee extension machine. On day 1, the one repetition maximum (1RM) was determined. Subjects performed 4 sets of 10 repetitions, with brief rests between sets, at ~80% of the 1RM on days 1 and 4. On day 6 they performed 5 sets of 10 repetitions. The exercises were done between 1500 and 1530 on day 6, so that the muscle biopsies at the end of the protein synthesis determination were taken ~23 h after the final exercise session. Subjects were asked not to perform any strenuous activities involving the other leg but otherwise to continue with their normal activities and diet.

**Feeding protocol.** Subjects went to the University of Rochester General Clinical Research Center immediately after exercising on day 6. There they received a standard meal containing 10 kcal/kg body weight, with 10–15% of energy from protein. The meal was consumed before 2100, after which subjects were not fed until the protein synthesis determination was started on day 7.

Starting at 0700 on day 7, subjects consumed liquid meals every 30 min until the end of the study. The energy content of each meal was 6% of the Harris-Benedict daily basal metabolic rate (based on height, weight, and age) or ~4% of the daily energy requirement for weight maintenance. Thus each subject consumed ~60% of his or her daily energy requirement by the time the final muscle biopsy was taken. The subjects were divided into three groups of six (3 men and women in each group) according to the level of protein in the meals (as % of energy): 7%, 14%, or 28%. In a typical 80-kg subject consuming 2,800 kcal/day, the 14% protein level corresponds to a protein intake of  $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  and is typical of the United States diet. The 7% protein intake

Table 1. Anthropometric data and energy intake during tracer infusion

Group	n, (M/F)	Age, yr	Height, cm	Weight, kg	Body Mass Index, kg/m <sup>2</sup>	Intake, kcal·kg <sup>-1</sup> ·h <sup>-1</sup>
Low protein	3/3	66 ± 1	169 ± 3	80 ± 5	28 ± 2	2.26 ± 0.08
Normal protein	3/3	71 ± 2	172 ± 6	81 ± 5	28 ± 2	2.28 ± 0.07
High protein	3/3	68 ± 1	168 ± 4	80 ± 8	28 ± 2	2.29 ± 0.06

Values are means ± SE; n, no. of subjects.

corresponds to a level ( $0.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) below the recommended allowance of  $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  (26). The 28% protein intake corresponds to about three times the minimum recommended intake. Table 1 shows the mean age, height, weight, body mass index ( $\text{kg}/\text{m}^2$ ), and energy intake during the tracer infusion for each diet group.

Ensure (Ross Laboratories, Columbus, OH) was used for the 14% protein meals and as the base product for the other meals. Polycose (Ross Laboratories), Trisun oil (SVO Specialty Products, East Lake, OH), and Promod (Ross Laboratories) were added to Ensure as required to produce the 7 and 28% protein meals. All meals had a ratio of carbohydrate to fat of 1.73 (as % of energy). The protein source for Ensure is casein, and the source for Promod is whey. Both of these protein sources have high biological value.

**Protein synthesis determination.** At 0800, a priming dose of L-[1-<sup>13</sup>C]leucine (Mass Trace, Woburn, MA) was given intravenously, followed by a 6.5-h intravenous infusion of this tracer. The priming dose was always 70% of the amount infused each hour during the continuous infusion. The continuous infusion was varied from 84 to 196 mg/h, depending on the size of the subject and the amount of protein fed, to produce similar plasma [<sup>13</sup>C]leucine enrichments in all subjects.

Plasma samples were obtained immediately before the start of the tracer infusion (*time 0*) and at 0.25, 0.5, 1, 2, 3, 4, 5, and 6 h after start of the infusion. Note that *time 0* in Figs. 1 and 2 is 1 h after the start of meal feeding. Isotopic enrichment of plasma leucine and α-ketoisocaproate (KIC) and leucine concentrations were measured in all samples by gas chromatography (GC)-mass spectrometry (25). Plasma insulin and amino acid levels were determined in the 0-, 2-, 4-, and 6-h samples. Insulin was determined by radioimmunoassay with a commercial kit (Linco Research, St. Charles, MO). Amino acids in sulfosalicylic acid-deproteinized samples were determined by HPLC (Beckman System Gold; Beckman Instruments, Brea, CA) with a  $0.3 \times 25\text{-cm}$  sulfonated polystyrene-divinylbenzene column (lithium form). Amino acids were eluted with Beckman lithium buffers and then detected fluorometrically after postcolumn reaction with *ortho*-phthalaldehyde. Norleucine was used as an internal standard for calculation of amino acid concentrations.

Muscle biopsies of the vastus lateralis were obtained bilaterally, with the subject under local anesthesia with lidocaine, 6–6.5 h after the start of the tracer infusion. The myofibrillar protein fraction was isolated, leucine was extracted from the protein hydrolyzate by HPLC, and the <sup>13</sup>C enrichment of the carboxyl group of the leucine was determined by isotope ratio mass spectrometry as described previously (36, 37). The supernatant from the muscle homogenate, containing the free leucine, was acidified with an equal volume of 50% acetic acid and then applied to a cation exchange resin (AG 50W-X8; Bio-Rad, Hercules, CA). The resin was washed with water and then amino acids were eluted with 4 N NH<sub>4</sub>OH. The solvent was removed by vacuum centrifugation. Enrichment of the free leucine in the residue was determined by GC-mass spectrometry, with the same method used for the plasma leucine analyses.

In subjects who never have received an infusion of [<sup>13</sup>C]leucine, the enrichment of the leucine in the myofibrillar protein fraction is  $+0.005 \pm 0.002$  atom % excess (APE) (mean ± SD,  $n = 5$ ) relative to a lot of reference leucine obtained from Sigma Chemical (St. Louis, MO). This SD can be accounted for by the analytic error of the method, indicating that biological variability in baseline [<sup>13</sup>C]leucine enrichment is negligible relative to the enrichments (0.025–0.05 APE) induced by the tracer infusion in the present study. Thus 0.005 APE was added to the reference value (always done in duplicate or triplicate each time samples from the study were analyzed) to estimate baseline <sup>13</sup>C enrichment in the myofibrillar leucine. By using an estimated baseline enrichment we could obtain tissue from exercised and sedentary muscle that never had been biopsied. This procedure increased the precision of the study, because in our experience myofibrillar synthesis is much more variable in muscles that have been biopsied previously (unpublished data). For example, in six subjects over 60 yr old (4 men, 2 women), the between-subject coefficient of variation for myofibrillar protein synthesis was 20% in the initial study and 65% in muscles that had been biopsied 3–4 mo earlier ( $P < 0.01$ ). A similar trend was found for mixed muscle protein synthesis in eight young men (24% vs. 35% coefficient of variation,  $P < 0.10$ ). In both groups, the increased variation in previously biopsied muscles was related to outliers (% more than 2.5 SD from mean in older subjects, % more than 2.5 SD from mean in younger subjects), which were not observed in the initial studies. The result of this increased variability on repeated muscle sampling is that the within-subject error in a study with a repeated-measures design can be greater than the between-subject error in an unpaired study design.

The fractional rate of protein synthesis was calculated as sample enrichment (APE relative to the calculated baseline value) divided by the area under the curve (APE·time) of plasma KIC enrichment from the start of the tracer infusion to the removal of the biopsy. For calculation of the area under the KIC enrichment curve, it was assumed that enrichment was constant from 6 h until removal of the biopsy.

**Data analysis.** Results are presented as means ± SE. The statistical significance of the effect of exercise, the level of protein intake, and their interaction on myofibrillar synthesis was determined by analysis of variance (NCSS 6.0; NCSS, Kaysville, UT). Other variables were measured only for descriptive purposes, so no hypothesis testing was performed for these variables.

## RESULTS

Average insulin concentrations during the tracer infusion were ~200–300 pmol/l in all groups, but mean insulin levels were ~70% higher in the high-protein group than in the other groups during the last 2 h of feeding (Fig. 1). Amino acid concentrations generally were proportional to the level of protein intake, as expected (Fig. 1). Because the HPLC column did not

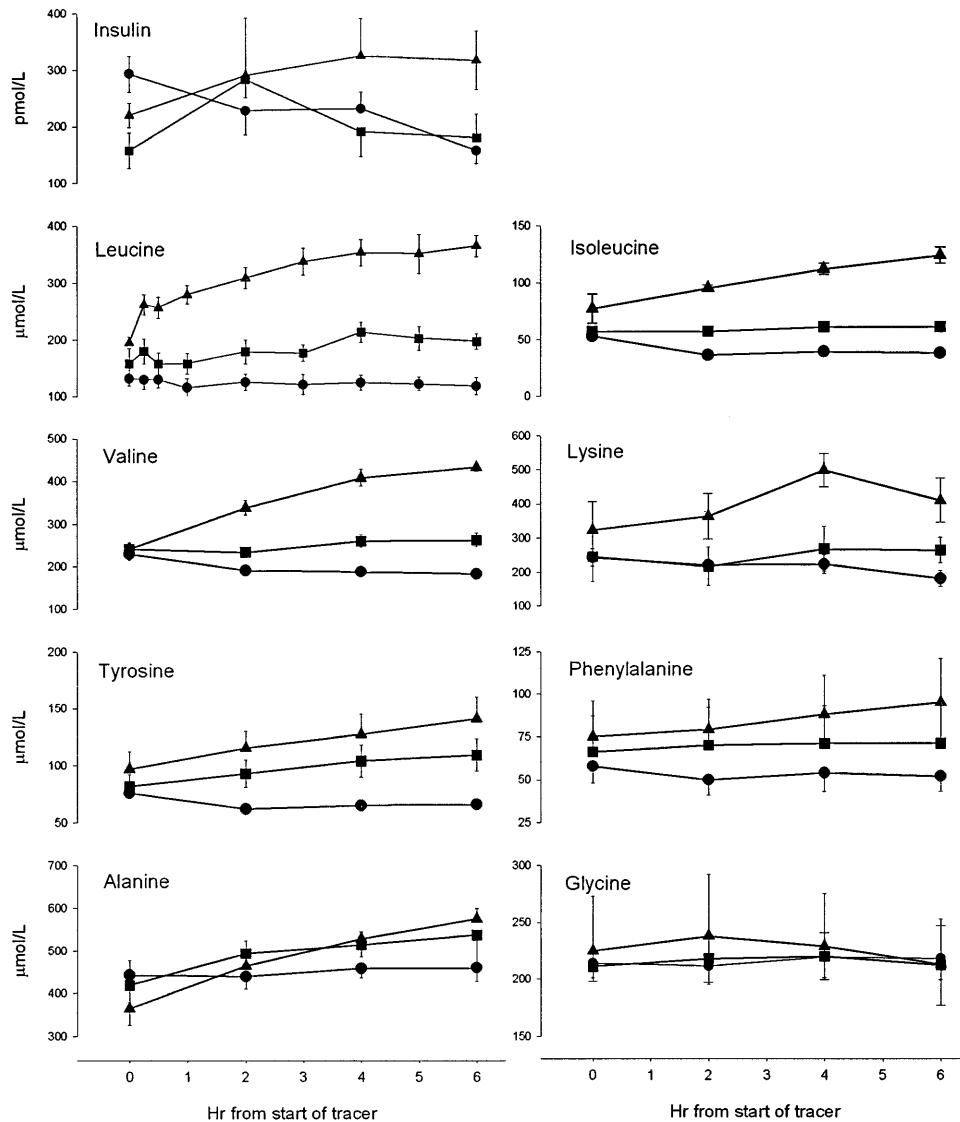


Fig. 1. Insulin and amino acid concentrations (means  $\pm$  SE) during tracer infusion in subjects fed every 30 min with meals containing a low-protein content (7% of energy, ●), normal-protein content (14% of energy, ■), or high-protein content (28% of energy, ▲). For insulin and leucine concentrations,  $n = 6$  per group. For other amino acids,  $n = 6$  for low protein,  $n = 4$  for normal protein, and  $n = 5$  for high protein, because of technical problems with HPLC system.

allow adequate resolution of some of the amino acids, results are not given for all of the amino acids. Leucine, isoleucine, valine, tyrosine, phenylalanine, and lysine concentrations were markedly affected by the level of protein intake. However, glycine and alanine concentrations were only marginally affected by the level of protein intake.

Figure 2 shows the isotopic enrichments of plasma leucine and plasma KIC throughout the tracer infusion, and of muscle tissue free leucine and leucine extracted from myofibrillar proteins at the end of the tracer infusion. We were generally successful in our goal of keeping plasma leucine and KIC enrichments approximately the same in all subjects. The mean plasma leucine enrichments were 8–10%, and the mean KIC enrichments were 6–8%. As expected, KIC enrichment was closer to leucine enrichment in subjects with the highest protein intake. In subjects fed the low-protein and normal-protein meals, the free leucine enrichment in muscle at the end of the tracer infusion was only slightly less than the plasma KIC enrichment and was  $\sim 10\%$  higher in the exercised muscle than in

the sedentary muscle. In subjects fed the high-protein meals, the free leucine enrichment was the same in sedentary and exercised muscle and was nearly identical to the plasma leucine and KIC enrichments at the end of the infusion. Tracer enrichment of the leucine extracted from myofibrillar proteins was greater in the exercised muscle than in the sedentary muscle in all subjects.

Mean myofibrillar protein synthesis rates are shown in Fig. 3. There was no evidence for an effect of the level of protein intake on the rate of myofibrillar protein synthesis either in sedentary muscle ( $P = 0.42$ ) or in exercised muscle ( $P = 0.84$ ). The exercised muscle had a faster synthesis rate than the sedentary muscle in every subject, with an average increase of 27% for all diet groups combined ( $P < 10^{-6}$ ). Men and women had similar fractional rates of myofibrillar synthesis in both sedentary ( $1.75 \pm 0.10\%/\text{day}$  in men;  $1.61 \pm 0.06\%/\text{day}$  in women,  $P = 0.29$ ) and exercised muscles ( $2.18 \pm 0.10\%/\text{day}$  in men;  $2.08 \pm 0.07\%/\text{day}$  in women,  $P = 0.43$ ). The exercise-induced increase in myofibrillar synthesis tended to be greater in the subjects fed the

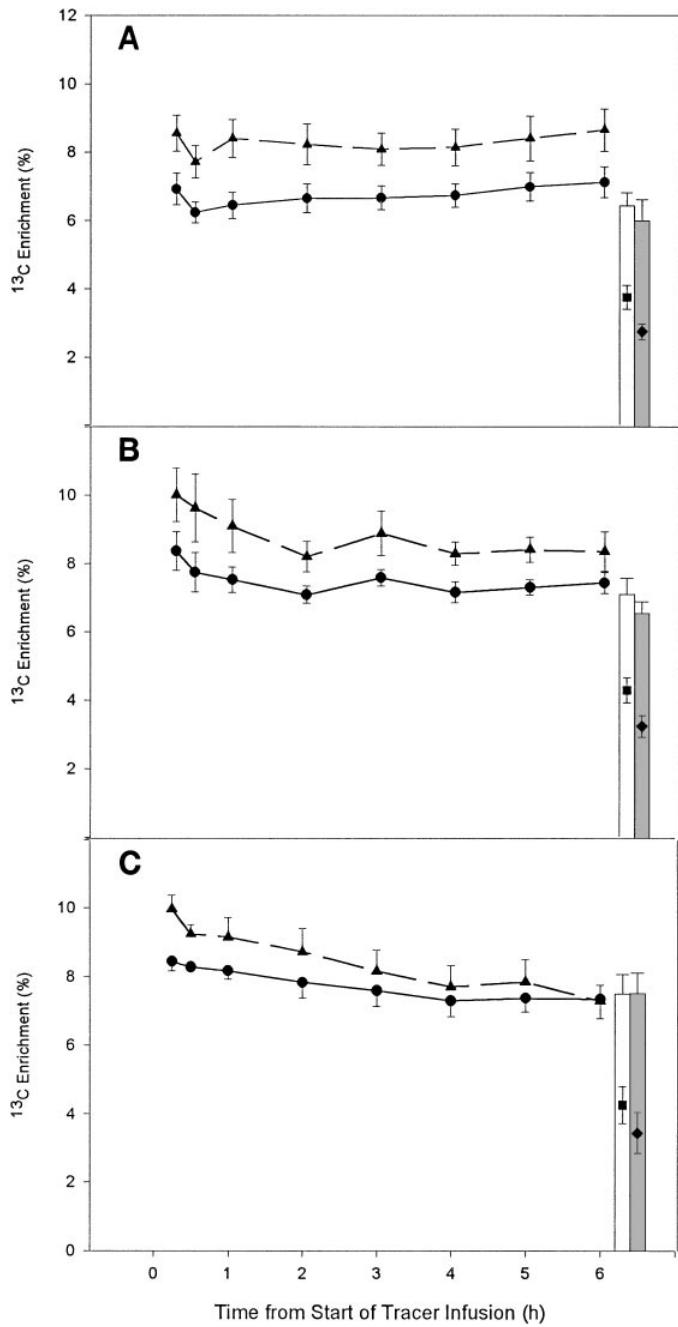


Fig. 2.  $^{13}\text{C}$  enrichments (means  $\pm$  SE) of plasma  $\alpha$ -ketoisocaproate (KIC, ●) and plasma leucine (▲) during infusion of  $[^{13}\text{C}]\text{leucine}$  and  $^{13}\text{C}$  enrichments of tissue free leucine and leucine in myofibrillar proteins in muscle biopsies taken near end of tracer infusion. Data are from low-protein (A), normal-protein (B), and high-protein (C) diet groups. Open bars, tissue free leucine from exercised muscle; gray bars, myofibrillar leucine from exercised muscle; ■, myofibrillar leucine from sedentary muscle; ♦, myofibrillar leucine from sedentary muscle. Myofibrillar leucine enrichments were multiplied by 100, so the same scale could be used.

low-protein meals ( $37 \pm 9\%$ ) than in those fed normal-protein meals ( $28 \pm 5\%$ ) or high-protein meals ( $20 \pm 3\%$ ), but the trend did not approach statistical significance ( $P = 0.31$ ). There was no difference between men and women in the failure of increasing protein intake to enhance the response to exercise ( $P = 0.14$  for diet  $\times$

gender  $\times$  exercise interaction). However, the power to detect a significant gender influence on the interaction between diet and exercise was low (~25% to detect a 1 SD difference between men and women in the effect of diet on the response to exercise).

## DISCUSSION

The present study confirms previous reports that muscle protein synthesis increases after resistance exercise (3, 4, 7, 11, 29, 40, 41). The magnitude of the effect of resistance exercise on muscle protein synthesis has been quite variable and probably depends on the intensity of the exercise more than any other factor. In studies done the day after a resistance exercise session, the percent increase in muscle protein synthesis ranged from 2% (not statistically significant) to over 100% (7, 29, 38, 40). The stimulation of muscle protein synthesis after resistance exercises persists for  $\geq 24$  h. In subjects who had been performing resistance exercises for several years, the average increase in muscle protein synthesis after a resistance exercise session was 50% 4 h after the exercise, 109% 24 h after exercise, and only 14% (not statistically significant) 36 h after exercise (7, 22). In young subjects who had not previously engaged in resistance training, a single resistance exercise session increased mean muscle protein synthesis 112% after 3 h, 65% after 24 h, and 34% after 48 h (29). The 27% stimulation of myofibrillar protein synthesis in the present study, after only three exercise sessions, was greater than the nonsignificant 10% mean increase observed in age-matched subjects after 3 mo of training in our previous study (38). However, the subjects in the present study performed twice as many repetitions (5 sets of 10 repetitions) the day before protein synthesis was measured than the subjects in the previous study (3 sets of 8 repetitions).

The major problem in determining protein synthesis rates in muscle is the uncertainty about the tracer enrichment of the aminoacyl-tRNA, the immediate

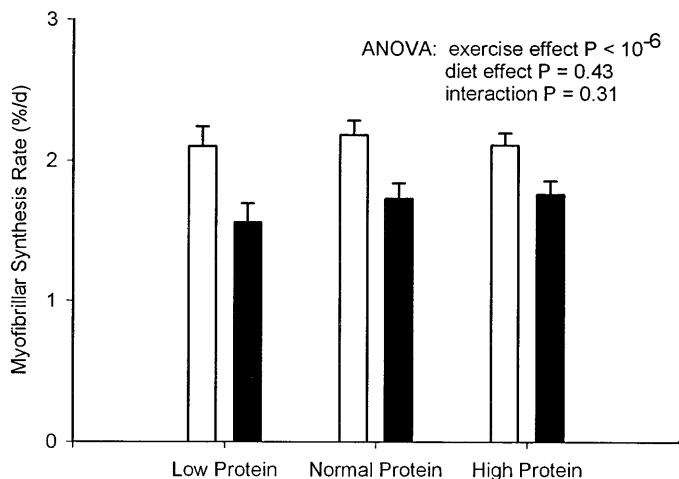


Fig. 3. Rate of myofibrillar protein synthesis (means  $\pm$  SE) in exercised (open bars) and sedentary (solid bars) muscles obtained from subjects fed meals containing a low-protein content (7% of energy), a normal-protein content (14% of energy), or a high-protein content (28% of energy).

precursor for incorporation of the tracer into proteins. Insufficient tissue is obtained by needle biopsy to directly measure this enrichment, so surrogate markers must be used. The plasma enrichment of KIC is a good index of leucyl-tRNA enrichment in resting muscle in postabsorptive dogs, pigs, and humans (1, 13, 34), but the effect of resistance exercise on the relation between intramuscular leucyl-tRNA enrichment and plasma KIC enrichment has not been studied. Recently it was reported that the tissue fluid free leucine enrichment is a good index of leucyl-tRNA enrichment in human muscle under various feeding conditions (17), but the effect of exercise was not specifically tested. We found that, for all diet groups combined, the mean enrichment of free leucine in the exercised muscle was 7% higher than that of the sedentary muscle (95% confidence interval of 0–13%). Thus we might have overestimated the effect of exercise on myofibrillar synthesis by using the same precursor enrichment (plasma KIC) for both sedentary and exercised muscle. Nevertheless, if the tissue free leucine enrichment at the end of the tracer infusion is an accurate index of leucyl-tRNA enrichment throughout the infusion, there was a significant increase of myofibrillar protein synthesis in the exercised muscle.

Even though older subjects have a slower rate of protein synthesis in untrained muscle than young adults (11, 30, 37–39, 41), there is no evidence that muscle protein synthesis is stimulated less by exercise in older muscle. To the contrary, some data (11, 41) suggest that the increase in protein synthesis after resistance exercises is greater in older muscle, at least in the early stages of training. Nevertheless, we observed that myofibrillar protein synthesis remained significantly slower in muscles of older subjects than in muscles of young adults after 3 mo of resistance training (38). These results prompted us to examine whether myofibrillar protein synthesis could be enhanced in older subjects in either sedentary or exercised muscles. Several lines of evidence suggested that increasing the protein intake might stimulate myofibrillar protein synthesis. Acute elevations of amino acid concentrations increase muscle protein synthesis *in vitro* (9, 16, 21) and *in vivo* (2, 4, 24, 31, 34). In fasting dogs, amino acid infusion markedly increases hindlimb protein synthesis (mostly muscle), whereas dextrose infusion is ineffective (5). Whole body protein synthesis and turnover in humans are more rapid in subjects consuming a high-protein diet than in those on a normal-protein diet (27, 28), an effect that is more pronounced in strength athletes (32). Increasing protein intake makes nitrogen balance more positive in both young and old subjects, including those performing resistance exercises (6, 8, 15, 27, 28, 32). It has been suggested that the minimum requirement for protein intake in persons performing heavy resistance exercises is about twice that of sedentary persons (14, 32).

Contrary to our hypothesis, there was no evidence that the stimulation of myofibrillar synthesis by resistance exercise was enhanced by increasing the protein intake over the range of 7–28% of energy intake as

protein. This conclusion is based on the assumption that any differences in leucyl-tRNA <sup>13</sup>C enrichment between sedentary and exercised muscles were not influenced by the level of protein intake. Although we could not measure leucyl-tRNA enrichment directly, the tissue free leucine enrichment may provide a good index. With the low-protein and normal-protein meals, the ratio of free leucine enrichment in exercised muscle to free leucine enrichment in sedentary muscle was ~1.1, whereas it was 1.0 with the high-protein meals. Thus the trend (which was not statistically significant) toward less stimulation of myofibrillar synthesis by exercise in the high-protein group might be explained by a slight overestimate of the exercise effect in the low-protein and normal-protein groups. Because free leucine enrichment in exercised and sedentary muscle was the same in the high-protein group, the exercise effect in this group is most likely to be quantitatively accurate. The high-protein feeding produces a situation similar to the flooding dose method, in which high doses of tracer are infused to equalize the tracer enrichment in all of the free amino acid pools (10). Future studies of the factors regulating the effect of exercise on muscle protein synthesis could use high-protein meals to minimize uncertainty about the tracer enrichment of the aminoacyl-tRNA.

We cannot exclude the possibility that protein intake would have influenced myofibrillar synthesis immediately after exercise. Biolo et al. (4) found that, in young subjects, the increase in muscle protein synthesis immediately after resistance exercises was >200% when amino acids were infused. This response was more than twice the stimulation in postabsorptive subjects in a similar study by the same group (3). The present study differed from that of Biolo et al. not only in examining protein synthesis after a longer postexercise delay, but also in the age of the subjects, provision of ordinary meals instead of intravenous amino acids, and examination of myofibrillar synthesis instead of total mixed muscle protein synthesis. Thus it is not particularly surprising that we did not verify that elevated amino acid levels enhance the stimulation of muscle protein synthesis by resistance exercise. The possibility that older subjects may not respond to the combination of exercise and high amino acid levels as much as young subjects is intriguing. The slower protein synthesis in older muscle seems to be caused by slower translation of mRNA, with no decline in total RNA or mRNA concentrations (35). The effects of both exercise and amino acids on protein synthesis are mediated, at least in part, by increased translation (7, 16). Thus the reduced translational efficiency in older muscle may prevent myofibrillar synthesis rates from exceeding the values observed in the present study.

It would be premature to conclude from our results that a high-protein intake could not enhance the increase in muscle mass induced by resistance training, because muscle mass is also determined by the rate of muscle protein breakdown. There is some evidence that high levels of branched-chain amino acids reduce the rate of protein breakdown in human skeletal muscle in

vivo (19, 20, 25). Whereas mixed muscle protein breakdown increases immediately after resistance exercises in postabsorptive subjects (3), it does not increase when subjects are given an amino acid infusion (4). However, there is no evidence that a high-protein diet inhibits the increase in the total daily degradation of myofibrillar proteins induced by resistance training, as reflected by 3-methylhistidine excretion in urine (6, 12). Campbell et al. (6) reported that a high-protein diet ( $1.62 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) did not enhance the effect of resistance training on nitrogen balance or muscle mass in healthy older subjects relative to effects observed at the minimum recommended intake ( $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ), even though nitrogen balance was more positive on a high-protein diet both before and after training. Meredith et al. (23) reported that a protein-containing supplement enhanced the hypertrophic response to resistance exercises in older subjects. The supplement increased overall energy intake as well as protein intake, so it is not possible to evaluate the specific effect of protein in that study. The protein requirement for muscle growth during resistance training is likely to be higher when energy intake is low, and lower when energy intake is high.

The present study confirms our previous finding (39) that feeding increases myofibrillar protein synthesis. The myofibrillar synthesis rate of  $\sim 1.7\%/\text{day}$  in sedentary muscle is  $\sim 70\%$  faster than the rate in postabsorptive subjects of similar age in our previous studies (37, 38). On the basis of data of Ljungqvist et al. (17), the ratio of muscle tissue free leucine enrichment to plasma KIC enrichment is  $\sim 0.68$  in postabsorptive subjects. In the present study this ratio ranged from 0.88 to 1, depending on the protein intake. Thus about one-half of the apparent effect of feeding could be an artifact of assuming that the relation between plasma KIC enrichment and muscle leucyl-tRNA enrichment is the same in postabsorptive and fed subjects, but there still is a substantial effect of feeding. A recent abstract indicated that meals did not increase muscle protein synthesis when the aminoacyl-tRNA enrichment was used to determine the rate of protein synthesis, but energy intake ( $1.26 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) was only  $\sim 55\%$  of the energy intake of subjects in the present study (18). In that study, muscle protein synthesis tended to decrease ( $\sim 10\%$ ) when the meals were pure carbohydrate and tended to increase ( $\sim 10\%$ ) when they contained protein, but the differences were not statistically significant. Another study, using the phenylalanine disposal method to determine protein synthesis, confirmed that meals with a high energy content ( $3.75 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , 22% of energy as protein) increase muscle (forearm) protein synthesis (33).

It is somewhat surprising that the high-protein meals did not increase protein synthesis in the sedentary muscle more than the low-protein meals did, because raising levels of amino acids, without providing other energy sources, increase muscle protein synthesis in humans (2, 4, 31). Apparently, the level of energy intake used in the present study was adequate to maximize myofibrillar synthesis in sedentary muscle

even when amino acid levels were not increased (low-protein group). Even though the plasma amino acid levels in the low-protein group were not elevated above values we usually observe in postabsorptive subjects, it is very likely that the flux of amino acids into the muscle was stimulated by the elevated insulin concentrations.

The present findings should not be generalized to long-term changes in dietary protein intake or to individuals consuming inadequate amounts of energy. It is important to note that the low-protein meals were given to subjects who were well nourished before the study. Chronic consumption of a low-protein diet, resulting in abnormally low levels of essential amino acids, could result in a reduced rate of muscle protein synthesis. Further research is needed to define the extent to which dietary protein intake can influence muscle protein metabolism.

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Address for reprint requests: S. Welle, Monroe Community Hospital, 435 East Henrietta Rd., Rochester, NY 14620.

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