

Collagen synthesis in human musculoskeletal tissues and skin

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¹Division of Molecular Physiology, School of Life Sciences, University of Dundee, Dundee, Scotland; ²Division of Clinical Physiology, Graduate Entry Medical School, University of Nottingham School of Biomedical Sciences, City Hospital, Derby, England, United Kingdom; and ³Sports Medicine Research Unit and Department of Orthopaedic Surgery, Copenhagen University Hospital at Bispebjerg, Copenhagen, Denmark

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Babraj, J. A., D. J. R. Cuthbertson, K. Smith, H. Langberg, B. Miller, M. R. Krogsgaard, M. Kjaer, and M. J. Rennie. Collagen synthesis in human musculoskeletal tissues and skin. *Am J Physiol Endocrinol Metab* 289: E864–E869, 2005. First published June 21, 2005; doi:10.1152/ajpendo.00243.2005.—We have developed a direct method for the measurement of human musculoskeletal collagen synthesis on the basis of the incorporation of stable isotope-labeled proline or leucine into protein and have used it to measure the rate of synthesis of collagen in tendon, ligament, muscle, and skin. In postabsorptive, healthy young men (28 ± 6 yr) synthetic rates for tendon, ligament, muscle, and skin collagen were 0.046 ± 0.005 , 0.040 ± 0.006 , 0.016 ± 0.002 , and $0.037 \pm 0.003\%/h$, respectively (means \pm SD). In postabsorptive, healthy elderly men (70 ± 6 yr) the rate of skeletal muscle collagen synthesis is greater than in the young ($0.023 \pm 0.002\%/h$, $P < 0.05$ vs. young). The rates of synthesis of tendon and ligament collagen are similar to those of mixed skeletal muscle protein in the postabsorptive state, whereas the rate for muscle collagen synthesis is much lower in both young and elderly men. After nutrient provision, collagen synthesis was unaltered in tendon and skeletal muscle, remaining at postabsorptive values (young: tendon, $0.045 \pm 0.008\%/h$; muscle, $0.016 \pm 0.003\%/h$; elderly: muscle, $0.024 \pm 0.003\%/h$). These results demonstrate that the rate of human musculoskeletal tissue collagen synthesis can be directly and robustly measured using stable isotope methodology.

stable-isotope tracers; musculoskeletal collagen synthesis; nutrition; aging

THE EXTRACELLULAR MATRIX of skeletal muscle, tendon, ligament, and bone is important for maintaining tissue structure and vital for transmission of force during muscular contraction (15). Collagen is the major protein in the extracellular matrix of the musculoskeletal tissue, but despite its importance to tissue function, knowledge of the physiological regulation of the amount and turnover of collagen in human beings is poor, partly due to the absence of direct methods for determination of collagen turnover in vivo under different conditions. The overall contribution of collagen to whole body protein turnover is unknown, despite the fact that collagen probably contributes 3.5 kg to the lean body mass compared with 12 kg for muscle myocellular protein, about which much more is known (22).

It has been demonstrated that the concentration of collagen in skeletal muscle increases (16, 29) in aging rats, whereas in tendon and ligament there is a decrease (13). In the collagen found in tendon, ligament, and skeletal muscle, there is an

increase in the nonreducible collagen cross-linking with aging, possibly resulting in stiffer, less compliant tissues (17, 23, 13). Direct measurements of collagen synthesis in rats (6 and 15 mo old), using a flooding dose of radioactive [¹⁴C]proline (19), have shown increased turnover, including both a greater collagen fractional synthetic rate and an increased degradation rate of newly synthesized collagen, with maturity. It might be expected that a similar temporal change might exist in human beings. Unfortunately, little is known about human musculoskeletal collagen metabolism and aging in vivo.

Feeding of protein or amino acids is a major, dose-dependent anabolic stimulus for skeletal muscle myofibrillar and sarcoplasmic protein (3, 4, 5, 7). Despite the large amount of evidence of nutritional regulation of skeletal muscle protein metabolism, the relative importance of nutrition in musculoskeletal collagen metabolism has not been elucidated. We have shown in separate studies that bone collagen synthesis is acutely regulated by mixed intravenous feeding (2), so we hypothesized that collagen from other musculoskeletal tissue, such as tendon and intramuscular connective tissue, might also be nutritionally regulated.

To test this, we have measured rates of collagen synthesis in human skeletal muscle, tendon, ligament, and skin in the postabsorptive states and, also, for tendon and skeletal muscle after nutritional intervention, either complete oral liquid feeds or essential amino acid (EAA) solutions. In addition, we have also investigated the effect of age on human skeletal muscle collagen synthesis. To accomplish our aims, we applied a newly developed method for the direct measurement of the rate of collagen synthesis by the incorporation of stable isotope-labeled proline or leucine into musculoskeletal tissue over time.

MATERIALS AND METHODS

Subjects

Subjects were recruited to ongoing research projects occurring in the Division of Molecular Physiology, University of Dundee, and the Sports Medicine Research Unit, Copenhagen University Hospital. All subjects gave informed consent, and the studies were carried out according to the Declaration of Helsinki under the auspices of the Ethics Committee for Copenhagen and Fredriksberg Municipalities and Tayside Regional Ethics Committees.

A summary of the proteins analyzed, tracers, and mode of delivery and number of subjects for each protocol is presented in Table 1.

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Table 1. Protocol summary

	Protocol 1		Protocol 2		Protocol 3		Protocol 4		
	Young PA (n = 8)		Young PA, Intra-operatively (n = 8)		Young PA (n = 4)	Young EAA (n = 4)	Elderly PA (n = 4)	Elderly EAA (n = 4)	Young Mixed Meal (n = 8)
Myofibrillar protein	✓				✓	✓	✓	✓	
Muscle collagen	✓				✓	✓	✓	✓	
Ligament collagen			✓						
Tendon collagen			✓						
Skin collagen	✓								✓
Tracer and delivery									
Constant infusion	[¹³ C]leucine				[¹³ C]KIC	[¹³ C]KIC	[¹³ C]KIC	[¹³ C]KIC	
Flooding dose	[¹³ C]- or [¹⁵ N]proline		[¹³ C]- or [¹⁵ N]proline						[¹³ C]proline

PA, postabsorptive; EAA, essential amino acids.

Fasted Studies

Protocol 1: muscle and skin. Eight healthy young men (age 25 ± 6 yr, BMI 24 ± 4 kg/m²; means \pm SD) had cannulae introduced into forearm veins, with one arm used for the introduction of tracers and the other arm for collection of blood samples. Subjects were given a primed constant infusion of [^{1-¹³C}]leucine (99 atom percent; Cambridge Isotope Labs, Woburn, MA; 0.8 mg/kg prime; 1.0 mg·kg⁻¹·h⁻¹ infusion rate), and after 120 min a flooding dose of [^{1-¹³C}]proline or [¹⁵N]proline (20 atom percent) was given (0.75 g of [¹³C]- or [¹⁵N]proline; 99 atom percent; Cambridge Isotope Labs) and 3 g of unlabeled proline (Sigma-Aldrich, Dorset, UK). Blood samples were taken every 30 min for the first 120 min and every 10–20 min after the flooding dose. Biopsies (150–300 mg) of vastus lateralis muscle were taken at the start of the study and after 4 h by use of the conchotome technique (8), with skin and fascia incisions made under local anesthesia (1% lignocaine). Skin biopsies were taken after 4 h, using the punch biopsy technique. The muscle and skin biopsies were blotted, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

Protocol 2: tendon and ligament. Eight young men (age 24 ± 8 yr, BMI 23 ± 4 kg/m²; means \pm SD) undergoing reconstructive surgery had cannulae introduced into forearm veins as above. Subjects were given a flooding dose of either [^{1-¹³C}]proline or [¹⁵N]proline (20 atom percent, as in protocol 1) 120 min before surgery. Basal blood samples were taken and then every 30 min thereafter. Surgical biopsies of tendon and ligament were taken 120 min postflood after induction of general anesthesia in patients who were undergoing reconstructive surgery for torn anterior cruciate ligament. The patella tendon was used as a reconstructive tissue, and part of the patella tendon piece dissected for this was used for this study; the ligamentous tissue that was found, perioperatively, to remain after the previous ligament rupture was used as ligament sample. The tissue was stored as in protocol 1.

Nutritional Studies

Protocol 3: skeletal muscle. Eight healthy young men (age 28 ± 6 yr, BMI 24 ± 3 kg/m²; means \pm SD) and 8 healthy elderly men (age 70 ± 6 yr, BMI 26 ± 4 kg/m²; means \pm SD) had cannulae introduced into forearm veins as above and were given a primed constant infusion of [^{1-¹³C}]ketoisocaproate [KIC, 99 atom percent (Cambridge Isotope Labs), prime 1.1 mg/kg and infusion rate 1.7 mg·kg⁻¹·h⁻¹] to label the tissue free leucine pools via transamination with the aim of delivering labeled leucine directly to the protein synthetic apparatus (6); the fibroblast also carries out transamination (27) and any enriched leucine in the plasma pool has originated within the fibroblast or myoblast and as such may reflect the true precursor enrichment, i.e., leucyl-tRNA, in both cell types.

Octreotide (Sandoz, Basel, Switzerland) was infused at 1.8 mg·kg⁻¹·h⁻¹ (sufficient to inhibit the postprandial secretion of hormones, including insulin and growth hormone), and insulin (Actrapid, Novo Nordisk) was replaced by infusing at 360 mIU·m body surface

area⁻²·h⁻¹ throughout the investigation to maintain plasma insulin concentration at ~ 10 m IU/l. The tracer infusion and insulin clamp were started 30 min before feeding. Subjects were randomly assigned to either group 1 (placebo) or group 2 (EAA), where group 1 contained four young and four elderly subjects who ingested a solution containing 0 g of EAA and group 2 contained four young and four elderly subjects who ingested a solution containing 20 g of EAA in a composition representative of muscle protein (27). Blood samples were taken at 20- to 30-min intervals throughout the study. Muscle biopsies were taken before the start of the study and 3 h after and stored as in protocol 1.

Protocol 4: tendon. Eight healthy young men (age 25 ± 1 yr, BMI 22 ± 2 kg/m²; means \pm SD) had cannulae introduced into forearm veins as above. Subjects were given a commercially available nutrient drink (15% protein, 64% carbohydrate, and 21% fat; Semper, Frederiksberg, Denmark) over 280 min to provide the equivalent of 1.4 \times basal metabolic rate. One hundred twenty minutes after the subjects started feeding, they were given a flooding dose of [^{1-¹³C}]proline (20 atom percent, as in protocol 1). Blood samples were taken every 30 min before the flood and every 10–20 min thereafter. Tendon biopsy was taken, under the guidance of ultrasound, 120 min after the flood and stored at -80°C .

Blood Processing and Analysis

Plasma was separated from whole blood by spinning at 1,600 g for 15 min at 4°C and extracted as previously described (24). The labeling of leucine, proline, and KIC in plasma was determined by gas chromatography-mass spectrometry after conversion to the *tert*-butyldimethylsilyl derivative.

Muscle Processing

These methods have been discussed previously (1). Briefly, muscle was powdered under liquid nitrogen, extracted with 0.15 M NaCl buffer, and centrifuged and the supernatant removed. KCl (0.7 M) was added to the pellet containing myofibrillar proteins and collagen and centrifuged, and the pellet containing collagen was washed with acetic acid and acetic acid-pepsin (0.1% wt/vol), dissolving immature collagen and leaving an insoluble collagen pellet. The myofibrillar, soluble, and insoluble collagens were hydrolyzed in 0.1 M HCl-Dowex H⁺ slurry at 110°C overnight and the liberated amino and imino acids separated using Dowex 50W-X8 H⁺ ion-exchange resin.

Tendon, Ligament, and Skin Processing

Skin was separated in dermis and epidermis by dissection before extraction of collagen. Tissue was powdered under liquid nitrogen and homogenized in 0.15 M NaCl buffer. Sample was centrifuged, supernatant was removed, and pellet was washed again with 0.15 M NaCl. The pellet was washed with 70% ethanol and centrifuged as before. The isolated collagen pellet was acid hydrolyzed, and amino and imino acids were separated as above.

Calculation of Fractional Synthetic Rates

Liberated amino and imino acids were derivatized as their *N*-acetyl-*n*-propyl esters for measurement of tracer incorporation by gas chromatography-combustion-isotope ratio mass spectrometry. Fractional synthetic rates (FSR, %/h) were calculated by comparing the incorporation of tracer over time into isolated protein fractions with the plasma leucine, where [¹³C]KIC was the tracer, or plasma KIC, where [¹³C]leucine was the tracer, or the area under the proline decay curve following the flooding dose of [¹³C]- or [¹⁵N]proline.

Measurement of Collagen Hydroxyproline and Proline Concentration

An internal standard, norleucine, was added to the soluble and insoluble collagen fractions for quantification of hydroxyproline and proline content prior to derivatization as their *N*-acetyl-*n*-propyl esters. Gas chromatography-mass spectrometry was used to determine collagen hydroxyproline and proline concentration using a standard curve of known hydroxyproline and proline concentrations. The collagen concentration was calculated using the assumption that hydroxyproline accounts for 13% of collagen (28).

Statistics

The results were analyzed with InStat (v3.0) for Windows (GraphPad Software, San Diego, CA). We chose to apply one-way ANOVA with multiple data sets with Bonferroni post test procedures for comparison of group means. All values in the text and in the tables are means \pm SD. *P* was taken as significant at 0.05 or less.

RESULTS

Protein Synthesis in Musculoskeletal Tissues of Young Men in the Postabsorptive State

FSRs of skeletal muscle collagen in young healthy men were the same irrespective of tracer or mode of delivery (0.018 ± 0.005 , 0.016 ± 0.004 , 0.016 ± 0.004 , and 0.016 ± 0.003 %/h [means for [¹³C]proline, [¹⁵N]proline, [¹³C]leucine (data from subjects participating in *protocol 1*) and [¹³C]KIC (data from subjects participating in *protocol 3*), respectively; Fig. 1A]).

The use of the flooding dose of [¹³C]proline had no effect on [¹³C]leucine incorporation into myofibrillar protein with the FSR identical with both tracers (0.038 ± 0.009 vs. 0.037 ± 0.007 %/h, respectively, (data from subjects participating in *protocol 1*); Fig. 1B). When a flooding dose of [¹³C]- or [¹⁵N]proline was used to measure tendon, ligament, and dermal collagen synthesis, the observed rates were identical for each tissue irrespective of tracer label [tendon: 0.045 ± 0.007 vs. 0.047 ± 0.004 %/h, respectively; ligament: 0.040 ± 0.007 vs. 0.039 ± 0.007 %/h, respectively; dermis: 0.037 ± 0.003 vs. 0.037 ± 0.004 %/h, respectively (tendon and ligament data from subjects participating in *protocol 2*, dermis data from subjects participating in *protocol 1*); Fig. 1C].

Skeletal Muscle Collagen Synthesis and Concentration in Young and Elderly Men

In the postabsorptive state, FSR of skeletal muscle collagen was found to be significantly higher in the elderly compared with the young, i.e., 0.024 ± 0.002 vs. 0.017 ± 0.004 %/h, *P* < 0.05, respectively (data from subjects participating in *protocol 3*); Fig. 2A).

Despite the increased rate of synthesis of skeletal muscle collagen in the elderly subjects, the total amounts of protein

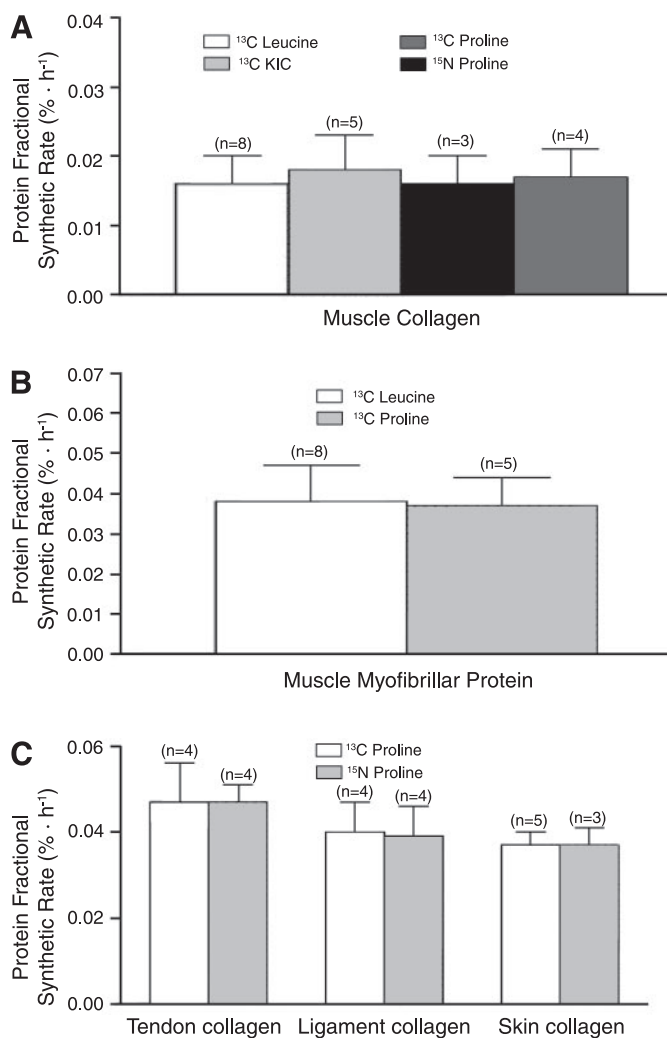


Fig. 1. Measurement of postabsorptive protein synthesis using different tracers. A: skeletal muscle collagen. B: skeletal muscle myofibrillar protein. C: tendon, ligament, and skin collagen. Data are from subjects participating in *protocols 1* and *2*.

and collagen within muscle were found to be similar (Table 2). There was a slight, but not significant (*P* = 0.08), increase in the total insoluble collagen concentration present in the elderly subjects (Table 2). Within the pepsin-insoluble collagen fraction there is also a significant (*P* < 0.05) reduction in the ratio of proline to hydroxyproline within the elderly, i.e., increased hydroxyproline, suggesting increased hydroxylation of proline.

Effect of Nutrition

Skeletal muscle collagen synthesis was unresponsive to an oral dose of 20 g of EAA in both elderly and young men [0.023 ± 0.002 and 0.016 ± 0.002 %/h, respectively (data from subjects participating in *protocol 3*); Fig. 2A]. There was no difference in basal synthetic rates of myofibrillar proteins between the young (0.032 ± 0.004 %/h) and elderly men (0.029 ± 0.005 %/h). However, 20 g of EAA caused a significant stimulation of myofibrillar protein synthesis in both the young and elderly (Fig. 2B), rising to 0.105 ± 0.009 %/h (*P* < 0.001) in the young and 0.074 ± 0.008 %/h (*P* < 0.001) in the elderly. The increase in myofibrillar protein synthesis

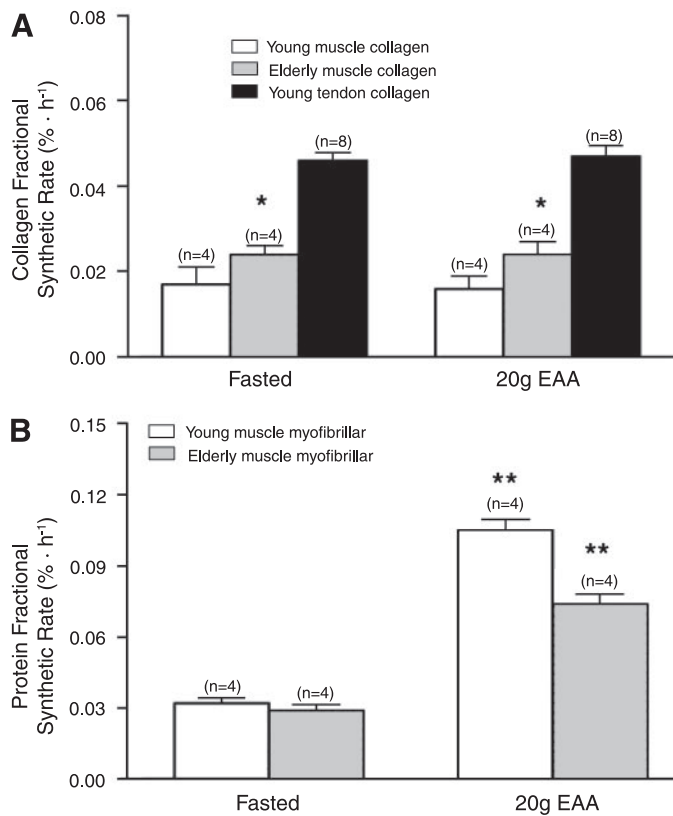


Fig. 2. Postabsorptive and fed protein synthetic rates in musculoskeletal collagen (A; * $P < 0.05$, young vs. elderly) and skeletal muscle myofibrillar protein (B; ** $P < 0.01$, fasted vs. fed). EAA, essential amino acids. Data are from subjects participating in *protocols 3 and 4*.

was lower in the elderly than in the young ($P < 0.001$). In tendon, semicontinuous oral feeding of mixed nutrients at 1.4 times the basal metabolic rate had no stimulatory effect on collagen synthesis [0.045 ± 0.008 %/h (data from subjects participating in *protocol 4*); Fig. 2a].

DISCUSSION

Our results demonstrate that musculoskeletal collagen synthesis can be measured with identical results with either [¹³C]- or [¹⁵N]proline or [¹³C]leucine, with delivery via either a flooding dose (proline) or a constant infusion (leucine). Furthermore, in the fasted state, skeletal muscle collagen synthesis is higher in healthy elderly men than in healthy young men, unlike myofibrillar protein synthesis, which is identical in both. Finally, skeletal muscle and tendon collagen synthesis is not regulated by feeding, unlike myofibrillar protein synthesis.

METHODOLOGICAL CONSIDERATIONS

In skeletal muscle, there were no differences in determined rates of collagen synthesis obtained with the three different tracers utilized. This was hardly surprising using the differently labeled variants of proline, but it was of some surprise using leucine, with [¹³C]KIC as the surrogate precursor, because previous results on skin collagen synthesis suggested that the prolyl tRNA labeling was only ~20% of the plasma proline labeling (11). However, it seems that fibroblasts like muscle may actively catabolize leucine to its keto acid (27) and that,

unlike in bone and skin cells, leucine and KIC can sufficiently equilibrate between plasma and the cytoplasm of fibroblasts so that KIC labeling is a good index of leucyl tRNA labeling. We also discovered that the application of a flooding dose of the imino acid proline has no detectable effect on the simultaneous measurement of myofibrillar protein synthesis by use of a constant infusion of leucine, which is useful information suggesting that, generally, proline flooding doses are unlikely to perturb protein metabolism. The ability to use different tracers and modes of infusion to measure collagen synthesis allows for a greater flexibility in study design and provides an ability to carry out repeated measures in the same subjects.

Postabsorptive Musculoskeletal Collagen Synthesis

Skeletal muscle collagen has a much lower fractional synthetic rate than the myofibrillar proteins. This, together with the fact that skeletal muscle collagen concentration is only 15–20% of myofibrillar protein, indicates a much lower absolute rate of synthesis of collagen than that of other muscle proteins. However, although the fractional synthesis rate of collagen in muscle is lower than that of myofibrillar protein, the rate of protein synthesis is markedly elevated within 12 h of previous exercise in both (20, 21). This emphasizes the potential of intramuscular connective tissue to adapt acutely to exercise and thereby to modify the intramuscular connective tissue structure to altered morphology of the muscle cell after, e.g., resistance training resulting in muscle cell hypertrophy.

Tendon and ligament collagen had similar synthetic rates, which were higher than those of muscle collagen or myofibrillar protein. This suggests a higher rate of remodeling in the tendon and ligament than hitherto thought, which is consonant with reports that tendon and ligament are lively structures that undergo rapid morphological adaptation to physical training (18). With regard to ligament, it is interesting that such a pronounced synthesis rate was found in tissue samples taken interoperatively from a ruptured anterior cruciate ligament (ACL), suggesting that ruptured parts of the ACL remain vital for several months after the injury. This finding should be of major surgical interest, due to the fact that cruciate ligaments have been shown to have afferent neural signaling to skeletal muscle in the thigh of humans (10). The fact that ruptured ligament maintains the ability to synthesize collagen raises the possibility that, if this tissue is used in combination with the tendon reconstruction graft when ACL repair is performed, this might not only ensure good mechanical reconstruction of the ACL but also potentially could provide the basis for maintaining some afferent neural signaling from the reconstructed ligament.

The rate of dermal collagen synthesis in the postabsorptive state was found to be larger than that of skeletal muscle

Table 2. Collagen concentration in young and elderly men

	Total Protein, $\mu\text{g}/\text{mg}$ muscle wet wt	Total Collagen, $\mu\text{g}/\text{mg}$ muscle wet wt	Pepsin-Insoluble Collagen, $\mu\text{g}/\text{mg}$ muscle wet wt	Pepsin Insoluble Collagen (Pro/OHPro ratio)
Young	134 ± 4	9.54 ± 2.0	2.26 ± 1.0	0.98 ± 0.14
Elderly	131 ± 21	9.92 ± 1.1	3.94 ± 0.7	$0.70 \pm 0.03^*$

Values are means \pm SD, $n = 4$ for each group. Data are from subjects participating in *protocol 3*. Pro, proline; OHPro, hydroxyproline. * $P < 0.05$.

collagen and of a similar magnitude to that of myofibrillar protein. The rates of dermal collagen synthesis reported here (0.037 ± 0.003 %/h) are not significantly different from those reported by El Harake et al. (0.076 ± 0.063 %/h) (11), although the precision of the estimate is greater. Given the differences in sample site, isolation, and preparation and likely collagen type composition, the fact that the two results are of the same order of magnitude suggests that our method is not subject to very large errors.

Effect of Age on Skeletal Muscle Collagen Synthesis and Concentration

In elderly men, the rate of collagen synthesis is ~50% greater than in young men. Previously, it was found that there is a faster rate of skeletal muscle collagen synthesis in middle-aged (15 mo old) rats than in young (6 mo old) rats (19), which is consistent with our results. The increase in collagen fractional synthesis rate in the elderly may be due to a subacute inflammatory condition, as we see a fourfold increase in NF- κ B protein expression (7) and increased levels of tumor necrosis factor- α (TNF- α ; 1.7-fold) and interleukin-6 (IL-6; 1.3-fold) mRNA in the muscle of the elderly compared with those of the young (Rennie MJ, Cuthbertson DJ, and Pedersen BK, unpublished data). Other researchers have shown an increased amount of transforming growth factor- β (TGF- β) and IL-6 protein in the blood of elderly subjects (12). Both IL-6 and TGF- β are potent stimulators of collagen metabolism and are regulated by TNF- α (26).

In rats, there is an increase in total collagen concentration in muscles with age (9, 16, 29). However, in our study we saw no increase in total protein or total collagen concentration per muscle wet weight in healthy elderly muscle. Because we see no change in total protein concentration in the young and elderly muscle, this suggests that there is no increased hydration of skeletal muscle in the elderly subjects. Therefore, because we detected a higher postabsorptive collagen synthetic rate without any increase in total collagen concentration in muscle of the elderly subjects, we are forced to conclude that collagen breakdown must also be elevated, as is seen with aging in rats (19).

In the muscle of the elderly subjects, we also found an increase in the amount of insoluble collagen, i.e., more mature, chemically resistant, and cross-linked collagen. In rat muscle, an increase in cross-linking with age has been reported (17, 29), and this increase has been correlated with decreased viscoelastic and plastic properties of the whole muscle (17). We also found a reduced ratio of proline to hydroxyproline in the elderly muscle insoluble collagen, which may be indicative of increased synthesis of type III collagen or type I collagen homotrimers (14) with a decrease in type I collagen heterotrimers.

Effect of Nutrition on Collagen Synthesis

Collagen synthesis in young or elderly skeletal muscle is not stimulated by ingestion of 20 g of EAA, even though this dose has been shown to maximally stimulate myofibrillar and sarcoplasmic protein synthesis in the young and significantly in the elderly (7). The lack of nutritional regulation of skeletal muscle collagen is not due to the lack of insulin response, since a similar study, in which the blood insulin was not controlled

(20) with the measurement of collagen synthesis in the fed state, provided values identical to those reported here. Tendon collagen synthesis, like skeletal muscle collagen synthesis, is not stimulated by the ingestion of a mixed meal over 4 h. Therefore, amino acids, fatty acids, and carbohydrate do not stimulate fibroblast collagen synthesis in skeletal muscle or tendon in vivo. In sharp contrast, bone collagen synthesis, after the intravenous delivery of a mixed meal over 4 h, was elevated by ~60% (2). The lack of response to feeding in skeletal muscle and tendon and the acute stimulation in bone of collagen synthesis may be indicative of the different roles that collagen plays in the tissues of the musculoskeletal system.

In summary, we have demonstrated the ability to directly measure collagen synthesis in musculoskeletal tissue and skin and have shown that physiological interventions result in teleologically believable responses in skeletal muscle and tendon collagen synthesis.

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We thank the subjects and their families for their participation. Current address of B. Miller: Department of Sport and Exercise Science, University of Auckland, Private Bag 92019, Auckland, New Zealand.

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