

## Short-term immobilization and recovery affect skeletal muscle but not collagen tissue turnover in humans

Britt Christensen,<sup>1</sup> Eva Dyrberg,<sup>1</sup> Per Aagaard,<sup>1,2</sup> Michael Kjaer,<sup>1</sup> and Henning Langberg<sup>1</sup>

<sup>1</sup>Institute of Sports Medicine Copenhagen, Bispebjerg Hospital and Faculty of Health Sciences, University of Copenhagen, Copenhagen; and <sup>2</sup>Institute of Sports Exercise and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark

Submitted 26 March 2008; accepted in final form 12 October 2008

**Christensen B, Dyrberg E, Aagaard P, Kjaer M, Langberg H.** Short-term immobilization and recovery affect skeletal muscle but not collagen tissue turnover in humans. *J Appl Physiol* 105: 1845–1851, 2008. First published October 16, 2008; doi:10.1152/jappphysiol.90445.2008.—Not much is known about the effects of immobilization and subsequent recovery on tendon connective tissue. In the present study, healthy young men had their nondominant leg immobilized for a 2-wk period, followed by a recovery period of the same length. Immobilization resulted in a mean decrease of 6% (5,413 to 5,077 mm<sup>2</sup>) in cross-sectional area (CSA) of the triceps surae muscles and a mean decrease of 9% (261 to 238 N·m) in strength of the immobilized calf muscles. Two weeks of recovery resulted in a 6% increased in CSA (to 5,367 mm<sup>2</sup>), whereas strength remained suppressed (240 N·m). No difference in Achilles tendon CSA was detected between the two legs at any time point. Local tendon collagen synthesis, measured as the peritendinous concentrations of PINP (NH<sub>2</sub>-terminal propeptide of type I collagen; indirect marker for collagen synthesis), was unchanged after 2 wk of immobilization. However, peritendinous levels of PINP were significantly elevated in the immobilized leg (15 to 139 ng/ml) following 2 wk of remobilization compared with preimmobilization levels. In contradiction hereto, systemic concentrations of PINP remained unchanged throughout the study. Immobilization reduced muscle size and strength, while tendon size and collagen turnover were unchanged. While recovery resulted in an increase in muscle size, strength was unchanged. No significant difference in tendon size could be detected between the two legs after 2 wk of recovery, although collagen synthesis was increased in the previously immobilized leg. Thus 2 wk of immobilization are sufficient to induce significant changes in muscle tissue, whereas tendon tissue seems to be more resistant to short-term immobilization.

collagen type I turnover; disuse; muscle strength; cross-sectional area

IMMOBILIZATION IS KNOWN TO result in substantial muscle atrophy, bone degeneration, joint stiffness, and functional limitations (10, 30, 31). In addition to bone and muscle, tendon tissue is also affected by immobilization, but it is still not known to what extent. It has been proposed that immobilization alters the ultrastructure in a negative direction; also the biochemical and biomechanical properties of the tendon are influenced (30). Most studies on immobilization and remobilization of tendon tissue have been performed on animals (7, 11, 19, 22, 28), and thus not much is known about the adaptation of human tendons to immobilization.

To our knowledge, only one study has investigated the effects of a shorter period of immobilization on the collagen synthesis rate in tendon in humans. This study demonstrated

that collagen synthesis in patellar tendon was decreased as a result of 10 and 21 days of immobilization measured by stable isotopes, although no changes in tendon cross-sectional area (CSA) was observed (4). From animal studies, it is known that the activity of enzymes involved in the biosynthesis of collagen is decreased in rat tendons in response to immobilization, suggesting an adaptive change in the biosynthesis of collagen in tendons in response to unloading (11). However, no significant change in the overall concentration of hydroxyproline, a measure of total collagen content, was found in relation to a short-term period of immobilization (1 wk) in these animal studies (11, 28). In contrast to these results, other studies, although on rabbits, have shown that both synthesis and degradation within the tendon are increased in response to immobilization, when measured by stable isotopes (7). Taken together, these studies indicate that tendons most likely respond to a reduction in mechanical loading by reducing the rate of collagen turnover, although no changes could be found in the overall architecture of the tendon, measured as CSA. In a study by Reeves et al. (27), a 90-day bed rest did not result in any changes in tendon CSA. This was confirmed by two recent human studies, where tendon CSA was unaltered, despite 3 wk of immobilization of the patellar tendon (4), or 7 wk of immobilization of the Achilles tendon (2).

The primary purpose of the present study was to investigate the effects of 2 wk of immobilization followed by a 2-wk recovery period on collagen metabolism of the human Achilles tendon *in vivo*. The length of the immobilization period was chosen to mimic the immobilization after a sports injury or after a period of hospitalization. The microdialysis technique was used to measure *in vivo* concentrations of indirect markers of collagen synthesis related to the Achilles tendon. The microdialysis technique was chosen over the biopsy technique, since the effects of taking repeated biopsies from healthy tendon are presently unknown and thereby preclude the possibilities for direct measurements of tendon hydroxyproline (marker for total collagen content) cross-links in the present study. The concentration of PINP (NH<sub>2</sub>-terminal propeptide of type I collagen) was used as an indirect marker of collagen synthesis. PINP is the NH<sub>2</sub>-terminal propeptide that is removed by enzymatic cleavage from the procollagen molecule, an absolute prerequisite for collagen fiber formation (21). The removal of these propeptides does not, however, ensure that functionally collagen fibrils are formed, but it does tell us something about the rate of collagen synthesis. Concentrations

Address for reprint requests and other correspondence: H. Langberg, Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark (e-mail: hl02@bbh.regionh.dk).

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were measured both in serum and dialysate, representing the systemic (whole body) and the local peritendinous levels, respectively. The amount of PINP in serum is derived from whole body collagen type I synthesis, i.e., in tendon, muscle, skin, and bone. The systemic levels of collagen degradation were assessed by measurements of CTx (COOH-terminal telopeptide of type I collagen) in the urine and thus provides a measure for total degradation of collagen type I in tendon, muscle, skin, and bone tissue. CTx is a telopeptide cleaved of during degradation off the collagen molecule (6) and can thus be used as an indirect marker of collagen degradation. Collagen molecules are degraded through several pathways, and the measured peptides only give us an estimate of the rate of collagen degradation. Finally, clinical parameters, such as muscle strength and CSA of both the muscle and tendon, were measured as secondary effect variables to demonstrate and monitor the unloading and reloading of the tendon tissue. It was hypothesized that collagen synthesis would decrease with immobilization and increase in response to recovery. The opposite was expected for collagen degradation, and we hypothesized that overall tendon size would remain unchanged.

## METHODS

**Subjects.** Twelve healthy young men ( $24 \pm 0.86$  yr,  $185 \pm 2$  cm,  $86 \pm 4$  kg, body mass index  $25 \pm 0.7$  kg/m<sup>2</sup>, means  $\pm$  SE) were included in the present study, evaluating the effects of 2 wk of immobilization with plaster of Paris from below the knee to the toes (Table 1). The ankle joint was immobilized in a 90° angle, leaving the tendon in a neutral position. The subjects were all moderately trained ( $4 \pm 0.6$  h/wk) and did not suffer from any present or previous tendon symptoms or injuries in the lower extremity. All subjects gave, after receiving oral and written information, written, informed consent to participate in the study, in adherence to the declaration of Helsinki. The study was approved by the local Human Subject Ethics Committee of Copenhagen and Frederiksberg [(KF) 11 2006-1743].

**Study design.** Baseline measurements were performed 1 wk before applying the plaster of Paris, to minimize the risk of infections from the invasive methods used in the present protocol. The nondominant leg was always the one immobilized. After 2 wk of immobilization, the plaster of Paris was checked for evidence of weight-bearing (any visible damage or marks after load bearing), the plaster was removed, and the subjects tested again. Two weeks following the removal of the plaster, with only normal daily activities allowed, the test procedure was repeated. Both legs were tested on all experimental days.

**Strength test.** The strength test was performed as previously described (18). Subjects were seated in a rigid steel frame with one knee fully extended and the hip flexed at 90°. The foot rested in a neutral position (90°) against a steel plate. The position of the heel was adjusted so that the mechanical axis of rotation corresponded to the lateral malleolus. The back of the seat was adjusted to prevent displacement during the test. Plantar flexion force was measured with a strain gauge load cell attached between the footplate and the steel frame. Force signals were sampled at 50 Hz by an analog-to-digital

converter, and the data were stored on a computer for subsequent analysis. The control leg was at all time points tested first. Subjects performed five forceful plantar flexion efforts that served as warm-up. Thereafter, three force ramp contractions were performed and two maximum isometric plantar flexions. Two different types of contractions were used, since some people reach their maximum force best during a ramp contraction, while others do so during a maximum effort. The contractions lasted for ~5 s with a 1-min rest in between. Data were analyzed for maximum isometric plantar flexion force [maximum voluntary contraction (MVC)], and the best out of the five efforts was used.

**Measurements of CSA (muscle and tendon).** CSA of both triceps surae muscle and the Achilles tendon were determined by MRI [General Electric Sigma Horizon LX, 1.5 T, protocol: echo time 16 ms, repetition time 305 ms, echo tog length 4 (number of echoes per repetition time interval), bandwidth 15.63 MHz, field of view 30 cm, slice thickness 6 mm, frequency 384 pixels, phase 256 pixels, nex 4, and frequency direction right/left] for both legs at all time points. Calf muscle CSA was measured 10 cm distally to the top of caput fibula (corresponding to the largest CSA), and Achilles tendon CSA was measured at the level of the distal part of the medial malleolus with the ankle joint at 90° (corresponding to the area where the tendon is most prone to rupture).

All scans were blinded, and the measurements of CSA were performed in randomized order by the use of the software Web1000. All scans were measured twice on 2 separate days, and the average was used as the CSA. Because of difficulties in separation of the different muscles in the calf (resolution of the pictures were not high enough) and to minimize the measurements error, CSA of the calf muscles were measured by drawing a straight line between the dorsal part of tibia and the dorsal part of fibula and then follow the edge of the soleus and gastrocnemius muscles, leading to a small but systematic overestimation of the total CSA of triceps surae muscle. The mean coefficient of variation for repeated measures was 0.9% for muscle CSA and 6.5% for tendon CSA.

**Microdialysis.** Microdialysis was used to determine local collagen turnover and performed in principle, as described previously (15). Under local anesthesia (Lidocaine, 10 mg/ml), the microdialysis catheter was placed ventral to the Achilles tendon in the peritendinous space in both legs, as close to the tendon as possible. The active part of the catheter covered the area from 3 to 6 cm proximal to the Achilles tendon insertion on the calcaneus bone. The catheter was perfused with a Ringer-acetate solution containing radioactive labeled D-[3-<sup>3</sup>H]glucose (specific activity 250  $\mu$ Ci, Perkin Elmer) at a rate of 2  $\mu$ l/min by a high-precision syringe pump (CMA 100). The interstitial peritendinous concentrations of PINP was calculated using the internal reference calibration method (29), as previously described (15). Radioactive labeled glucose was used, since no labeled PINP was commercially available. To evaluate the use of glucose as reference for PINP, an in vitro experiment was performed (data not shown), showing that calculated concentrations of PINP would be slightly underestimated when using labeled glucose as the reference substance. Dialysate was collected for a total of 5 h, one sample every hour, weighed to verify the absence of ultrafiltration, and immediately frozen at -80°C until analysis was performed. The first sample was discharged to minimize the risk of an effect of the insertion procedure.

The microdialysis catheters were custom made in the laboratory, as previously described (14). The catheters were sterilized using ethylene oxide before usage.

**Blood and urine sampling.** The sampled blood was used for measurements of systemic levels of PINP. All blood samples were taken in the morning when the subjects arrived fasting (since midnight). Blood was collected using a butterfly (Terumo, cannula 0.8  $\times$  19 mm, 18 cm) from the antecubital vein and left on ice for 10–15 min before they were centrifuged for 10 min with 3,800 rpm at 4°C and stored at -80°C for subsequent analysis.

Table 1. Anthropometric data

Number of Subjects	12 (males)
Age range, yr	19–30
Mean age, yr	24 $\pm$ 0.86
Mean height, m	1.85 $\pm$ 0.02
Mean weight, kg	86 $\pm$ 3.6
Mean body mass index, kg/m <sup>2</sup>	25 $\pm$ 0.75

Values are means  $\pm$  SE.

Urine was collected after an overnight fast and immediately frozen at  $-80^{\circ}\text{C}$  until analysis of urinary concentrations of CTx and creatinine. Concentrations of creatinine were measured to evaluate the filtration rate.

**Measurements of collagen turnover.** Samples were analyzed for PINP using a sandwich ELISA, as previously described (24). Serum samples were diluted 1:50 before analysis, and dialysate was diluted 1:8. Samples from the same subject were analyzed in the same assay. The detection level was 41 pg/ml, and the intra-assay variation (coefficient of variation) was 4.9% at 4.2 ng/ml (24).

Concentrations of CTx were measured by the Urine Crosslaps ELISA assay from Nordic bioscience. The urine samples were diluted 1:2 before analysis, and the results were corrected for creatinine in the urine sample. The detection level was 50  $\mu\text{g/l}$ , according to the manufacture and the intra-assay variation (coefficient of variation) was 4.7% at 480  $\mu\text{g/l}$ .

**Statistics.** In all statistical analysis was the level of significance set to  $P < 0.05$ . All results are expressed as means  $\pm$  SE. To test for differences in serum and urine, concentrations of PINP, CTx, and creatinine, a one-way ANOVA followed by Tukey's multiple comparison tests was used. Changes in Achilles tendon CSA were only analyzed in between legs on the separate experimental days because of methodological problems (for further explanations see RESULTS section). A Student's paired *t*-test was used for these comparisons. A two-way ANOVA was used to analyze for differences in muscle CSA, MVC, and peritendinous concentrations of PINP over time and between legs. Data on PINP was log transformed before the ANOVA was performed to pass the normality test. The two-way ANOVA was followed by a Tukey's post hoc test. Graf Pad Prism and Sigma STAT were used for statistical analysis.

## RESULTS

**Immobilization.** In none of the subjects could any indications of loading of the immobilized leg be detected.

**Muscle CSA.** The two legs were similar, with no statistical significant difference in CSA of triceps surae muscle at baseline before the immobilization period (Fig. 1A). The CSA of the calf muscle on the immobilized leg was significantly decreased after 2 wk of immobilization by 6% (from  $5,413 \pm 262$  to  $5,077 \pm 238$   $\text{mm}^2$ ,  $P = 0.013$ ). Comparing the two legs after the immobilization period showed that the CSA of the immobilized leg was significantly smaller than that of the control leg ( $P = 0.012$ ). CSA of the immobilized leg increased significantly by 6% in response to 2 wk of recovery ( $5,367 \pm 235$   $\text{mm}^2$ ,  $P = 0.033$ ) (Fig. 1A) and reached baseline measurements. There were no change in calf muscle CSA of the control leg over the measured time period ( $5,431 \pm 228$ ,  $5,470 \pm 258$ , and  $5,523 \pm 248$   $\text{mm}^2$ ,  $P > 0.05$ ).

**Muscle strength.** No significant difference in muscle strength could be detected between the two legs before the immobilization period ( $P > 0.05$ ), and the strength of the control leg was unchanged throughout the study ( $259.8 \pm 16.8$ ,  $263.7 \pm 19.3$ , and  $270.1 \pm 18.1$   $\text{N}\cdot\text{m}$ ,  $P > 0.05$ ). Maximum isometric strength (MVC) of the calf muscles of the immobilized leg decreased significantly after 2 wk of immobilization compared with baseline measurements, with a 9% loss in muscle strength (from  $260.8 \pm 15.9$  to  $238.1 \pm 17.7$   $\text{N}\cdot\text{m}$ ,  $P = 0.043$ ). This resulted in a significant difference in muscle strength between the two legs after the immobilization period ( $263.7 \pm 19.3$  and  $238.1 \pm 17.7$   $\text{N}\cdot\text{m}$ ,  $P = 0.023$ ). Calf muscle strength of the immobilized leg did not change statistically in relation to remobilization ( $239.7 \pm 18.4$   $\text{N}\cdot\text{m}$ ), resulting in the maintenance of a significant difference in strength between the two

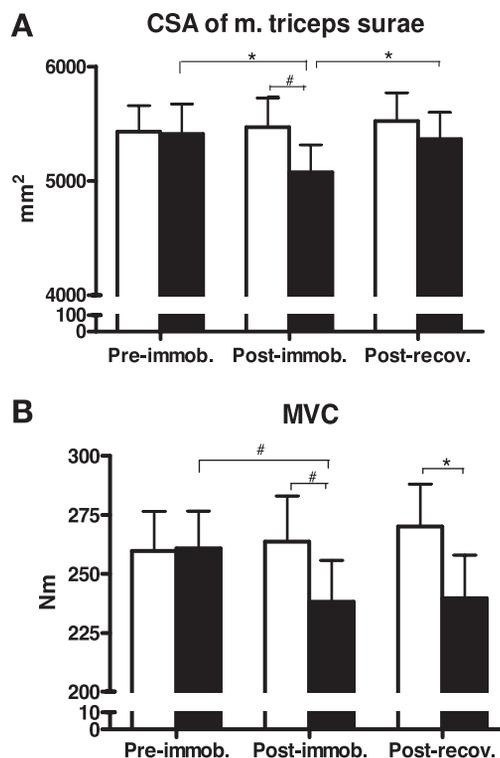


Fig. 1. Muscle cross-sectional area (CSA) and strength. Measurements are shown before and after immobilization and after recovery of CSA of triceps surae muscle ( $n = 12$ ; A) and isometric maximum voluntary contraction (MVC) of the calf muscles ( $n = 12$ ; B). Open bars, control leg; solid bars, immobilized leg. Values are means  $\pm$  SE. \* $P < 0.01$  and # $P < 0.05$ .

legs, despite the reloading of the immobilized leg ( $P = 0.009$ ) (Fig. 1B).

**Achilles tendon CSA.** The tendon CSA was identical in the two legs at baseline ( $P > 0.05$ ). Only pairwise comparison of tendon CSA was done due to technical difficulties in placing the axial scans on exactly the same place on the Achilles tendon at all experimental days. At no point could any statistically significant difference in tendon CSA between the control leg and the immobilized leg be detected ( $P > 0.05$ ) [difference between control leg and immobilized leg (95% confidence interval); before immobilization  $-1.25$  ( $-12.8$ – $10.4$ )  $\text{mm}^2$ , after immobilization  $15.48$  ( $-10.5$ – $41.5$ )  $\text{mm}^2$ , and after recovery  $-2.21$  ( $-20.4$ – $15.9$ )  $\text{mm}^2$ ] (Fig. 2).

**Collagen metabolism.** Serum levels of PINP did not change significantly after immobilization or in response to remobilization ( $117.9 \pm 13.6$ ,  $116.9 \pm 19.8$ , and  $117.7 \pm 16.5$   $\text{ng/ml}$ ,  $P > 0.05$ ) (Fig. 3A). There were no significant differences between the two legs with regards to the peritendinous concentration of PINP before the immobilization period ( $P > 0.05$ ). A significant increase in PINP was found after the immobilization period in the control leg compared with pre-immobilization conditions ( $14.2 \pm 2.5$  and  $55.7 \pm 18.5$   $\text{ng/ml}$ ,  $P = 0.049$ ). However, an increase in PINP was seen in relation to recovery; the increase in the immobilized leg was significantly different from baseline ( $15.2 \pm 4.4$  and  $138.6 \pm 45$   $\text{ng/ml}$ ,  $P < 0.001$ ) and following the immobilization period ( $61.6 \pm 27.0$  and  $138.6 \pm 45$   $\text{ng/ml}$ ,  $P = 0.006$ ) (Fig. 3B). The same was the case for the difference between the legs after the recovery period ( $138.6 \pm 45$  and  $59.6 \pm 21$   $\text{ng/ml}$ ,  $P = 0.014$ ).

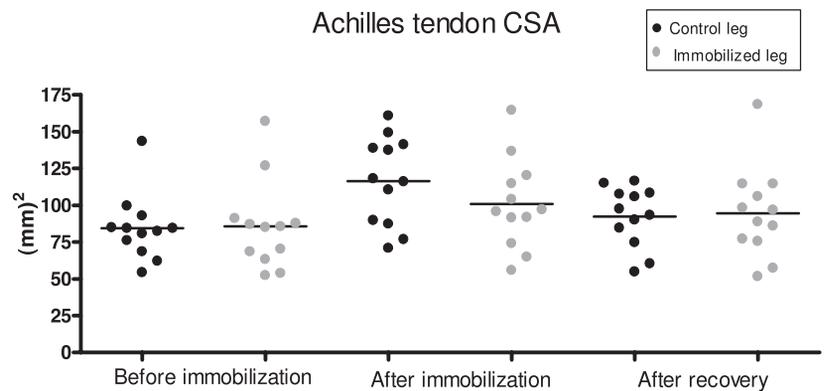


Fig. 2. Tendon CSA. CSA is shown of the Achilles tendon before and after immobilization and after recovery ( $n = 12$ ). Solid circles, control leg; shaded circles, immobilized leg.

No significant change in the concentration of urine CTx, corrected for creatinine, was found ( $224.9 \pm 57.7$ ,  $238.1 \pm 20.6$ , and  $315.6 \pm 67.3 \mu\text{g}/\text{mmol}$ ,  $P > 0.05$ ) (Fig. 3C). The creatinine levels did not change significantly throughout the experiment ( $P > 0.05$ ) (Table 2).

## DISCUSSION

The main findings of the present study are that Achilles tendon CSA and indirect markers of collagen synthesis are not changed as a result of a 2-wk period of immobilization. These findings support the notion that tendons are protected against loss of strength due to sudden unloading during periods of immobilization. Previous studies have shown that tendon tissue adapts to altered levels of physical activity by increasing the level of collagen synthesis (9, 13, 15, 20), but only two studies, apart from the present study, have analyzed the influence of unloading on collagen turnover in human tendon tissue so far (2, 4). In addition, only the present study has studied the effect of remobilization on collagen turnover after short-term immobilization.

**Muscle CSA and strength.** A significant decrease in both muscle CSA and strength following immobilization was found in the present study (Fig. 1, A and B, respectively), in line with data from earlier studies using short-term immobilization (8, 16, 26, 31–33). Muscle CSA increased back to baseline after the 2-wk remobilization period, whereas MVC remained decreased. These results support earlier studies underlining that the time period needed for full recovery of muscle strength is often longer than the length of the previous immobilization period (26, 31–33). Thus ordinary daily activities seem to be insufficient for restoring muscle strength after a 2-wk period of immobilization. The clinical implication of these data is that systematic strength training should always be part of the rehabilitation regime, even after a shorter period of immobilization.

**Tendon CSA.** The immobilization and thus unloading of the tendon tissue did not result in changes in the CSA of the tendons measured by MRI scans. Due to problems with the placement of the axial projections of the Achilles tendon, it was decided to use and compare only the control leg and the immobilized leg on each experimental day separately, instead of making comparisons over time. No significant difference in the CSA of the Achilles tendon was found between the two legs on any of the experimental days (Fig. 2).

The lack of change in tendon CSA over time in response to unloading found in the present study is in accordance with

previous studies. Reeves et al. did not find any change in tendon CSA after 90 days of bed rest (27), and recently de Boer et al. confirmed the data showing no change in patellar tendon CSA during 10 and 21 days of lower leg suspension (4). Likewise, we recently found that tendon CSA did not change in relation to a 7-wk period of immobilization (2). In a study on rabbits by Matsumoto et al. using 4 and 8 wk of immobilization, no significant change in CSA was seen after either 4 or 8 wk of immobilization or in the control group (19), a finding that has recently been confirmed (34). However, the effects of unloading of tendons may be more complicated than the results above indicate. A recent study by Eliasson et al. showed that tendon CSA increased to the same extent in both the unloaded (paralyzed by botulinum toxin) and control leg in growing rats, suggesting that hormonal growth stimulation has a somewhat greater impact on tendon size than the mechanical loading itself in immature tendons (5).

In addition, the joint angle during immobilization and thus the strain on the tendon seem to be of great importance. In the above-mentioned studies, all tendons were immobilized in a neutral position. In contrast, studies have shown that, if tendons are immobilized in a slack position (shortened), the concentrations of both prolyl-4-hydroxylase (PH) and galactosylhydroxylase-lysylglucosyltransferase (GGT) decrease significantly, indicating a decreased collagen synthesis. However, if stretch is applied to the tendon during immobilization (lengthened position), the tendon seems more resistant to atrophy, with PH and GGT levels comparable to control tendons. This may reflect a length-associated adaptive response, where the tendon reacts to a chronically lengthened position by elongation (11, 28). Measurement of CSA does not, however, detect changes in microarchitecture and collagen turnover. In accordance with this, it has been shown that both 4 and 8 wk of immobilization result in a significant decrease in tendon stiffness, without any changes in tendon CSA in rabbits (19). In conclusion, a review of the literature supports that a decrease in tendon CSA is only found when chronic and total unloading of the tendon is applied to the tendon, as during paralysis in humans (17).

**Immobilization and tendon collagen turnover.** In accordance with the data on CSA of the tendons, no change was found in local peritendinous concentrations of PINP, an indirect marker for collagen synthesis, in relation to immobilization (Fig. 3B). Concentrations of PINP are unchanged after 2 wk of immobilization in both serum and dialysate (Fig. 3, A and B). A significant increase in PINP in the peritendinous tissue was

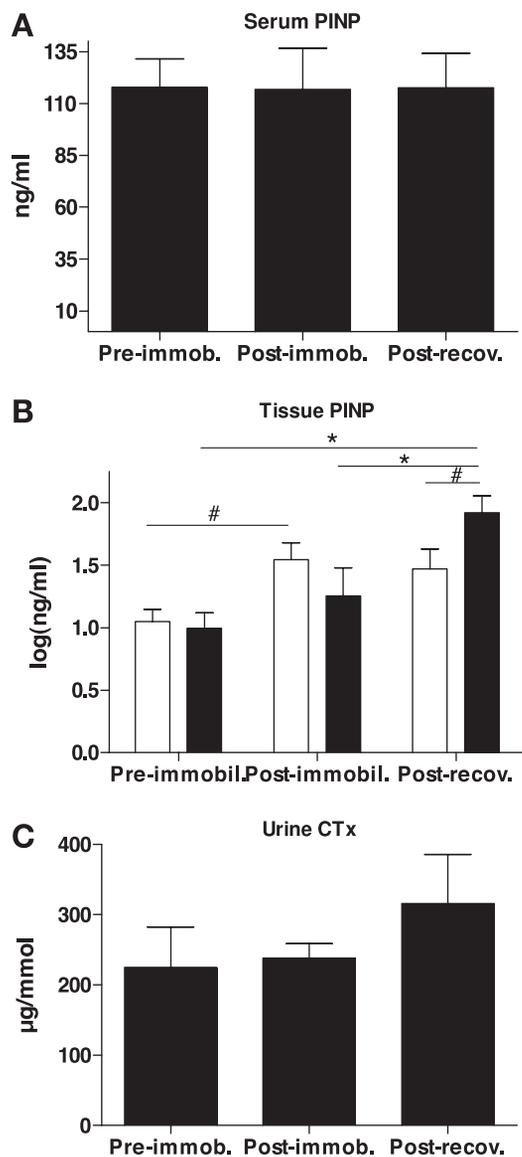


Fig. 3. Collagen turnover. Measurements are of markers of collagen metabolism before and after 2 wk of immobilization and after further 2 wk of rehabilitation. *A*: serum concentrations of PINP (NH<sub>2</sub>-terminal propeptide of type I collagen;  $n = 12$ ). *B*: peritendinous concentrations of PINP ( $n = 12$ ). *C*: urinary concentrations of CTx (COOH-terminal telopeptide of type I collagen;  $n = 12$ ). Open bars, control leg; solid bars, immobilized leg. Values are means  $\pm$  SE. \* $P < 0.01$  and # $P < 0.05$ .

found in the control leg in relation to the immobilization period. This increase could reflect an increased load applied to the control leg during the immobilization period, where the patients were instructed not to apply any load to the immobilized leg.

Along the same line, the indirect degradation marker ICTP (COOH-terminal telopeptide region of type I collagen) was measured in the dialysate from the peritendinous tissue around the Achilles tendon. The sensitivity of the commercially available assay was, however, not high enough to detect any changes in ICTP throughout the study. Almost all measurements were above the detection limit (0.3  $\mu\text{g/l}$ ) but below the range for measurements (1–50  $\mu\text{g/l}$ ), with no systemic difference. This does not, however, preclude that minor changes,

undetected by the present method, could occur. The systemic concentrations of CTx, corrected for creatinine levels, were found unchanged by immobilization (Fig. 3C). It has previously been shown that creatinine levels change in relation to immobilization (25), as a result of increased muscle breakdown; however, this was not the case in the present study. Correction of creatinine levels in the present study did not change the results. One explanation could be that the intervention was not drastic or long enough to induce changes in creatinine levels.

The overall conclusion from the present study seems to be that tendons are resistant to decreased load during a shorter period of immobilization. This may be beneficial for tendon tissue to preserve the tissue during reduced loading, as collagen synthesis is slower to adapt to training than muscle. This mechanism thus prevents the tendons from being overloaded during the reloading period when muscle power returns quickly. Supporting this hypothesis, Matsumoto et al. found that 8 wk of immobilization did not induce tendon atrophy and that immobilization did not constitute a risk factor for midsubstance Achilles tendon rupture in rabbits (19).

Almost all previous studies on immobilization and collagen turnover have been performed in animals. However, a recent study by de Boer et al. (4), the effects of 10 and 21 days of lower leg suspension on patellar tendon collagen synthesis in humans, was investigated. They found that tendon fractional synthesis rate decreased during the immobilization period (4). They did not, however, measure collagen degradation rate, which makes it difficult to determine total collagen turnover. In the present study, no change in markers of collagen synthesis was detected after 2 wk of immobilization. One explanation for these discrepancies could be the different measurement techniques.

Previous animal studies have measured the level of enzymes (PH and GGT) involved in the synthesis of collagen in tendons in relation to immobilization (1–3 wk) in rats, showing a decrease in the concentration of these enzymes, but only when the Achilles tendon is immobilized in a shortened position. Immobilized in the lengthened position, the concentrations were unaltered (12, 28). However, in two other studies by Amiel and colleagues, a small but significant increase in collagen synthesis was found on the effect of immobilization (9 and 12 wk) on patella tendon in rabbits (1, 7). In fact, both the synthesis and degradation of type I collagen were increased, but it was not until after 12 wk of immobilization that the increased degradation rate surpassed the increase in collagen synthesis, resulting in a net loss of collagen tissue (7). The previous animal studies fit well with the results found in the present study, where only longer periods of immobilization in a shortened position resulted in significant changes in collagen turnover.

Table 2. Creatinine levels

	Creatinine, mmol/l
Preimmobilization	16.6 $\pm$ 1.9
Postimmobilization	20.0 $\pm$ 2.1
Postrecovery	14.5 $\pm$ 1.5

Urine concentrations of creatinine before and after immobilization and after the recovery period. Values are means  $\pm$  SE;  $n = 12$  subjects.

**Recovery and tendon collagen turnover.** An increase in PINP was found in both legs during the recovery period, but only the increase in the immobilized leg was significant relative to baseline (preimmobilization) (Fig. 3B). The increase most likely reflects the effect of reloading after a period of inactivity, with an increased synthesis rate as the response. However, the effect seems to be local, since the systemic levels of PINP did not change. This is supported by previous studies showing that both an acute bout of exercise (9, 15, 23) and longer periods of training result in increased levels of PINP in the peritendinous space in humans (13). Likewise, it has been found that the rate of new collagen deposition, measured as labeled hydroxyproline-specific activity, increased in trained chickens compared with controls. In that study, no changes were found in either tendon dry weight or total hydroxyproline concentration (3), which correlates well with the present study, where no changes in tendon CSA were found, although the concentrations of PINP increased.

Systemic concentrations of CTx did not change during the recovery period (Fig. 3C). All together, these data imply that a recovery period is accompanied by a local increase in indirect markers for collagen synthesis in the tissue around the tendon, probably reflecting the increased load applied to the tendon tissue.

**Limitations.** There are some limitations associated with the microdialysis technique, but there is no better technique available at the moment when several sequential observations are required. Tendon biopsies, using stable isotopes, would be an alternative, which would ensure measurements from within the tendon, but this technique is, at present, limited in number and the size of the biopsies that can be taken from each subject, and there is an ethical problem with taking repeated tendon biopsies from healthy tendons. We have tried to correct for the influence of the insertion of the microdialysis catheter by performing the same measurements on the control leg and expressing results as the difference between the control and the immobilized leg, thereby overcoming the effect of the repeated insertion. It seems, from the present study, that the levels of PINP increased with repeated measurements, which probably reflects an increased collagen synthesis due to the microtrauma after the insertion of the microdialysis fiber; this is supported by a recent study (23). The present study design with a control leg overcomes this problem. The relative recovery was also measured to correct for variations in diffusion properties. The weight of each sample was measured to ensure that the setup function was optimal and that no ultrafiltration occurred.

**Conclusion.** From the present study, it can be concluded that a shorter period of immobilization results in a decrease in muscle CSA and strength, but that a 2-wk unload of tendon tissue is not enough to induce changes in indirect markers for collagen metabolism, as well as no changes in CSA of the Achilles tendon could be detected.

The subsequent recovery period after the immobilization period resulted in an increased muscle CSA, an unchanged tendon CSA, but a significant increase in indirect markers of collagen synthesis in the peritendinous tissue. The muscle strength was found not to return to basal levels during the 2 wk of remobilization. It should be stressed that ordinary daily activities may not be sufficient to restore muscle strength after a 2-wk period of immobilization, and that strength training

should be a recommended part of the rehabilitation, even after a shorter period of immobilization.

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