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Katja Heinemeier, Henning Langberg, Jens L. Olesen, and Michael Kjaer
Sports Medicine Research Unit, Department of Rheumatology H, Bispebjerg Hospital, DK-2400 Copenhagen, Denmark

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Heinemeier, Katja, Henning Langberg, Jens L. Olesen, and Michael Kjaer. Role of TGF-β_1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *J Appl Physiol* 95: 2390–2397, 2003. First published August 15, 2003; 10.1152/japplphysiol.00403.2003.—Mechanical loading of tissue is known to influence local collagen synthesis, and microdialysis studies indicate that mechanical loading of human tendon during exercise elevates tendinous type I collagen production. Transforming growth factor-β_1 (TGF-β_1), a potent stimulator of type I collagen synthesis, is released from cultured tendon fibroblasts in response to mechanical loading. Thus TGF-β_1 could link mechanical loading and collagen synthesis in tendon tissue in vivo. Tissue levels of TGF-β_1 and type I collagen metabolism markers [procollagen I COOH-terminal propeptide (PICP) and COOH-terminal telopeptide of type I collagen (ICTP)] were measured by microdialysis in peritendinous tissue of the Achilles' tendon in six male volunteers before and after treadmill running (1 h, 12 km/h, 3% uphill). In addition, blood levels of TGF-β_1, PICP, and ICTP were obtained. PICP levels increased 68 h after exercise ($P < 0.05$). Dialysate levels of TGF-β_1 changed from 303 ± 46 pg/ml (at rest) to 423 ± 86 pg/ml 3 h postexercise. This change was nonsignificant, but the decay of tissue TGF-β_1 after catheter insertion was markedly delayed by exercise compared with the decay seen in resting subjects. Plasma concentrations of TGF-β_1 rose 30% in response to exercise ($P < 0.05$ vs. pre). Our observations indicate an increased local production of type I collagen in human peritendinous tissue in response to uphill running. Although not conclusive, changes in circulating and local TGF-β_1, in response to exercise, suggest a role for TGF-β_1 in mechanical regulation of local collagen type I synthesis in tendon-related connective tissue in vivo.

extracellular matrix; tendon; mechanical loading; transforming growth factor-β_1

FIBROUS TYPE I COLLAGEN IS an important tensile strength-carrying part of the extracellular matrix (5), allowing for transmission of force generated by, e.g., skeletal muscle into movement (22). One mechanism known to regulate collagen homeostasis is the mechanical loading of tissue (7), and collagen expression and/or production has been shown to rise in several different cell types and tissues, including renal (53), cardiac (6, 49), vascular (38), pulmonary (3, 47), bone (19), ligament (24), and tendinous (36) cells and/or tissue, in response to strain. These findings are almost entirely based on in vitro studies, and only recently it has been indicated, by microdialysis studies, that the type I collagen accumulation increases in the peritendinous tissue of human Achilles tendon in the days after acute exercise involving mechanical loading of the tendon (29). The regulation of this increased collagen production is only partly understood. Regulatory hormones and growth factors are, together with integrins, cytoskeletal proteins, and certain ion channels, thought to be responsible for mechanically induced cell signaling (7). Studies have indicated that mechanical loading increases the expression of several growth factors and cytokines, such as IGF-I (39), IL-6 (8, 26), and transforming growth factor-β_1 (TGF-β_1) (18). With regards to TGF-β_1, in vitro experiments with human and animal cell cultures as well as in vivo animal studies have shown an increased expression of TGF-β_1 in response to mechanical stimuli in a number of cell and tissue types (8, 9, 18, 38, 40, 42, 45, 49). Furthermore, it has been found that mechanically induced type I collagen expression in ligament fibroblasts, cardiac fibroblasts, and human intestinal smooth muscle cells is directly dependent on TGF-β_1 activity (18, 24, 33). Thus it is possible that TGF-β_1 is involved in the regulation of type I collagen synthesis in human tendon tissue in a similar manner and that the increase in local type I collagen production found in human tendinous tissue in response to exercise (27, 29) is based, at least in part, on a local increase in the concentration of TGF-β_1 caused by mechanical stimuli of tendon fibroblasts. To investigate this hypothesis in humans, it was studied whether exercise that induced mechanical loading of the Achilles tendon resulted in any increase in systemic and local tissue TGF-β_1 concentrations along with an increase in local type I collagen production, as determined by microdialysis, in the peritendinous area of the human Achilles tendon.

MATERIALS AND METHODS

Microdialysis for Determination of Tissue TGF-β_1 and Markers of Type I Collagen Turnover

Microdialysis catheters were constructed of an 80-cm inlet of polyethylene tubing (0.76 mm ID), connected to 30 mm of permeable membrane [hollow plasma separator tubes

Address for reprint requests and other correspondence: K. Heinemeier, Sports Medicine Research Unit, Bispebjerg hospital-Bldg. 8, 1st floor, 23 Bispebjerg Bakke, DK-2400 Copenhagen NV, Denmark (E-mail: kh08@bbh.hosp.dk).

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(Asahi, Japan); diameter 4 mm; molecular mass cutoff 3,000 kDa], by 7.5 mm of nylon tubing (0.50 mm ID, 0.63 mm OD). Identical nylon tubing was used for the outlet (8 cm). A stainless steel wire was placed inside the catheter to increase mechanical stability. Before use the catheters were sterilized by use of ethylene oxide (ETO-sterilization; ABATOX, Herlev, DK & Mærsk Medical a/s, Lynge, Denmark). During each microdialysis run, two microdialysis catheters were placed diagonally, with an angle of 45° to the tendon and just ventral to both left and right Achilles tendon under ultrasound guidance, leaving the outlet of catheter at the medial side of the tendon. The catheters were perfused at a rate of 5 μl/min, by use of a high-precision syringe pump (CMA/100, Carnegie, Solna, Sweden), with Ringer-acetate containing ^{3}H-labeled human type IV collagen (130 kDa; NEN, Boston, MA) with a perfusate activity of 5.6 kBq/ml. The in vivo relative recovery for TGF-β_1 was not measured, because no radioactive TGF-β for human use was commercially available. Therefore the microdialysis results on TGF-β are presented as dialysate concentrations. As for the measurement of type I collagen turnover, levels of procollagen I COOH-terminal propeptide (PICP) and COOH-terminal telopeptide of type I collagen (ICTP) were measured in the dialysate. The level of PICP is correlated to type I collagen production, as one PICP is cleaved from every newly secreted type I collagen triple helix, whereas ICTP is a degradation product of type I collagen breakdown. The relative loss of ^{3}H-labeled human type IV collagen was used to estimate the in vivo relative recovery of PICP and ICTP according to the internal reference method (44), and, on the basis of the relative recoveries, the tissue level of PICP and ICTP was estimated as described previously in detail by Langberg et al. (29).

Control Experiment

To investigate possible fluctuations in basal tissue levels of TGF-β_1 during the microdialysis experimental procedure (described above), separate control experiments were carried out. Dialysate fluid was sampled from probes placed along the Achilles tendon for a period of 3.5 h at rest in six healthy, moderately trained male volunteers, with a mean age of 25 yr (range 23–27 yr) and a mean body mass of 81 kg (range 72–86 kg). All subjects gave informed consent, and the study was approved by the Ethical Committee of Copenhagen (KF 11-088/01). Subjects fasted from 10 PM on the day preceding the experiment and were told not to engage in any type of exercise or training for 48 h before the study. To rule out any influence of dietary collagen on circulating and local levels of type I collagen metabolism markers, the participants were given a list of gelatin-rich foods and informed not to consume any of these for 4 days leading up to the experiment and during the experiment. The experiment lasted for a total time of 3.5 h after insertion of the microdialysis fibers, and dialysate was sampled at 30-min intervals for the first 1.5 h and at 1-h intervals for the last 2 h. Samples were weighed to eliminate the possibility of ultrafiltration, and afterward they were immediately frozen at −80°C until analysis.

Exercise Experiment

Six healthy, moderately trained male volunteers with a mean age of 26 yr (range 25–28 yr) and a mean body mass of 77 kg (range 70–80 kg) were included in the study. All subjects gave informed consent, and the study was approved by the Ethical Committee of Copenhagen [KF 11-088/01].

Experiments were started at 8 AM on all days. Subjects fasted from 10 PM on days preceding experiment days and were told not to engage in any type of exercise or training for 48 h before the first day of the study and for the entire length of the experiment. Dietary intake of gelatin was restricted as in the control experiment. The first day of the experiment consisted of a 3-h preexercise rest period, a 1-h exercise period, and a postexercise recovery period of 6 h. Additionally, microdialysis was sampled for 4-h periods at 20 and 68 h after exercise (Fig. 1). The exercise intervention consisted of a 10-min warm-up treadmill run at 10 km/h, followed by 1 h of 3% uphill treadmill running at 12 km/h. No direct determination of the individuals maximal oxygen uptake was made, but considering that all individuals were moderately trained and young and that they were all completely exhausted after 1 h indicates a relative work intensity of 70–80% of maximal oxygen uptake (1). Microdialysis was performed throughout the rest periods, during which subjects were placed in a supine position at room temperature. Microdialysis catheters were always removed before running, and afterward new catheters were inserted. During microdialysis, dialysate samples were collected at 30-min intervals for the first hour and at 60-min intervals for the remaining periods. Samples were weighed to eliminate the possibility of ultrafiltration, and afterward they were immediately frozen at −80°C until analysis. To minimize the influence of the insertion trauma on measured concentrations of TGF-β, only samples collected after the first 2 h of microdialysis were used for analysis in the exercise experiment.

Blood Samples

On the basis of preliminary experiments (results not shown) and on recommendations given by Wakefield et al. (50), a reliable method for sampling and handling of blood for TGF-β determination was established. Blood samples were obtained before exercise, immediately after, and 6, 20, and 68 h after exercise (Fig. 1) by use of a wide-gauge butterfly needle (21-G) and removing the tourniquet immediately after needle insertion. Samples were collected in tubes containing 70 μl K_3EDTA, immediately cooled on ice for 30 min, and then centrifuged at 3,000 *g* for 15 min, whereafter the supernatant was collected and frozen at −80°C until analysis. Blood used for determination of type I collagen metabolism

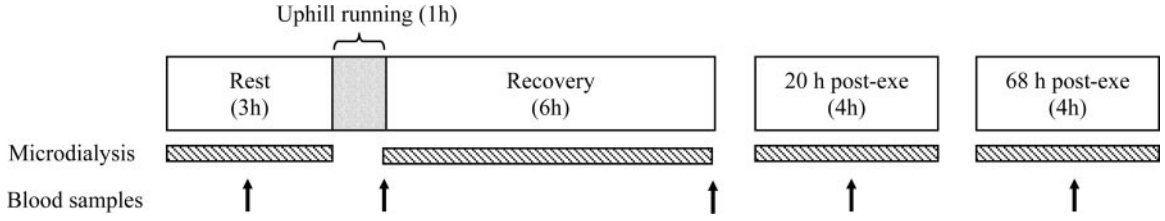

Fig. 1. Experimental design. In the exercise experiment, microdialysate was sampled from the peritendinous tissue of the Achilles tendon during rest periods before and after 1 h of uphill treadmill running (hatched bars). Additionally, blood was sampled before (Rest) and at different times after (post-exe) exercise (arrows).

markers (PICP and ICTP) was sampled as described above and collected in 10-ml vacutainers, allowed to coagulate for 30 min, and then centrifuged at 3,000 *g* for 15 min. The resulting supernatant (serum) was stored at −80°C until analysis. Hematocrit values were determined by the microhematocrit method at all times of blood sampling, and the measured blood concentrations of TGF-β_1, PICP, and ICTP were corrected for changes in plasma volume according to the Van Beaumont formula (48).

Sample Analysis

TGF-β_1. Samples were analyzed for TGF-β_1 by using a commercially available ELISA kit (DuoSet, R & D Systems, Minneapolis, MN). Representative dialysate samples were tested for the presence of active TGF-β_1, but the concentration was under the detection limit of the assay. Accordingly, all samples were activated by acidification to separate TGF-β_1 from its binding proteins, allowing for measurement of total TGF-β_1. For activation of plasma samples, 2.5 M acetic acid-10 M urea were used, and 1 M HCl was used for microdialysis samples (as recommended by the supplier). Activated samples were neutralized by use of 2.7 M NaOH-1 M HEPES for plasma samples and 1.2 M NaOH-0.5 M HEPES for microdialysis (as recommended by the supplier). Samples were loaded on ELISA plates immediately after neutralization. All samples were measured in duplicate, and samples obtained from each subject were measured in the same assay. The average coefficient of variation on duplicate measurements was found to be 2.0% (range 0.02–10.6%). The intra-assay precision was found to be 5.9% at 1,619 pg/ml (plasma), whereas the interassay precision was 7.6% at 1,724 pg/ml (plasma).

To evaluate the degree of degradation of TGF-β_1 in dialysate, we performed repeated measurements of samples, which were frozen and thawed between measurements (and left for up to 2 h at room temperature before refreezing); no change was observed over time in the TGF-β_1 level of these dialysate samples. This indicates that the degradation of TGF-β_1 in dialysate is negligible, at least at room temperature, and we would not expect a considerable degradation even at the slightly higher temperature of the dialysate when it is sampled from the subjects.

Type I collagen metabolism markers. Microdialysis and serum samples were analyzed for PICP and ICTP with a commercially available RIA kit (Orion Diagnostica, Espoo, Finland). Samples from each subject were measured in the same assay. According to the manufacturer, the detection level of the kit is 1.2 μg/l for PICP and 0.5 μg/l for ICTP. Intra-assay precision is given as 3.1% for PICP at 54 μg/l and 6.2% for ICTP at 3.8 μg/l, whereas the interassay precision is given as 5.8% for PICP at 52 μg/l and 7.9% for ICTP at 3.3 μg/l.

Statistics

All data are presented as means ± SE. When a significant difference was found over time (determined by the Friedman test), the Wilcoxon signed-rank test was used to compare paired observations. $P < 0.05$ was considered significant. Microdialysis results for TGF-β_1, PICP, and ICTP are given as the average of values observed in paired samples from the right and left leg of all subjects. No systematic significant difference was seen between legs with regards to the obtained values of TGF-β_1, PICP, and ICTP.

RESULTS

Exercise-Induced Changes in TGF-β_1

To evaluate the influence of exercise on systemic and local TGF-β_1 levels, blood and dialysate samples (from peritendon tissue) were obtained from the participating subjects before, and at several times after, 1 h of uphill treadmill running (Fig. 1). A significant increase in plasma TGF-β_1, from 992 ± 49 pg/ml (at rest) to 1,301 ± 39 pg/ml, was found immediately after the exercise bout ($P < 0.05$), and levels tended to remain elevated at 6 h postexercise (1,114 ± 29 pg/ml), although this was not significant compared with the basal level ($P = 0.08$) (Fig. 2). In dialysate, no significant changes were seen between resting values of TGF-β_1 and the concentrations measured postexercise, although a value of 423 ± 86 pg/ml was observed 3 h postexercise compared with the resting value of 303 ± 46 pg/ml ($P > 0.05$) (Fig. 3). Analysis of successive microdialysis samples obtained during a 6-h recovery period after exercise revealed that the dialysate level of TGF-β_1 was unchanged until 4 h postexercise, whereafter a significant decrease was seen relative to the initial concentration at 0.5 h (from 509 ± 91 to 244 ± 91 pg/ml) ($P < 0.05$) (Fig. 4). In comparison, an ongoing significant decrease was found in a control experiment in which dialysate was obtained over 3.5 h in subjects who had not performed exercise (Fig. 5). Taking this into account, the constant TGF-β_1 level found in dialysate up to 4 h after exercise could indicate an exercise-induced increase in tissue TGF-β_1. This is further illustrated by the relative changes over time in dialysate levels of TGF-β_1 found in the postexercise recovery period compared with the relative changes seen at in the control experiment (Fig. 6).

Changes in Type I Collagen Turnover

The concentration of PICP in dialysate was below the detection level (1.2 μg/l) in samples obtained before, immediately after, and 20 h after exercise, whereas a tissue level of 52 ± 12.6 μg/l was observed at 68 h postexercise ($P < 0.05$) (Fig. 7*A*). Tissue ICTP

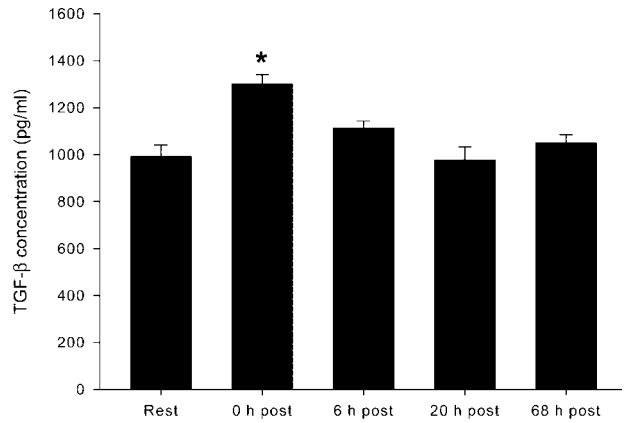

Fig. 2. Hematocrit-corrected concentrations of transforming growth factor-β_1 (TGF-β_1) in plasma before and after (post) exercise ($n = 6$). *$P < 0.05$. Error bars represent SE.

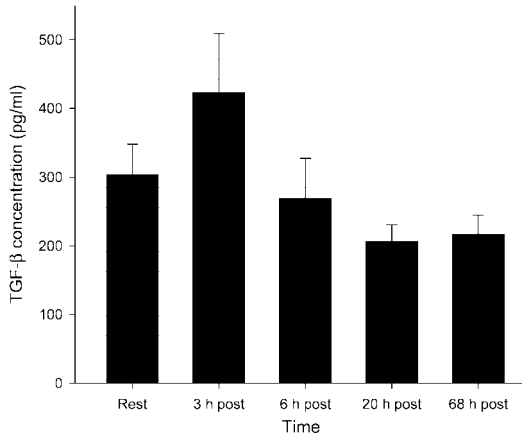

Fig. 3. Concentrations of TGF-β_1 in dialysate obtained from the peritendinous tissue of the Achilles tendon before and after exercise. Values are based on average concentrations observed in paired dialysate samples (from right and left leg) obtained minimum 2 h after insertion of microdialysis probes ($n = 6$). Error bars represent SE.

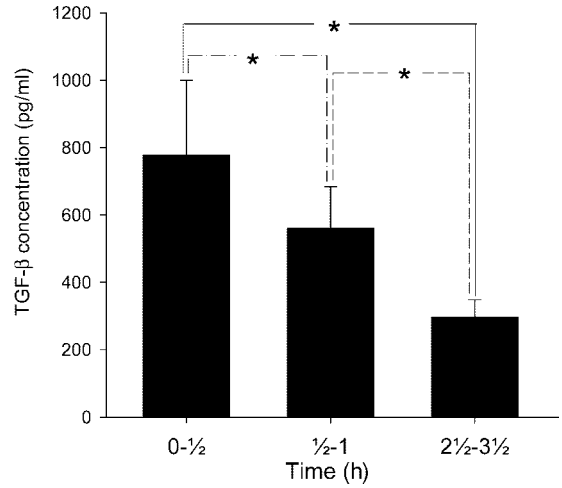

Fig. 5. Concentration of TGF-β_1 in dialysate obtained at different times after insertion of microdialysis catheters in resting subjects (control experiment) ($n = 6$). *$P < 0.05$. Error bars represent SE.

levels remained unchanged ($P > 0.05$) (Fig. 7*B*). In serum, PICP levels were significantly decreased 6 h after exercise ($P < 0.05$), whereas serum ICTP levels were significantly elevated immediately after exercise ($P < 0.05$) (Table 1).

DISCUSSION

The present study is to our knowledge the first to report detection of tissue TGF-β by use of the microdialysis method. Employing high-molecular-mass cutoff microdialysis probes, we measured levels of TGF-β_1 in dialysate obtained from connective tissue in the peritendinous region of the human Achilles tendon before and after exercise, involving mechanical loading of the tendon. The magnitude of the changes in dialysate concentrations of TGF-β_1 after exercise, compared with the results obtained in control studies, indicates a possible exercise-induced increase in local TGF-β_1. This could play a role for the increase in local peritendinous type I collagen production observed 3 days after exercise. Furthermore, a postexercise increase in systemic TGF-β_1 concentrations indicates a release of the cytokine in response to exercise and may further support a role for TGF-β_1 in relation to exercise-induced collagen synthesis in regions that are subjected to mechanical loading.

The increase in formation of procollagen propeptides for type I collagen (PICP) indicates an increased accumulation of collagen in response to exercise and fits with the general view that mechanical loading influences ECM gene expression (7). Mechanically induced ECM remodeling has been observed in several tissue types, including bone (35) and cardiac tissue (52), and experiments with cell cultures and in vivo animal studies indicate that the tissue adaptations to mechanical loading consist in part of an increased type I collagen expression and/or production (3, 6, 19, 49, 53). A num-

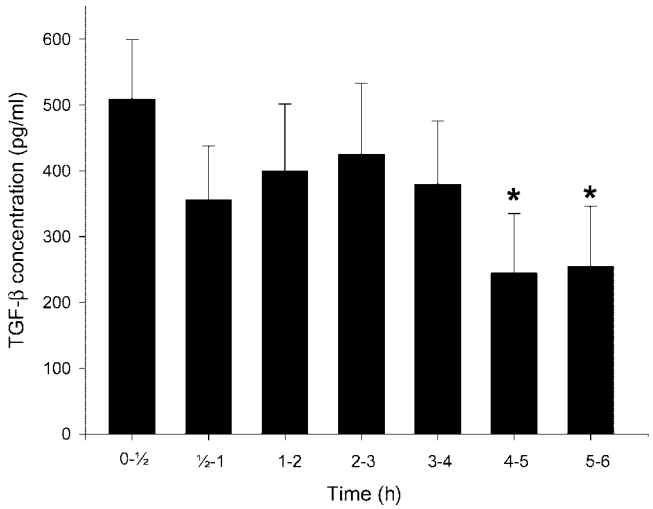

Fig. 4. Concentration of TGF-β_1 in dialysate samples obtained during 6 h of postexercise recovery ($n = 6$). *$P < 0.05$. Error bars represent SE.

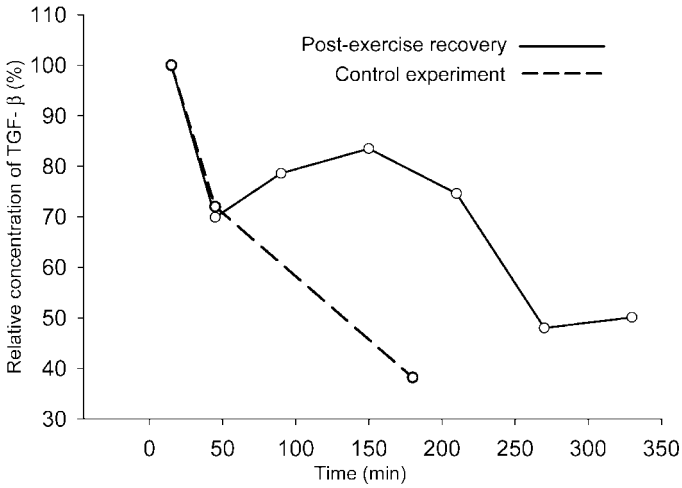

Fig. 6. Relative changes over time in the concentration TGF-β_1 in dialysate sampled in the postexercise period of the exercise experiment (solid line) and in the control experiment (dotted line).

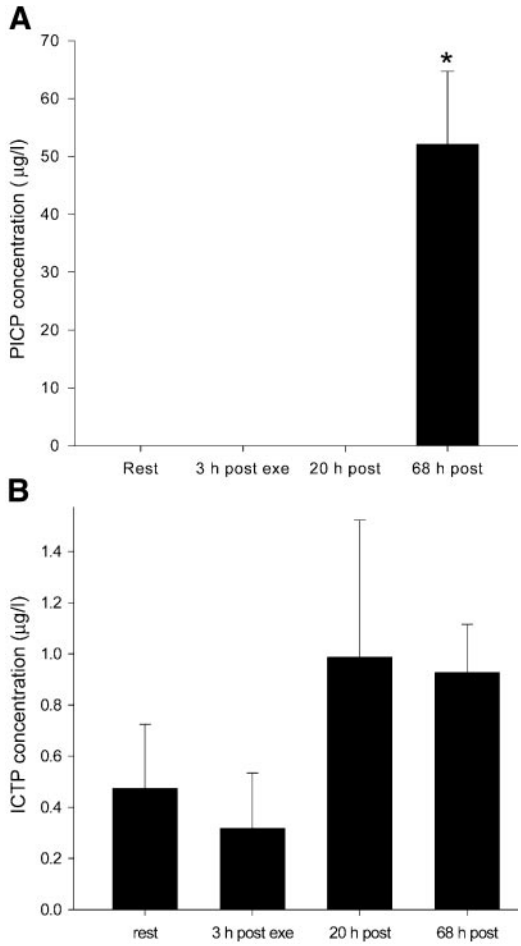

Fig. 7. Levels of procollagen I COOH-terminal propeptide (PICP; *A*) and COOH-terminal telopeptide of type I collagen (ICTP; *B*) in peritendinous tissue in response to uphill running. Values are based on the averages of paired observations (from right and left leg) corrected for relative recovery ($n = 6$). Levels of PICP were lower than the detection level in dialysate obtained before and at 3 and 20 h postexercise. *$P < 0.05$. Error bars represent SE.

ber of animal studies show an increase in total collagen content or collagen synthesis in tendon tissue in response to training (10, 51), whereas immobilization evidently results in a decreased collagen synthesis (23, 43) and loss of total collagen (20). Furthermore, human subjects performing frequent weight-bearing exercise have been found to have Achilles tendons with larger cross-sectional areas than normal control subjects (41, 54).

Recent microdialysis experiments, along with the present study, support the existence of an adaptation response in human tendon tissue in vivo. In response to both long-term strength training and acute long distance running (36 km), Langberg and coworkers (27, 29) found a local PICP increase in Achilles peritendon tissue independent of changes in circulating PICP levels, and, given that no changes were found in local ICTP levels, these results point toward an exercise-induced net increase in the local tendinous production of type I collagen. In the present study we saw a similar reaction, although the increase in tissue PICP in response to 1 h of uphill running was moderate compared with the response found to 36 km of running by Langberg et al. (29). This could indicate a dose-response relationship in the exercise-induced local tendinous collagen production. The mechanisms linking mechanical loading to type I collagen synthesis remain to be explained in detail, and in the present study it was investigated whether TGF-β_1 is involved in exercise-induced type I collagen production in human tendon tissue in vivo. In vitro studies with human cell cultures show stretch-induced TGF-β_1 expression in tendon, ligament, and cardiac fibroblasts (24, 42, 45), smooth muscle cells (18, 38), and osteoblast-like cells (8); also in vivo and in vitro animal studies support a connection between mechanical strain and TGF-β expression (9, 30, 32, 49, 55). Moreover, studies indicate that mechanically induced type I collagen synthesis can be ablated by inhibiting TGF-β_1 activity (18, 24, 33). In conflict with the hypothesis of TGF-β_1 as a mediator of collagen synthesis in tendon tissue, Fenwick and coworkers (13) were not able to detect the TGF-β receptor I or TGF-β_1 in human Achilles tendon tissue. However, the normal tendon specimens analyzed by Fenwick et al. were from cadavers of very elderly people and from nondiseased parts of tendon from patients with chronic Achilles tendinopathy, and thus it must be assumed that such tendon tissue has not been subjected to a normal degree of mechanical loading. This may influence the presence of both TGF-β_1 and receptor I, because human and animal ex vivo and/or in vitro studies indicate that TGF-β_1 and its receptors are in fact present in tendon tissue and that their degree of expression is highly variable and inducible (15, 34, 37). Furthermore, in vitro studies with animal tenocytes show a marked response to stimulation with TGF-β_1, β_2, and β_3, indicating that the TGF-β signaling is successful and that the TGF-β receptors are present in these cells (2, 25, 40).

On the basis of the results obtained in the present study we cannot be fully conclusive with regard to TGF-β_1's role in exercise-induced type I collagen production in tendon tissue, because several methodological complications are related to microdialysis measurement of local TGF-β_1. Especially the large fluctuations in basal dialysate levels of TGF-β_1, presumably caused by initial trauma-induced platelet release of TGF-β_1, represent a problem. When analyzing the ex-

Table 1. *Serum levels of type I collagen metabolism markers*

Time point	Serum PICP, μg/l	Serum ICTP, μg/l
Preexercise	189 ± 19	3.5 ± 0.4
0 h Postexercise	196 ± 19	5.3 ± 0.5*
6 h Postexercise	160 ± 17*	3.9 ± 0.5
20 h Postexercise	189 ± 20	4.1 ± 0.4
68 h Postexercise	204 ± 28	4.0 ± 0.3

Values are means ± SE. PICP, procollagen I COOH-terminal propeptide; ICTP, COOH-terminal telopeptide of type I collagen. *Significant change from preexercise value ($P < 0.05$).

ercise-induced changes in local TGF-β_1, it is clear that these could be masked by changes caused exclusively by the microdialysis intervention itself. In view of the ongoing decrease in dialysate levels of TGF-β_1 seen at rest after insertion of the catheters, the comparatively stable level of dialysate TGF-β_1 observed in the postexercise recovery period could well indicate a relative exercise-induced increase in the local TGF-β_1 concentration as expressed by the difference in values between the two curves shown in Fig. 6.

The fact that no estimation was made of the actual tissue concentration of TGF-β_1, owing to the lack of a suitable tracer for estimation of the in vivo recovery, could represent an additional bias in the investigation of exercise-induced changes in local TGF-β_1. However, all microdialysis measurements were done on subjects in the resting state, and therefore it would be expected that the recovery of TGF-β_1 was relatively constant. This is so because relative recovery of several other substances, e.g., PGE_2, glucose, and lactate, when determined in relation to exercise, has been found to remain constant during rest periods before and after exercise, whereas changes in recovery are seen when measurements are performed during exercise (28).

The increase found in circulating TGF-β_1 levels in response to exercise could, along with the microdialysis findings, support a role for the cytokine in relation to exercise-induced changes in type I collagen synthesis. Meanwhile, the time lapse between the increase in TGF-β_1 and the increase in local PICP may indicate alternative signaling pathways, possibly involving other growth factors, or at least the contribution of a downstream mediator of TGF-β_1. Studies indicate that connective tissue growth factor, acting downstream of TGF-β_1, is largely responsible for TGF-β_1-mediated type I collagen expression in fibroblasts (12, 17), and such a two-step signaling cascade could explain the latency in collagen production. In line with this, type I collagen mRNA levels have been shown to reach maximum as late as 24 h after stimulation with TGF-β_1 in human fibroblasts (14). Considering this, the latency in activation of type I collagen metabolism does not rule out the involvement of TGF-β_1. Moreover, it should be considered that potential changes in the expression of, e.g., TGF-β receptors and procollagen C-proteinase, which cleaves PICP from procollagen (31) could influence the timing of type I collagen expression and accumulation.

In view of the strong relationship between mechanical loading and TGF-β_1 synthesis seen in various cell types (3, 8, 9, 38, 45, 53), the increase in circulating levels of TGF-β_1 may indicate a release of TGF-β_1 from tissues that are mechanically loaded during exercise, including bone, muscle, tendon, and perhaps cardiac and vascular tissues. Considering the tendency to an increase in dialysate-TGF-β_1 after exercise in the present study, the exercise-induced increase in systemic TGF-β_1 could well be a reflection of locally synthesized TGF-β_1 acting in an autocrine manner to induce collagen synthesis. Alternatively, it is possible that the increased plasma TGF-β_1 concentration is caused by exercise-induced platelet release of TGF-β_1, because prolonged exercise has been shown to induce platelet activation (11). Such a platelet activation could be linked to local exercise-induced tissue damage, and in this manner TGF-β_1 may function to induce local type I collagen synthesis in relation to mechanical loading during exercise. This hypothesis is supported by in vivo studies that show immediate increases in levels of TGF-β_1 in rat muscle in response to endurance exercise (4, 16). On the basis of the present study it cannot be concluded whether the increase in plasma TGF-β_1, seen in response to exercise, reflects a release of the cytokine from platelet α-granules or whether it originates from an increased production in tissues subjected to mechanical loading during exercise. The present study is the first to report an increase in plasma-TGF-β_1 in response to acute exercise, although pre- and postexercise levels of TGF-β_1 have been measured earlier. Suzuki et al. (46) found a small rise in TGF-β_1 in response to a marathon race, but the change did not obtain statistical significance. The high basal level of TGF-β_1 observed by Suzuki and coworkers ($\sim$13 ng/ml) could indicate that plasma samples were contaminated with TGF-β_1 from platelet granules because of inefficient inhibition of platelet activation (50). Such a contamination could well mask a significant increase in response to a marathon race, because a moderate increase in plasma-TGF-β_1, like the one observed in the present study of 0.3 ng/ml, could easily be overlooked in samples containing amounts of TGF-β_1 in the range of 13 ng/ml. Results reported by Hering et al. (21) support the possibility of an exercise-induced increase in TGF-β_1, because they showed an increase in plasma-TGF-β_1 after 14 and 21 days of strength training.

Taken together, the present study shows an increase in circulating levels of TGF-β_1 in response to acute exercise and indicates a local elevation of TGF-β_1 in the peritendinous tissue of the Achilles tendon. The TGF-β_1 response is followed by an increase in indicators of local type I collagen formation. Although microdialysis measurement of TGF-β_1 is novel and associated with technical difficulties, these findings suggest that TGF-β_1 may play a role in initiation of local peritendinous collagen formation after mechanical loading of tendon structures in humans.

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DISCLOSURES

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