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## Age and aerobic exercise training effects on whole body and muscle protein metabolism

Kevin R. Short,<sup>1</sup> Janet L. Vittone,<sup>2</sup> Maureen L. Bigelow,<sup>1</sup> David N. Proctor,<sup>3</sup> and K. Sreekumaran Nair<sup>1</sup>

Divisions of <sup>1</sup>Endocrinology and <sup>2</sup>General Internal Medicine, Department of Internal Medicine, and <sup>3</sup>Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota 55905

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**Short, Kevin R., Janet L. Vittone, Maureen L. Bigelow, David N. Proctor, and K. Sreekumaran Nair.** Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab* 286: E92–E101, 2004. First published September 23, 2003; 10.1152/ajpendo.00366.2003.—Aging in humans is associated with loss of lean body mass, but the causes are incompletely defined. Lean tissue mass and function depend on continuous rebuilding of proteins. We tested the hypotheses that whole body and mixed muscle protein metabolism declines with age in men and women and that aerobic exercise training would partly reverse this decline. Seventy-eight healthy, previously untrained men and women aged 19–87 yr were studied before and after 4 mo of bicycle training (up to 45 min at 80% peak heart rate, 3–4 days/wk) or control (flexibility) activity. At the whole body level, protein breakdown (measured as [<sup>13</sup>C]leucine and [<sup>15</sup>N]phenylalanine flux), Leu oxidation, and protein synthesis (nonoxidative Leu disposal) declined with age at a rate of 4–5% per decade ( $P < 0.001$ ). Fat-free mass was closely correlated with protein turnover and declined 3% per decade ( $P < 0.001$ ), but even after covariate adjustment for fat-free mass, the decline in protein turnover with age remained significant. There were no differences between men and women after adjustment for fat-free mass. Mixed muscle protein synthesis also declined with age 3.5% per decade ( $P < 0.05$ ). Exercise training improved aerobic capacity 9% overall ( $P < 0.01$ ), and mixed muscle protein synthesis increased 22% ( $P < 0.05$ ), with no effect of age on the training response for either variable. Fat-free mass, whole body protein turnover, and resting metabolic rate were unchanged by training. We conclude that rates of whole body and muscle protein metabolism decline with age in men and women, thus indicating that there is a progressive decline in the body's remodeling processes with aging. This study also demonstrates that aerobic exercise can enhance muscle protein synthesis irrespective of age.

leucine; phenylalanine; amino acid kinetics; resting metabolic rate; fractional synthesis rate; aging

AGING IN HUMANS is associated with alterations in body composition, leading to a loss of lean tissue and a corresponding gain in body fat (34, 35). This shift in body composition is considered a risk factor for disease and disability, yet the mechanisms for lean tissue loss in older persons are not yet fully understood. The quantity of lean tissue mass depends on protein turnover and the balance between protein synthesis and breakdown. Protein kinetics at the level of the whole body, body regions, or individual tissues can be readily measured in vivo with the use of labeled amino acid tracers (24). Although these methods have been used to determine whether protein metabolism is altered with aging, several questions remain.

Several studies have compared whole body protein turnover data in younger and older people (Table 1) but have provided widely mixed results. At least three factors may account for these varied outcomes. First, some studies have relied on small sample sizes, often comparing eight or fewer people grouped as “young” and “old” (7, 11, 12, 14, 15, 23, 33, 45, 49, 51). Unless the variability among people is small, there may not be adequate power to detect a difference between age groups. Small sample sizes have also limited the ability to compare men and women. Some differences in protein metabolism between young men and women have been reported (17, 39), but age and gender interactions have not been examined. Second, all but one (3) of the previous studies have compared groups of young (typically aged <35 yr) and old (typically aged >60 yr) people without considering people of intermediate ages. Thus it cannot be determined whether the change in protein turnover with aging, if it occurs, is linear. Third, by convention, and to account for differences in body size, rates of whole body protein metabolism are typically expressed per unit of body mass, lean mass, or both. In some studies, older people had lower protein metabolism per kilogram body mass but not per kilogram lean mass, suggesting that differences with age are a reflection of reduced lean mass rather than age per se (7, 22, 23, 45). However, two recent investigations that included either a larger sample size (28) or intermediate age groups (3) reported that protein turnover was reduced with age even after division by fat-free mass. Calculating a simple ratio standard, however, assumes that protein turnover is linearly related to body mass or lean mass, with a slope of 1.0 and an intercept of 0. This is seldom the case for biological variables, so there is potential to make erroneous conclusions when comparing different groups, unless a regression-based method for normalization is used (27, 37).

The loss of lean mass with aging primarily affects skeletal muscle, referred to as the sarcopenia of aging (34). It has been reported that the synthesis rate of mixed (total) muscle proteins is reduced in elderly people (3, 15, 45, 46, 49), although not all studies are in agreement (40, 41, 43). The reasons for this discrepancy are not entirely clear but have been debated elsewhere (42, 43, 48). More importantly, many studies examining the age effect on mixed muscle protein synthesis have the same limitations already noted above: comparisons have typically been between small groups of young and older people, as only one study has included a middle-aged group (3) and only one has studied more than nine people per group (43).

Protein metabolism, especially in muscle, can be strongly affected by physical activity (36). Most of the work in this area

Address for reprint requests and other correspondence: K. S. Nair, Endocrinology Research Unit, Mayo Clinic, 200 First St. SW, Rochester, MN 55905 (E-mail: nair.sree@mayo.edu).

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Table 1. *Studies comparing whole body amino acid kinetics in younger and older people*

First Author (Ref)	Number of Subjects by Age			Tracer Method	Dietary Control, days	Activity Control, days	Body Composition Method	Age Effect	
	Younger	Middle	Older					per body wt	per FFM
Sharp (33)	2M		4M	Gly	NA	NA	None	↓	NA
Young (51)	4M/F		4 F	Gly	NA	NA	None	↓	NA
Yarasheshi (49)	6M/F		6M/F	Leu	3 (old only)	NA	None	↓	NA
Uauy (38)*	8M/F		11M/F	Gly	5	NA	TBK	↔	↑
Winterer (47)	8M/F		9M/F	Gly	5	NA	TBK	↔	↑
Gersovitz (14)	5M		6M	Gly	7	NA	TBK	↔	↔
Fukagawa (11)	5M		5M	Leu	3	NA	TBW	↔	↔
Fukagawa (12)	6M		6M	Leu	3	NA	TBW	↔	↔
Benedek (6)	26M		26M	Gly	None	None	BIA	↔	↔
Volpi (43)	26M		22M	Phe	None	None	None	↔	NA
Welle (46)†	9M/F		7M/F	Leu	3	3	TBK	NA	↔
Hasten (15)	7M/F		7M/F	Leu	3	NA	DEXA	NA	↔
Boirie (8)	14M/F		12M/F	Leu	3	NA	TBW	NA	↔
Robert (31)	15M/F		10M/F	Leu	3	NA	TBW	↓ F↔M	↔
Welle (45)	8M/F		8M/F	Leu	3	3	TBK	↓	↔
Boirie (7)	6M		6M	Leu	3	NA	BIA	↓	↔
Morais (22)	15M/F		16M/F	Gly	7–9	NA	BIA	↓	↔
Morais (23)	7M/F		8M/F	Gly	7	NA	MRI	↓	↔
Pannemans (28)	29M/F		26M/F	Gly	7	NA	TBW	↓	↓
Balogopal (3)	8M/F	8M/F	8M/F	Leu	5	3	DEXA	↓	↓

M and F, males and females, respectively, with M/F referring to inclusion of both sexes. Tracer methods are either the single dose [<sup>15</sup>N]glycine approach (Gly) or the primed, continuous infusion of [<sup>13</sup>C]leucine (Leu) or [<sup>2</sup>H<sub>5</sub>]phenylalanine (Phe). Dietary control refers to the number of days before the study that a standardized meal plan was performed. Activity control refers to screening for habitual level of physical activity and control of activity before the study. Body composition methods are BIA, bioelectrical impedance; DEXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; TBK, total body potassium content; TBW, total body water content using <sup>2</sup>H<sub>2</sub>O or H<sub>2</sub><sup>18</sup>O. Symbols under age effect columns indicate whether older people were higher (↑), lower (↓), or the same (↔) as younger people after simple ratio-standard normalization for body wt or an index of lean mass/fat-free mass (FFM). NA, data not available. \*Young subjects studied in Refs. 38 and 47 are the same. †The study by Welle et al. (46) was performed during meal consumption; all other Leu or Phe tracer studies were performed in the fasted state.

has been performed using resistance exercise. In both older and younger people, muscle protein synthesis has been shown to increase in response to resistance training (4, 15, 36, 49, 50). Current recommendations suggest that all people maintain a program of regular aerobic and resistance exercise (1). However, there is almost no information about the effect of regular aerobic exercise training on protein metabolism. One study reported that whole body protein turnover was not different during rest or exercise in a group of young endurance-trained people compared with sedentary controls (17). The effect of aerobic exercise training on muscle protein synthesis has not yet been determined.

The current investigation tested the hypotheses that, with healthy aging in the absence of overt disease, there is a decline in the rate of whole body protein turnover, even after adjustment for lean mass, and in the rate of mixed muscle protein synthesis. We studied a large number of men and women across the adult age span in whom diet and exercise had been controlled. We also determined whether aerobic exercise training could affect protein metabolism by retesting participants after 4-mo bicycle exercise training or a similar period of control (flexibility training) activity.

## METHODS

**Participants.** Healthy men and women who exercised <30 min twice per week during the previous 9 mo were recruited. Health status was assessed by medical history, physical exam, blood chemistries (liver enzymes, creatinine, electrolytes, and glucose), complete blood count, urinalysis, and electrocardiogram. Exclusion criteria included tobacco use, beta-blockers, diabetes or other endocrine disorders, and debilitating chronic illness. Forty women and 38 men between the

ages of 19 and 87 yr met these criteria and were enrolled after providing written and oral consent. Characteristics of the participants are shown in Table 2. Participants gave their informed oral and written consent before any tests were performed. The Mayo Foundation Institutional Review Board approved the study.

**General study protocol.** Participants were randomized to either a 16-wk aerobic exercise ( $n = 41$ ) or a control ( $n = 37$ ) program. A similar 5-day protocol was completed at baseline and again after the training or control phases. In exercisers, the follow-up protocol was started on the day after completion of the last exercise session. Participants were asked to refrain from vigorous physical activity during the 5 days but otherwise maintained their normal daily living patterns. The one exception was that the test of aerobic exercise capacity was performed on *day 1* or *2*. During each study period, a weight-maintaining diet (55:30:15% carbohydrate, fat, and protein, respectively) was provided for the first 4 days, the first 3 days of which were conducted on an outpatient basis. On the morning of *day 4*, subjects were admitted to the General Clinical Research Center (GCRC) after an overnight fast. Body composition was then measured. On the following morning (*day 5*), resting metabolic rate and protein turnover were measured in the postabsorptive state.

**Exercise and control programs.** The exercise program was performed on a stationary bicycle. Training started with three sessions per week, lasting 20 min each, at an intensity eliciting 70% of maximal heart rate. Intensity, duration, and number of sessions were gradually increased so that the final month of training consisted of four sessions per week at 80% of maximal heart rate for 40 min. Exercise specialists supervised each session and recorded heart rates with heart rate monitors. Compliance with the target workloads and number of sessions was >90%. The control group was taught a series of flexibility exercises at the beginning of their program. They were encouraged to perform these stretching exercises on their own while maintaining a regular lifestyle pattern, but no other intervention was

Table 2. Baseline physiological characteristics of participants

		Age Group, yr					
		20–29	30–39	40–49	50–59	60–69	70+
No. of participants	W	5	6	7	5	6	11
	M	6	6	6	5	7	8
Weight, kg	W	71.9±2.9	67.3±3.0	66.0±1.1	70.0±4.9	73.6±2.0	68.4±2.9
	M	82.8±4.5	92.2±2.3	90.4±3.8	90.4±4.7	92.5±1.9	80.4±4.2
BMI, kg/m <sup>2</sup>	W	24.3±0.9	23.8±0.8	24.2±0.4	25.6±1.5	27.5±0.6	25.7±0.8
	M	25.5±1.3	27.0±0.6	28.2±0.7	27.8±1.2	28.3±0.5	27.1±1.3
Body fat, %	W	33.4±3.1	34.1±1.7	36.1±0.8	36.6±2.4	40.3±1.4	38.6±1.4
	M	20.5±1.6	26.7±0.7	26.0±2.9	25.5±0.8	27.5±1.3	26.8±2.2
FFM, kg	W	43.5±1.4	40.6±1.1	38.5±0.9	40.0±2.0	40.1±1.5	38.4±1.2
	M	61.1±2.5	62.5±1.4	61.2±3.7	60.4±4.1	60.9±1.2	52.8±1.4
Leg muscle area, cm <sup>2</sup>	W	233±8	229±7	223±9	221±16	217±14	190±8
	M	344±21	347±17	366±20	285±40	286±17	263±16

Values are means ± SE. W, women; M, men; BMI, body mass index. Significant differences between men and women were present for each variable. Body fat increased ( $r = 0.40$ ) and FFM ( $r = -0.35$ ) and leg muscle area decreased ( $r = -0.50$ ) with age,  $P < 0.01$ .

performed. Postintervention data were not available on one member of the exercise group, a 50-yr-old male. After completion of follow-up testing, 24 control group members opted to complete the exercise program. Thus there were a total of 64 people studied before and after exercise training with complete data.

Because the goal of the study was to examine effects of the exercise program per se, participants were instructed to maintain their body weight. Weight was recorded weekly, and the GCRC dietary staff provided further guidance if weight changed >2%. Only one person in the exercise group (data not included) discontinued the study due to excessive weight loss.

**Measurements.** A standard treadmill stress test was performed initially to ensure cardiovascular health and was followed on another day with measurement of peak oxygen uptake ( $\dot{V}O_{2\text{ peak}}$ ) on a bicycle ergometer (30). Expired gases, heart rate, and blood pressure were continuously monitored throughout the tests, which lasted ~8–15 min (30). The posttraining assessment was made 1 or 2 days after the completion of the last training bout, and therefore 3 or 4 days before measurement of protein turnover.

Fat and fat-free masses were determined on *day 4* of the protocol with dual-energy X-ray absorptiometry (Lunar DPX-L, Madison, WI). Leg muscle cross-sectional area was measured with a computed tomography scan at the midthigh (18).

Resting metabolic rate (RMR) was measured on *day 5*, during the protein turnover studies, with subjects in a postabsorptive state soon after awakening. Oxygen uptake and carbon dioxide production were monitored for 30 min, and energy expenditure was calculated using DeltaTrac equipment and software (Sensormedics, Yorba Linda, CA).

Forearm and hand vein catheters were inserted in the evening of *day 4*. At 2:00 AM the next day, primed, continuous infusions of [<sup>1-<sup>13</sup>C</sup>]leucine (Leu, 7 μmol/kg body wt prime, 7 μmol·kg<sup>-1</sup>·h<sup>-1</sup> thereafter) and [<sup>15</sup>N]phenylalanine (Phe, 4.1 μmol/kg body wt prime, 4.1 μmol·kg<sup>-1</sup>·h<sup>-1</sup> thereafter) were started and continued for 10 h. Arterialized blood samples from a heated hand vein and collections of expired air were obtained at 30-min intervals between 5 and 10 h of the infusion (24). Biopsies of the vastus lateralis muscle were performed at 5 and 10 h of tracer infusion (26). The second biopsy of the day was collected from a fresh incision site ~10–12 cm proximal from the first biopsy. The tissue was quickly frozen in liquid nitrogen and stored with plasma samples at -80°C until analysis.

Isotopic enrichment of breath <sup>13</sup>CO<sub>2</sub> and plasma [<sup>13</sup>C]Leu, [<sup>13</sup>C]-ketoisocaproic acid (KIC), and [<sup>15</sup>N]Phe was determined using mass spectrometry as previously described (3, 20, 24). Average steady-state enrichment values from 5 to 10 h were used to calculate whole body rates of Phe flux, Leu flux, Leu oxidation, and nonoxidative Leu disposal (NOLD) with standard equations (3, 20, 24). A 25-mg piece of muscle tissue was used to obtain mixed (total) protein, as previ-

ously reported (2, 26). The protein-bound enrichment of [<sup>15</sup>N]Phe in mixed muscle protein was determined on the trimethylacetyl methane derivative by gas chromatography-combustion-isotope ratio mass spectrometry (3, 25). The fractional synthesis rate of muscle proteins was calculated using a standard equation (24, 26), with the enrichment of [<sup>15</sup>N]Phe in plasma used as the precursor pool.

**Statistical analysis.** Linear regression analysis was used to determine the strength of the relationships between age and body composition (fat-free mass) and the metabolic outcome variables (whole body protein dynamics and RMR). Univariate correlations were explored first, followed by multiple regression analysis. Covariate adjustment of the metabolic outcome variable for fat-free mass was performed as described elsewhere (37), so that the residual effect of age could be graphically represented.

Global differences between men and women were first analyzed using unpaired *t*-tests. Sex was not a significant component in multivariate modeling once fat-free mass was considered and after covariate adjustment for fat-free mass differences between men and women were no longer evident.

Treatment effects were first tested by performing separate paired *t*-tests within the control and exercise groups. This approach was necessitated by the fact that the exercise group contained several people who were also in the control group. Although this prevented direct comparison between the control and exercise groups, we used the data from the control subjects as a demonstration of the relative stability of the measurements and attributed any changes within the exercise group to the training protocol. When significant changes were detected in the exercise group, linear regression analysis was performed on the pre-to-post change ( $\Delta$  values) to determine whether there were interactions with either age or sex.

Grouped data are reported as means ± SE. *P* values <0.05 were considered statistically significant for all analyses.

## RESULTS

**Physiological characteristics.** Table 2 displays the baseline physiological characteristics of the subjects. The data are presented by decade of age to simplify the presentation, but analysis of age effects was performed by linear regression. As expected, there were significant differences between men and women for most variables. With the exception of body weight, there were significant age-related changes in body composition reflecting increasing adiposity and decreasing fat-free mass (FFM) and leg muscle size in both sexes. The peak aerobic capacity ( $\dot{V}O_{2\text{ peak}}$ ) declined with age 8% per decade in both men and women. After covariate adjustment for FFM,  $\dot{V}O_{2\text{ peak}}$

was not different between men and women but still declined 5.5% per decade ( $r = 0.82$ ,  $P < 0.01$ ), with a mean value of  $2.43 \pm 0.05$  l/min in 20- to 29-yr-olds and  $1.66 \pm 0.06$  l/min in those over 70 yr of age.

There were no changes in the physiological characteristics of the control group at the follow-up test. In exercisers, there were small but statistically significant ( $P < 0.001$ ) reductions in body weight ( $0.6 \pm 0.2$  kg) and body mass index (BMI;  $0.2 \pm 0.1$  kg/m<sup>2</sup>) but no change in body fat, FFM, or leg muscle size.  $\dot{V}O_{2\text{ peak}}$  improved by 9.5% on average ( $P < 0.001$ ). The magnitude of posttraining changes in body mass, BMI, and  $\dot{V}O_{2\text{ peak}}$  in the exercise participants was similar in men and women and among participants of different ages.

**Whole body protein turnover and RMR.** During the tracer studies at baseline, the average enrichment value of <sup>13</sup>CO<sub>2</sub> in expired breath was  $0.0189 \pm 0.0003$  atom percent excess (APE). In plasma, the average enrichment (in molar percent excess, MPE) of [<sup>13</sup>C]Leu was  $8.725 \pm 0.131$ , of [<sup>13</sup>C]KIC was  $7.547 \pm 0.109$ , and of [<sup>15</sup>N]Phe was  $10.411 \pm 0.179$ . Similar values were achieved during follow-up tests in both the control and exercise groups (not shown).

The baseline relationships between age and whole body Phe and Leu kinetics (in mmol/h) are shown in Fig. 1. The rates of Phe and Leu flux were positively correlated ( $r = 0.78$ ,  $P < 0.001$ ). On average, men had 26–38% higher values than women ( $P < 0.001$ ). In both sexes there was a decline of 3–7% per decade in these absolute rates with advancing age ( $P < 0.001$ ). When expressed as a ratio to Leu flux, Leu oxidation was 9% higher in men than in women ( $0.242 \pm 0.006$  vs.  $0.219 \pm 0.005$ ,  $P < 0.005$ ), and this difference did not vary with age.

To account for differences in body size, whole body protein turnover values are typically expressed per unit of total body

mass or per unit of lean (fat-free) mass. In the current study, there were significant positive correlations between the measurements of amino acid kinetics and total body mass ( $r$  values from 0.37 to 0.65,  $P < 0.01$ ). However, there were stronger associations ( $P < 0.001$ ) with FFM and Phe flux ( $r = 0.60$  for men, 0.61 for women), Leu flux ( $r = 0.82$  men, 0.60 women), Leu oxidation ( $r = 0.70$  men, 0.71 women), and NOLD ( $r = 0.78$  men, 0.79 women). The slopes of the regression equations between FFM and amino acid kinetics did not differ between men and women. More importantly, none of the regression lines passed through the origin. For this reason, simple ratio scaling of amino acid flux ( $x$ ) to FFM ( $y$ ) of the form  $x/y$  was not considered appropriate, for reasons previously described (27, 37). Thus a covariate approach was used to adjust the individual values of Phe and Leu kinetics for FFM (37). After this adjustment, there were no longer differences between men and women, but there was still a significant decline with age, 3–4% per decade ( $P < 0.01$ ), for each of the Phe and Leu measurements (Fig. 2).

Multiple regression analysis confirmed that both age and FFM made significant contributions to models explaining the variance in Phe and Leu kinetics. Table 3 shows that a greater percentage of the variance in the whole body Phe and Leu kinetics was explained after age had been added to the model that already included FFM as a variable. Interactive and quadratic terms were not significant. No other body composition terms, such as total body mass, fat mass, or BMI, made further significant contributions to these models.

RMR was higher in men than in women (25% on average,  $P < 0.001$ , when expressed in kcal/h) and declined with age (4% per decade) in both sexes (Fig. 3A). RMR was also strongly related to FFM in men ( $r = 0.71$ ) and in women ( $r = 0.59$ ), both  $P < 0.001$ . After covariate adjustment of RMR for

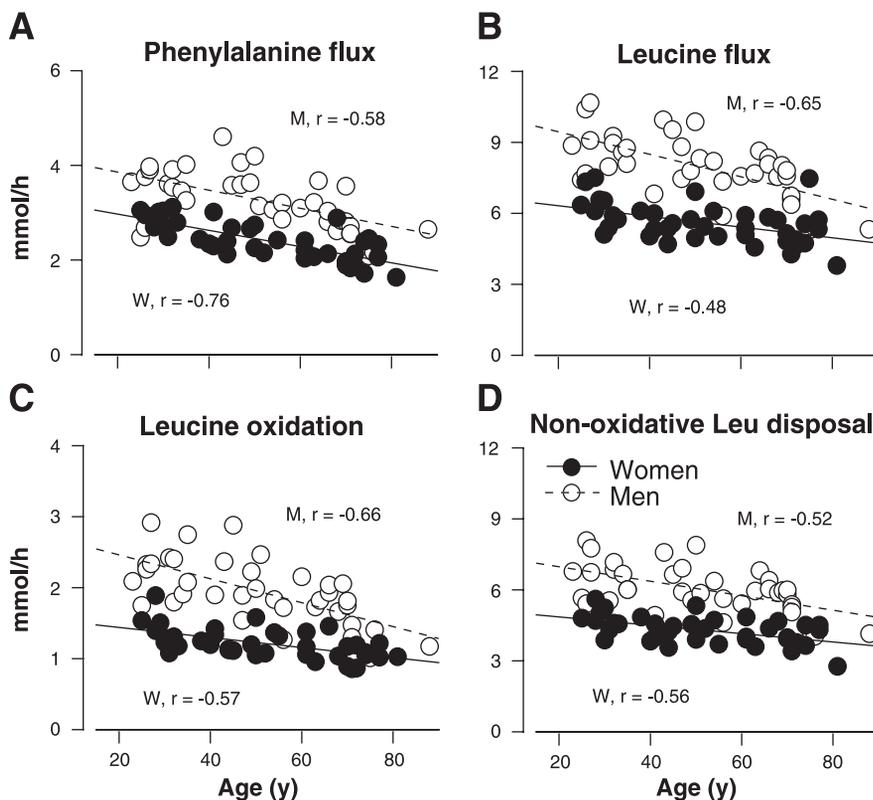


Fig. 1. Whole body amino acid kinetics decline with age. Separate symbols, regression lines, and correlation coefficients are given for women (W) and men (M). All correlation coefficients were statistically significant,  $P < 0.001$ .

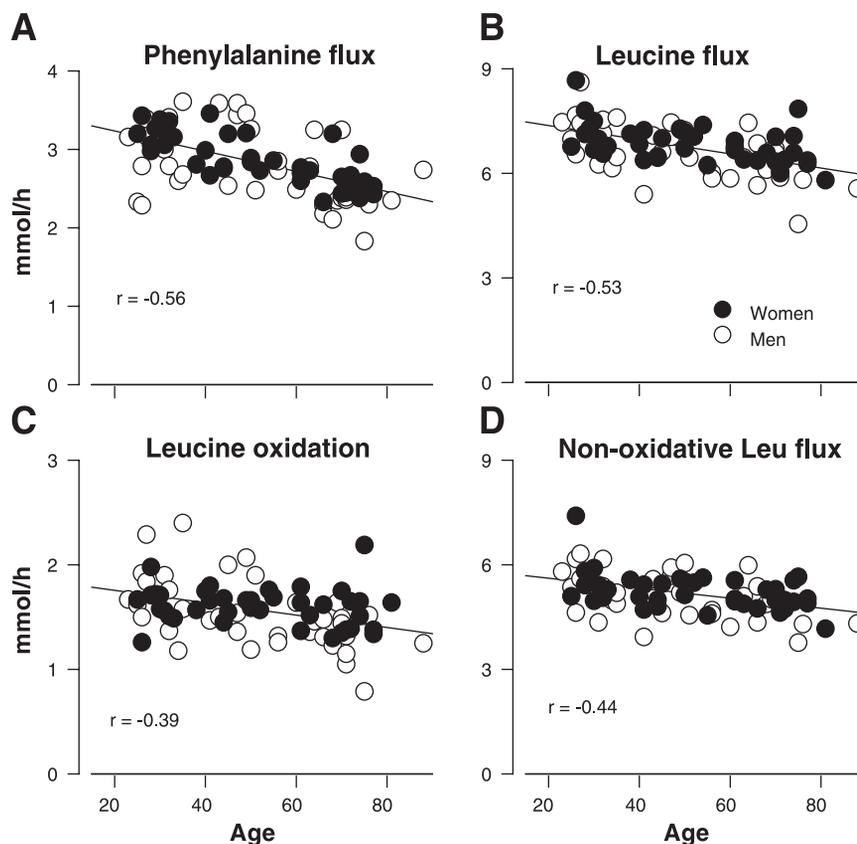


Fig. 2. Relationship between age and amino acid kinetics after covariate adjustment for fat-free mass. Regression lines are shown for the whole group, as there were no differences between men and women. The decline of each variable with age was significant,  $P < 0.01$ .

FFM, men and women were no longer different, but there remained a significant decline with age of 2.5% per decade (Fig. 3B).

RMR (in kcal/h) was closely correlated with the absolute rates (mmol/h) of Phe flux ( $r = 0.80$ ), Leu flux ( $r = 0.89$ ), Leu

Table 3. Univariate and multivariate relationships between amino acid kinetics, resting metabolic rate, FFM, and age

	FFM	FFM + Age
Phenylalanine flux		
Men	37	45
Women	36	74
Men and women	61	74
Leucine flux		
Men	67	76
Women	60	67
Men and women	82	87
Leucine oxidization		
Men	48	62
Women	50	49
Men and women	72	77
Nonoxidative leucine disposal		
Men	59	64*
Women	46	54
Men and women	75	80
Resting metabolic rate		
Men	48	51†
Women	27	53
Men and women	73	78

Adjusted  $R^2$  values are given  $\times 100$  so they indicate the % variation of the dependent variable explained by the independent variable(s). The overall model statistic in all cases was  $P < 0.002$ . The  $P$  value for the individual predictor variables, FFM and age, was  $< 0.01$  in all models except \*, where the age term was  $P = 0.026$ , and †, where the age term was  $P = 0.09$ .

oxidation ( $r = 0.87$ ), and NOLD ( $r = 0.86$ ), all  $P < 0.001$ . After adjustment for both RMR and protein turnover for FFM, RMR remained significantly correlated with Phe flux ( $r = 0.38$ ), Leu flux ( $r = 0.55$ ), Leu oxidation ( $r = 0.45$ ), and NOLD ( $r = 0.43$ ), all  $P < 0.01$ . When adjustment of the individual Phe and Leu kinetic values was performed with RMR as the covariate, differences between men and women in amino acid turnover were eliminated. Adjustment for RMR also reduced the effect of age on Phe flux, although there was still a small but significant 2.5% decline per decade ( $r = 0.31$ ,  $P < 0.01$ ). In contrast, the age effect on Leu kinetic values was no longer statistically significant after adjustment for RMR.

It should be noted that, if simple ratio scaling had been (incorrectly) used instead of covariate adjustment, the calculated rates of Phe flux, Leu flux, and NOLD per kilogram FFM would be 7–10% higher in women than in men ( $P < 0.001$ ), whereas Leu oxidation would be slightly (5%), but not significantly ( $P = 0.11$ ), higher in men. RMR per kilogram FFM would also be 12% higher in women ( $P < 0.001$ ). These differences between men and women when the simple ratio approach is used arise from the fact that men and women differ in FFM and the regression lines between FFM and amino acid kinetics or RMR do not pass through the origin.

After the control and exercise programs, there were no changes in any of the Phe or Leu kinetics measured. Figure 4 shows the difference between the pre- and postexercise values for men and women of different ages. There was also no change in RMR in the control group or after the exercise training program (not shown).

**Mixed muscle protein synthesis.** Because of technical limitations and prior use for other analyses, muscle samples for

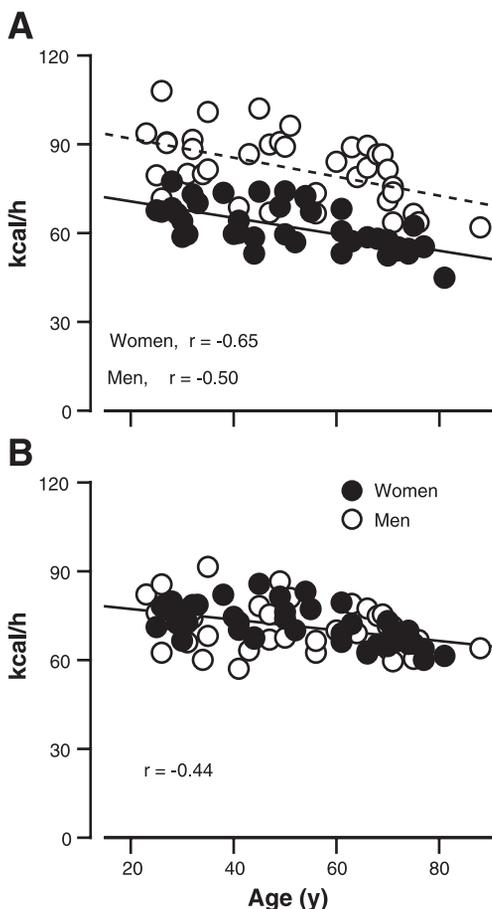


Fig. 3. Relationship between age and resting metabolic rate (RMR). A: a significant decline in RMR with age was evident in women (solid line) and men (dashed line). B: after adjustment of RMR for fat-free mass, there was still a significant decline with age. The regression line (B) is shown for the whole group, because there were no differences between men and women. All correlation coefficients significant,  $P < 0.01$ .

mixed muscle protein analysis were not available for all subjects. However, subjects on whom this analysis was performed were representative of the entire group in all other respects. At baseline ( $n = 51$  people; 28 women, 23 men), muscle protein fractional synthesis rate declined 3.5% per decade ( $P < 0.05$ ; Fig. 5A). Because most previous studies have compared groups of younger and older people, we also analyzed the data after assigning individuals to one of three age categories. The average rate for older people (60–74 yr,  $n = 20$ ;  $0.0374 \pm 0.0028\%/h$ ) was 19% lower ( $P < 0.025$ ) than that for younger people (19–38 yr,  $n = 20$ ;  $0.0460 \pm 0.0021\%/h$ ). The rate for middle-aged people (40–55 yr,  $n = 11$ ;  $0.0400 \pm 0.0032\%/h$ ) was intermediate but not significantly different from either younger or older groups.

There was no change in mixed muscle protein synthesis in subjects in the control group ( $n = 20$ ), whereas the aerobic exercise training ( $n = 35$ ) resulted in an average 22% increase ( $P < 0.05$ ; Fig. 5B). Within the exercise group, there was no effect of age on the change in muscle protein synthesis in either men or women (Fig. 5C). The average change in muscle protein synthesis rate was calculated for young ( $0.008 \pm 0.004\%/h$  for 7 women and 5 men), middle-aged ( $0.012 \pm 0.007\%/h$  for 5 women and 5 men), and older ( $0.008 \pm$

$0.005\%/h$  for 6 women and 7 men) groups, with the same age classifications used as before. Because of the variation within these smaller groups, however, statistically significant changes were not detected.

## DISCUSSION

The two main findings of the current study are 1) that the rates of whole body protein turnover and mixed muscle protein synthesis decline with normal aging and 2) that 4 mo of aerobic exercise training resulted in an increase in muscle protein synthesis but did not affect whole body protein turnover. The decline with age in whole body protein kinetics was evident in both men and women, was confirmed with two separate amino acid tracers, and persisted even after adjustment for differences in FFM. To our knowledge, this is the largest study to date to examine the effect of age on whole body protein turnover and the first to test a continuum of people between the third and ninth decades of life. Furthermore, this is the first study to prospectively examine the effect of aerobic exercise training on either whole body or muscle protein metabolism.

Although the effect of age on whole body protein turnover has been addressed in previous studies, no consensus has been reached (Table 1). Small sample sizes may have prevented detection of differences between younger and older people in some studies. Another limitation of all but one (3) of the previous investigations was the lack of data for people between young and old groups, i.e., ~45–65 yr of age. Both of these limitations were overcome in the current study by measuring Phe and Leu kinetics in 78 men and women with ages ranging from 19 to 87 yr. Like others (44), we found that FFM was a better predictor than total body mass of whole body amino acid flux, which reflects the fact that fat-free tissue is more metabolically active than adipose tissue and bone. Together, FFM and age explained up to 87% of the variance in amino acid kinetics (Table 3). With advancing age, there is a tendency to lose lean mass, as shown here by the reduction of total FFM and the reduction in cross-sectional muscle area of the thighs (Table 2). However, the fact that the age-related decline in protein turnover persisted even after covariate adjustment for FFM indicates that there is an overall decline in the ability to remodel body tissues. A potentially confounding factor is that lean tissue is comprised of many tissues with different sizes and protein turnover rates, and with aging, the reduction in lean mass is predominantly skeletal muscle (3). The relative contributions of the different lean tissues to whole body protein metabolism may therefore vary across age. When we consider that muscle protein synthesis is slower than many other lean tissues (5, 13), selective loss of muscle in elderly people might be expected to result in faster rates of whole body protein turnover, as other organs with faster metabolism would make greater contributions. Because there is a reduction in whole body protein metabolism, it appears that protein turnover in tissues besides skeletal muscle must also decline with aging in humans. This is an important issue, because protein turnover is necessary to maintain tissue quality by continuous replacement of damaged proteins. Older people may accumulate a greater number of damaged or modified proteins that contribute to the reduction in body functions, which could result in slower tissue repair in response to illness or trauma (21). Interventions that

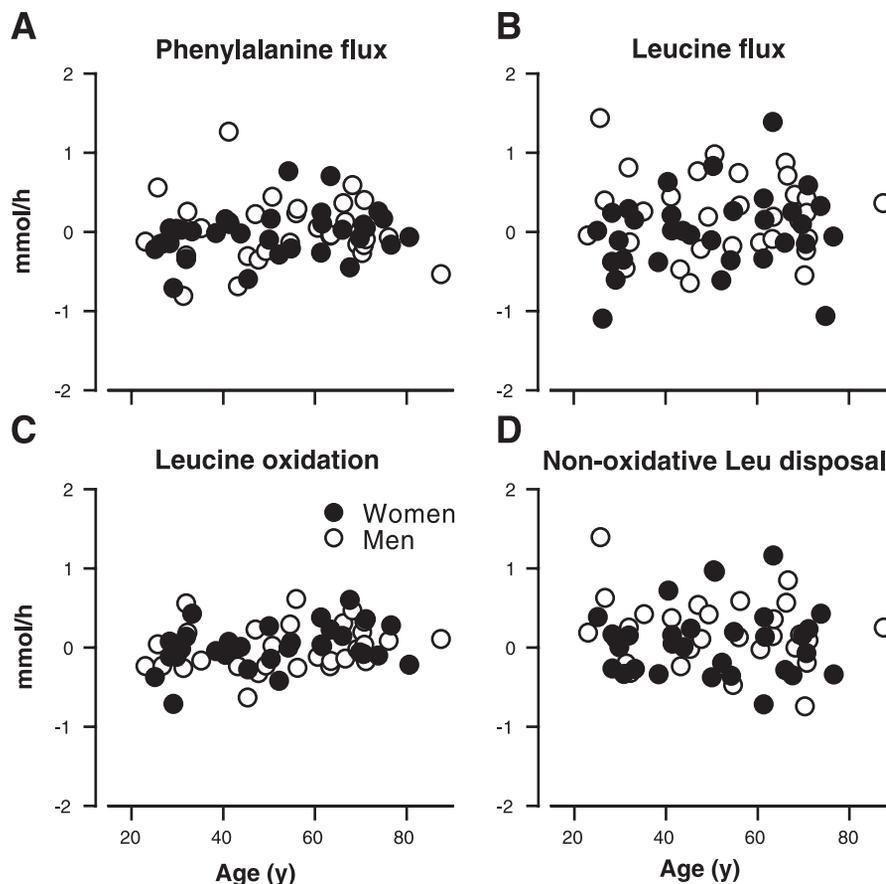


Fig. 4. Changes in whole body amino acid kinetics after 16 wk of aerobic exercise training. Posttraining minus pretraining values ( $\Delta$  values) are shown. There were no significant changes after the training program.

increase protein turnover could therefore have potential benefits for health and physical function.

Like whole body protein turnover, the postabsorptive RMR declined with age, even after adjustment for FFM, in agreement with previous reports (16, 29). This further establishes that, with aging, there is a fundamental change in the metabolic capacity of lean tissue, although additional work is needed to understand how this change arises. We also found that RMR was highly correlated with protein turnover. This could be expected, since protein turnover may account for  $\sim 20\%$  of the metabolic rate at rest (44). After whole body protein turnover rates were adjusted for RMR, the effect of age on Leu kinetics was eliminated and was greatly reduced for Phe flux. This indicates that amino acid metabolism rates are proportional to other components of energy expenditure in the body. Likewise, free fatty acid metabolism is more closely related to RMR than to FFM in young to middle-aged people (27). It was proposed that the body's energy needs at rest may determine the rate of fatty acid metabolism, because fat is a major fuel source (27). For protein metabolism the situation is likely to be different, since amino acids are normally used more for protein remodeling than as fuel. Therefore, it is possible that the age-related decline in both protein turnover and RMR is determined by other factors, such as hormones or sympathetic activity, that change with age.

We employed a 4-mo program of bicycle training to determine whether it could improve muscle and whole body protein turnover, particularly in the elderly. The posttraining measurements were performed 6 days after the last exercise session and

5 days after the  $\dot{V}O_{2\text{ peak}}$  test. This was done so that any changes that occurred could be considered training effects instead of acute effects of the last exercise bout. It has clearly been shown that resistance training can increase protein synthesis rates in older people (4, 15, 36, 49, 50), although none of those studies found an effect on whole body protein metabolism. Unlike resistance training, aerobic exercise is not expected to result in a significant change in muscle size or strength (9). Rather, there is an increase in endurance capacity as a result of increased muscle mitochondrial size and number, increased capillary density, and a shift toward the expression of slow-twitch muscle fibers (9). In accord, in the present study there was a 9% increase in  $\dot{V}O_{2\text{ peak}}$  but no change in either FFM or leg muscle size. We have reported in a separate publication from this study that there was a 45–76% increase in the activity of mitochondrial oxidative enzymes in muscle (35). The changes in  $\dot{V}O_{2\text{ peak}}$  and mitochondrial enzymes were similar in younger and older people, indicating that the ability of skeletal muscle to adapt to exercise training remains intact in healthy older people.

The aerobic exercise training resulted in an  $\sim 20\%$  increase in mixed muscle protein synthesis, with no significant effect of age on the training response. The increased fractional synthesis rate of muscle proteins is a marker of the remodeling process taking place as a result of the exercise stimulus. We have measured mixed muscle protein synthesis in the present study, which represents the average of all muscle proteins. It is not yet known how aerobic exercise training affects the synthesis rates of subfractions of the muscle, such as the mitochondrial or

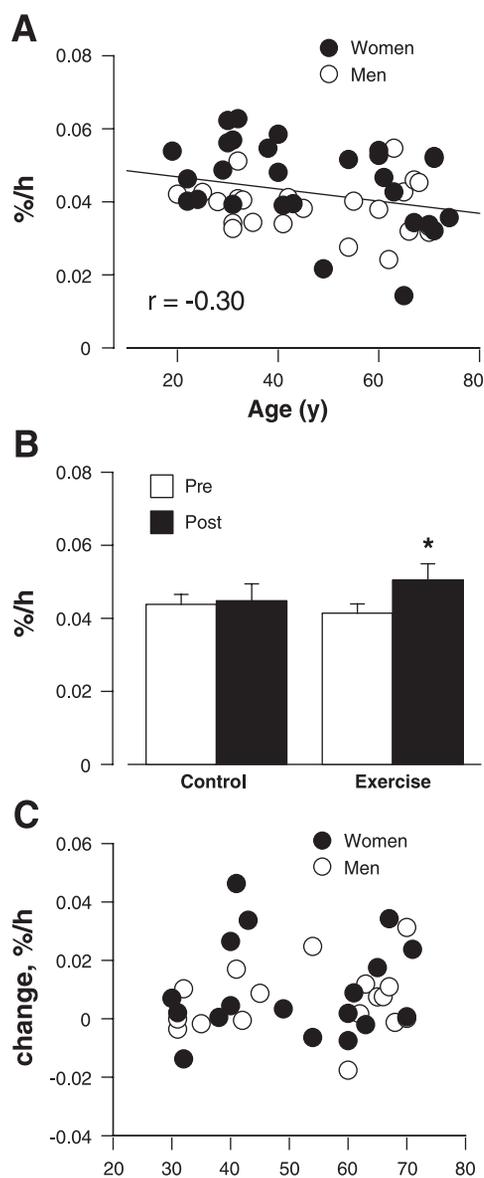


Fig. 5. Age and exercise effects on fractional synthesis rate of mixed muscle protein. A: at baseline there was a significant decline with age in muscle protein synthesis rate. B: exercise training resulted in an overall 22% increase in muscle protein synthesis,  $*P < 0.05$ , whereas there was no change in the control group. C: change in muscle protein synthesis rate ( $\Delta$  value) in the exercise group did not vary with age in men or women.

contractile proteins. The synthesis rates of mitochondrial proteins, mixed myofibrillar proteins, and the contractile protein myosin heavy chain have been reported to decline with age (3, 15, 32, 45). Resistance training can increase the synthesis rate of mixed myofibrillar proteins and myosin heavy chain in younger and older people (4, 15, 36, 49, 50). In the present study, the aerobic training program resulted in an increase in the abundance of mRNAs encoding mitochondrial proteins (ND4, COX4) and transcription factors that promote an oxidative phenotype (PGC-1, NRF-1, TFAM) in muscle, as reported elsewhere (35). These changes did not differ with age. The increase in mRNA template availability could be expected to promote an increase in mitochondrial protein synthesis, leading to the increase in oxidative enzymes in muscles of

exercise-trained individuals (9, 35). The available evidence suggests that older people respond in a manner similar to that of younger people in this regard. However, this possibility requires confirmation in future studies.

Although there was an increase in muscle protein synthesis, whole body protein turnover and RMR at rest were not changed after completion of the exercise training program. Bicycle training would be expected to recruit primarily the leg muscles, and therefore the exercise effect on protein synthesis may not occur, or may be lower, in other muscle groups or other tissues. Because whole body protein turnover represents the average contributions of many different body protein pools, the change in protein synthesis in the thigh muscle is probably too small to be detected at the whole body level. To significantly alter the age-related decline in whole body protein turnover and RMR may therefore require more frequent or vigorous exercise, a combination of resistance and aerobic exercise involving several muscle groups, or other types of interventions.

The large number of people studied in the present study allowed for comparisons between men and women. On average, the unadjusted rates of whole body protein turnover (Fig. 1) were higher in men than in women, as could be expected given the overall difference in body size, particularly in FFM. After adjustment for FFM (but not body mass), differences between men and women were no longer apparent. This appears to be in agreement with other studies that used a simple ratio normalization to divide leucine and/or lysine flux or nitrogen balance by FFM, which removed any differences between young men and women (17, 28, 44). However, the results of these earlier comparisons should be interpreted cautiously because of the potential problems that arise with simple ratio scaling. In the present study, for example, if whole body protein turnover and RMR data had been divided by FFM, we would have concluded erroneously that women have higher Leu and Phe flux and RMR than men.

There was only one difference in protein kinetics detected between men and women in the present study. Leu oxidation per se did not differ between sexes after covariate adjustment for either FFM or RMR, but Leu oxidation as a proportion of Leu flux was 9% higher in men. Volpi et al. (39) reported that Leu oxidation per unit of FFM was 18% higher in young men than in young women. Together, these data suggest that men oxidize a small, but significantly higher, proportion of Leu at rest, although the reason for this difference and the physiological significance are not yet clear.

To control for the potentially confounding effects of diet and physical activity, we provided the participants with a weight-maintaining diet and asked them to refrain from vigorous activity for 5 days before the studies. Normal daily activities were permitted. Control of diet and, to a lesser extent, physical activity before conducting protein turnover studies has been a regular practice (see Table 1) but has become a recent topic of debate (42, 43, 48). It has been proposed that protein turnover studies should be performed in younger and older people in their "free-living" conditions, without control of diet or exercise (42, 43), but a systematic comparison between these approaches has not been reported. In our experience, controlling the macronutrient content of the diet helps ensure highly reproducible measures of protein turnover (10). The results from the exercise-trained individuals in the current study indi-

cate that aerobic exercise had no residual effect on whole body protein turnover. However, muscle protein synthesis in the trained group was elevated when measured 6 days after the last exercise bout, and transcript levels for several muscle proteins were also 20–80% increased above pretraining levels (35). Likewise, when middle-aged and older people performed a 3-mo resistance training program, increased synthesis rates of mixed muscle and myosin heavy-chain proteins and alterations in myosin heavy-chain mRNA levels were detected 4 days after the last exercise session (4). Thus the effect of physical activity on muscle gene expression and protein synthesis should be carefully considered when these measurements are performed. Additional work is needed to determine whether exercise effects on whole body or muscle protein metabolism are detectable within several hours or days of exercise.

Finally, a potential concern in the present study was that we used plasma [<sup>15</sup>N]Phe enrichment instead of muscle tissue fluid or amino acyl-tRNA enrichment in the calculation of muscle protein synthesis rate. Amino acyl-tRNA is the immediate precursor pool for protein synthesis, but because of the technical demands in performing this measurement, it is used in only a limited number of studies. The use of plasma or tissue fluid as surrogates for amino acyl-tRNA results in lower calculated synthesis rates, with tissue fluid values coming closer to those obtained with amino acyl-tRNA (5, 19). Because of technical limitations and the use of muscle samples for other analyses (35), we were unable to obtain values for [<sup>15</sup>N]Phe enrichment in tissue fluid in this study. Thus the values for mixed muscle protein synthesis, based on a plasma precursor, probably underestimate the actual rates of protein synthesis. However, it has been shown that there is a stable relationship in enrichment values among the plasma, tissue fluid, and amino acyl-tRNA compartments during postabsorptive conditions, as used in the present study (5, 19). Because all of the participants were tested in a similar manner, we are confident that the changes in muscle protein synthesis with aging and exercise training reflect real physiological differences.

In conclusion, both men and women experience a decline in rates of whole body protein synthesis and breakdown and muscle protein synthesis as they age. There is a concurrent reduction in the amount of fat-free mass with aging, but adjustment for fat-free mass does not completely eliminate the effect of age on whole body protein turnover. This implies that the decline in protein turnover with aging is due to change in both the amount and metabolic activity of lean tissue in the body. A similar conclusion is drawn when the effects of age on resting metabolic rate are examined. These findings suggest that overall tissue remodeling rate is reduced in older people and could contribute to reduced function and repair in many organs. A 4-mo program of aerobic exercise involving primarily leg muscles had no impact on whole body protein turnover but did increase muscle protein synthesis. These results demonstrate that the effect of aging on muscle protein synthesis can be reversed by interventions such as exercise.

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Current address for D. N. Proctor: Noll Physiological Research Center, The Pennsylvania State University, University Park, PA 16802-6900.

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