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Effect of anti-inflammatory medication on the running-induced rise in patella tendon collagen synthesis in humans

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Christensen B, Dandanell S, Kjaer M, Langberg H. Effect of anti-inflammatory medication on the running-induced rise in patella tendon collagen synthesis in humans. *J Appl Physiol* 110: 137–141, 2011. First published October 28, 2010; doi:10.1152/japplphysiol.00942.2010.— NSAIDs are widely used in the treatment of inflammatory diseases as well as of tendon diseases associated with pain in sports and labor. However, the effect of NSAID intake, and thus blockade of PGE_2 production, on the tendon tissue adaptation is unknown. The purpose of the present study was to elucidate the possible effects of NSAID intake on healthy tendon collagen turnover in relation to a strenuous bout of endurance exercise. Fifteen healthy young men were randomly assigned into two experimental groups, with one group receiving indomethacin (oral 2×100 mg Confortid daily for 7 days; NSAID; $n = 7$) and a placebo group ($n = 8$). Both groups were exposed to a prolonged bout of running (36 km). The collagen synthesis NH_2-terminal propeptide of type I (PINP) and PGE_2 concentrations were measured before and 72 h following the run in the patella tendon by microdialysis. The peritendinous concentrations of PINP increased significantly in the placebo group as a result of the run, as shown previously. PGE_2 levels were significantly decreased 72 h after the run compared with basal levels in the subjects treated with NSAID and unchanged in the placebo group. The NSAID intake abolished the adaptive increase in collagen synthesis in the patella tendon found in the placebo group in response to the prolonged exercise ($P < 0.05$). The present study demonstrates that intake of NSAID decreased interstitial PGE_2 and abolished the exercise-induced adaptive increase in collagen synthesis in human tendons.

inflammation; NSAID; type I collagen

HUMAN SKELETAL MUSCLES AND tendons are both known to respond and adapt to altered levels of physical activity by, e.g., hypertrophy and increased collagen synthesis (18, 29). Several studies have shown that acute bouts of exercise, as well as prolonged training, induces changes in local metabolism, inflammatory activity, and collagen turnover in the Achilles tendon (14, 24), resulting in an increased formation of type I collagen in the hours and days following the loading (14, 24, 32). Along the same line, the human patella tendon has also been shown to demonstrate adaptive potential with markedly increased collagen synthesis in response to exercise (31). This transformation of mechanical forces to biochemical and structural responses (15) involves a number of different growth factors (18), such as IGF-I (1), transforming growth factor-β (TGF-β) (14, 36), PDGF-bb (5), IL-6 (Andersen MB, Pingel J, Kjaer M, Laugberg H, unpublished observation), and IL-1β

(10), which have been shown to stimulate the synthesis of collagen, at least in vitro. We have performed a number of studies on humans showing that several of the above-mentioned growth factors are increased in concentration in response to exercise (8, 19–21, 23).

Prostaglandins (e.g., the eicosanoid PGE_2) are known to be involved in the inflammatory response in humans (6). Newly performed studies have demonstrated that prostaglandin concentration in plasma or interstitial tissue can be blocked by ingestion or local infusion of NSAID (20, 30). Studies in skeletal muscle have shown that NSAID can block the adaptive activation of satellite cells, the stem cell of skeletal muscles, and thus reduce the hypertrophy of skeletal muscle in response to loading (27). Whether PGs play a role in the adaptive response in connective tissue is at present, however, not known. NSAID is often the drug of choice in the treatment of inflammation, e.g., tendinopathies, soft tissue, and ligamentous injuries (3). Considering the wide use of NSAID, the physiological effects of this drug on the tendon tissue are important to understand for optimizing the treatment of patients with tendinopathies and other tendon disorders (34). However, a full understanding of the effects of PGE_2 and the use of NSAID in relation to mechanical loading in healthy tendon tissue is needed before it is possible to understand the pathology fully.

The purpose of the present study was to analyze the effect of NSAID on the local peritendinous concentrations of PGE_2 and patella tendon collagen synthesis in response to an acute bout of endurance training. This was done to clarify the relationship between collagen synthesis and PGE_2 levels by monitoring the effect on collagen synthesis when PGE_2 release is blocked by NSAID. Based on previous findings, it was hypothesized that the treatment with NSAID would lead to a decrease in PGE_2 levels. Given that PGE_2 is a growth factor for collagen tissue, NSAID treatment would then lead to a decrease in the exercise-induced increase in collagen synthesis.

METHODS

Subjects. A total of 15 healthy young men were included in the present study (Table 1). They were randomly assigned into two groups (by envelope): one group ($n = 8$) receiving placebo (calcium tablets) and the other group ($n = 7$), indomethacin (oral intake starting 72 h prior to exercise and continuing 72 h postexercise; 100 mg Confortid twice a day). Indomethacin is an NSAID that inhibits both cyclooxygenase-1 (COX-1) and COX-2 and thereby the production of PGE_2. The included subjects were all experienced runners, were training for a marathon, and were able to run 36 km in less than 3 h. None of the subjects suffered from any tendon injuries within the last year or had been taking any kind of medication within the last half year. All subjects gave written informed consent to participate in the study after

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Table 1. –*Anthropometric data*

	Placebo Group	NSAID Group
Age, yr	26 ± 1	25 ± 1
Weight, kg	75.4 ± 2.7	84.5 ± 3.5
Height, cm	182 ± 2	185 ± 2
BMI, kg/m^2	22.8 ± 0.5	24.0 ± 0.8
$\dot{V}_{O_{2peak}}$, l/min	4.8 ± 0.3	4.9 ± 0.1

Values are means ± SE. BMI, body mass index. There were no statistical significant differences between the 2 groups ($P > 0.05$).

receiving both written and oral information, in adherence to the declaration of Helsinki. The local human subject ethics committee of Copenhagen and Frederiksberg approved the study.

Study design. Each subject completed a total of four experimental days. On the first day, the subjects had their aerobic capacity measured on a treadmill. A minimum of 1 wk later, the collagen synthesis was measured at rest by the microdialysis method. The amount of collagen synthesis was determined in the peritendinous tissue ventrally to the patellar tendon. It was randomized in whichever leg the collagen synthesis was determined. At least 1 wk later, the subjects performed the 36 km of running; a route of 12 km was run a total of three times. At 72 h after completion of the run, the collagen synthesis was measured again by microdialysis in the patella tendon.

Placebo/NSAID was taken the first time 3 days before the 36-km run and until the last experimental day was conducted (1 pill every morning and evening). Hence, the measurements of collagen synthesis before running (baseline) were conducted before treatment with NSAID and thereby not affected by the treatment. All subjects were interviewed at the last day of the project to ensure that the protocol for intake of medication was followed and full compliance was found.

Measurements of aerobic capacity. Aerobic capacity was measured as previously described (16). The participants performed a maximal run at a constant speed of 130% of the self-reported speed on a 10-km run, which should ensure that exhaustion would occur within 5 to 7 min. After the first 2 min of running, the incline of the treadmill was adjusted to 2% and then increased by 2% every 1.5 min until exhaustion. Respiratory variables were measured continuously (AMIS 2001 automated metabolic cart; Innovation, Odense, Denmark) and averaged for each 30-s period. The mean of the three highest measurements of $\dot{V}_{O_2}$ was used as the peak oxygen consumption ($\dot{V}_{O_{2peak}}$).

Microdialysis. The microdialysis method was used in the present study to determine collagen synthesis in the peritendinous tissue of the patella tendon and was performed in principle as described previously (26). Before insertion of the microdialysis catheter the skin on both sides of the patella tendon was anesthetized using local anesthesia (lidocaine). The microdialysis catheters were positioned ventral and as close to the patella tendon as possible using ultrasound guidance. During the experiment, the actual flow in the microdialysis catheters were monitored by weighing the veils used for collecting the samples before and after the experiment. For each sample a correction factor was calculated and used to determine the in vivo recovery of NH$_2$-terminal propeptide of type I collagen (PINP) and PGE$_2$ using the internal reference method (37). The factor was determined as the exchange rate over the membrane of the microdialysis fiber. Three nanomolar ^{3}H-labeled human type IV collagen (130 kDa; specific activity: 7.0 TBq mg^{-1}; NEN, Boston, MA) was added to the perfusate to determine the relative loss. A high precision syringe pump (model CMA100) insured a perfusion rate of 2 μl/min. Dialysate was collected for a total of 4 h of which the last 3.5 h were used for analysis minimizing the possible effects of the insertion of the microdialysis catheter. The dialysate was immediately frozen at −80°C until subsequent analyses were performed.

The microdialysis catheters used in the present study were custom-made as previously described (24). The catheters were sterilized

before usage (ETO sterilization). The peritendinous concentrations of the marker for collagen synthesis PINP and PGE$_2$ were calculated using the internal reference method (37) as previously described (24).

Measurements of PGE$_2$. PGE$_2$ concentrations in dialysate from the peritendinous tissue of the patella tendons were measured with the PGE$_2$ EIA kit (monoclonal, cat. no. 514010; Cayman Chemical). Samples were diluted 1:5 before analysis, and all samples from the same subject were analyzed in the same assay. Intra-assay variation (coefficient of variation) was 3.9%, and the interassay variation was 6.4% at 500 pg/ml. The detection level of the kit was 15 pg/ml.

Measurements of collagen synthesis. ELISA measured peritendinous concentrations of PINP, a marker for collagen synthesis, as previously described (33). Concentrations were measured in the dialysate (local peritendinous concentration), which were diluted (1:8) before analysis. 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 (33).

Statistics. The level of statistical significance was set to $P < 0.05$. All results are represented as means ± SE. A student's unpaired *t*-test was used to analyze for differences in anthropometric data between the two groups. Differences between the placebo and NSAID group in regard to PINP and PGE$_2$ levels, respectively, were analyzed by a two-way ANOVA on ln-transformed data with Tukey's post hoc test. SigmaPlot 11.0 was used for statistical analysis and graphical presentation.

RESULTS

Subjects. There were no significant differences in anthropometric data (height, weight, age, body mass index, and $\dot{V}_{O_{2peak}}$) between the two groups ($P > 0.05$) (Table 1).

PGE$_2$ blockade in the patella tendon. Local tissue concentrations of prostaglandin were measured in the peritendinous space of the patella tendon at rest and 72 h after the 36 km of running, both in relation to treatment with placebo and NSAID. There was a significant decrease in PGE$_2$ levels after the 36-km run in the NSAID group (497 ± 149 to 132 ± 26 pg/ml) ($P < 0.001$); however, PGE$_2$ levels were unchanged in the placebo group (317 ± 107 to 331 ± 111 pg/ml) ($P > 0.05$) (Fig. 1, *A* and *B*).

Effects of PGE$_2$ blockade on collagen synthesis in the patella tendon. The peritendinous concentrations of PINP increased significantly in the placebo group (39 ± 11 to 100 ± 20 ng/ml) ($P = 0.002$), but this increase was abolished in the NSAID group (18 ± 5 to 11 ± 3 ng/ml) ($P > 0.05$). The overall effect of the treatment was significant ($P = 0.004$). No statistical difference at rest ($P > 0.05$) was found between the placebo and NSAID group; however, a significant difference at 72 h postrunning ($P < 0.001$) was found between the groups (Fig. 2, *A* and *B*).

DISCUSSION

The main finding of the present study is the demonstration of a total blunting of the exercise-induced increase in collagen synthesis in the patella tendon following an intake of NSAID. The intake of NSAID leads to a reduction in the PGE$_2$ production and this was associated with a decreased collagen synthesis response.

The present study showed that an acute prolonged bout of endurance exercise (36 km running) induced an increase in collagen synthesis in the patella tendon (Fig. 2). This is in accordance with previous studies showing that Achilles tendon tissue responds upon an acute bout of different types of
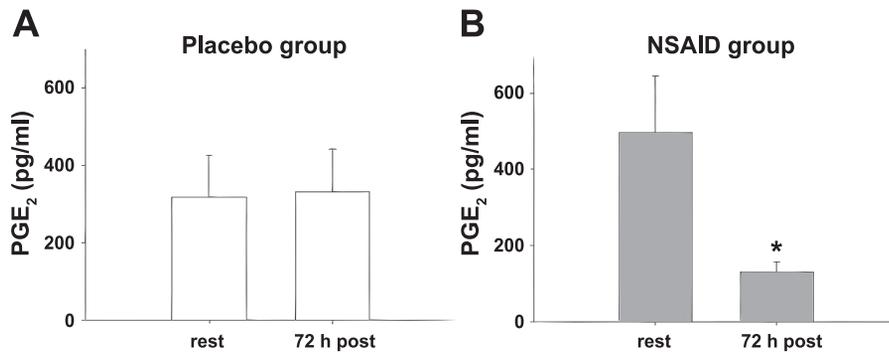
Fig. 1. PGE_2 blockade in the patella tendon. Measurements of patella tendon peritendinous concentrations of PGE_2 before and 72 h after a 36-km run after placebo treatment ($n = 5$) (*A*) and NSAID treatment ($n = 6$) (*B*). Bars represent means $\pm$ SE, $*P < 0.05$.

exercise (14, 24, 32) as well as prolonged training (22) by increasing collagen type I synthesis. In addition, studies using the infusion of stable isotopes, potentially a more direct measure of collagen synthesis, showed the same adaptive response to a 1-h kicking exercise in the patella tendon with increased collagen formation (31).

The adaptive response in collagen synthesis in human tendons to loading is thought to be mediated through a combination of a direct mechanical effect on the load on the fibroblasts and the release of various substances, such as different cytokines (e.g., IL-6) and growth factors (e.g., TGF-β, IGF-I) (28). PGE_2 levels have been shown to be elevated during and immediately after exercise locally in the peritendinous tissue (20, 23, 24) and thus potentially play a role in the exercise-induced adaptive response in collagen synthesis (14, 22, 24, 31, 32). As PGE_2 concentration can be manipulated by reducing the interstitial concentration through an intake of NSAID (13), it is possible to test this hypothesis. Several studies have stated that a prolonged run as the present one used leads to an increased release of various inflammatory factors (11, 21, 23). In addition, in vitro studies have shown that a regimen of cyclic mechanical stretching of human tendon fibroblasts results in an increased production of PGE_2 and COX by the fibroblasts in a stretching frequency-dependent manner (4, 25, 39).

The consequences of these elevated levels of PGE_2 during exercise have been addressed in previous studies, showing that rabbit tendons injected with PGE_2 had a predominant pattern of degeneration in the tendon matrix, with a decreased collagen fibril diameter and loss of parallel collagen fiber organization (17). This is supported by additional studies showing that exogenous PGE_2 decreased both the in vitro proliferation of human patellar tendon fibroblasts and the collagen production compared with the placebo group (7). Furthermore, PGE_2 and

collagenase levels increased, while the hydroxyproline content was unchanged, indicating a net increase in collagen degradation after stretching of avian flexor digitorum profundus tendons (8). This could indicate that the increased PGE_2 production seen in relation to exercise/stretching could play some role in tendon collagen degeneration. In support of PGE_2 being a growth factor for collagen synthesis, previous in vitro studies have shown that blockade of PGE_2 release by indomethacin results in a decrease in DNA synthesis (4), cell proliferation, and tendon glycosaminoglycan synthesis (35). Thus, the increase in collagen synthesis in the present study could be partly mediated through the increase in PGE_2. Several studies have analyzed the effect of PGE_2 blockade on the collagen tissue supporting the findings from the present study. In a study by Ferry et al. (9) it was found that COX-2 inhibitors given in the postoperative period after injury at the osteotendinous junction in rabbits, resulted in significantly decreased levels of hydroxyproline, a marker for collagen synthesis, compared with the placebo group. This resulted in a detrimental effect on tendon healing strength, with the tendons treated with COX-2 inhibitors being significantly weaker than the control tendons (9). In a rat study by Forslund et al. (12), it was found that indomethacin treatment resulted in a significantly reduced cross-sectional area of the tendon regenerate, but failure load was unchanged. On the other side, protein synthesis (measured as an increase in ^3H-proline incorporation) has been found to be increased, which could indicate that the synthesis of collagen molecules is actually stimulated by PGE_2 inhibition (4).

In the present study, the intake of NSAID lead to a significant reduction in the exercise-induced collagen synthesis in the patella tendon (Fig. 2). Unfortunately, no measurements were performed immediately after exercise in the present study, but a significant lowering of the PGE_2
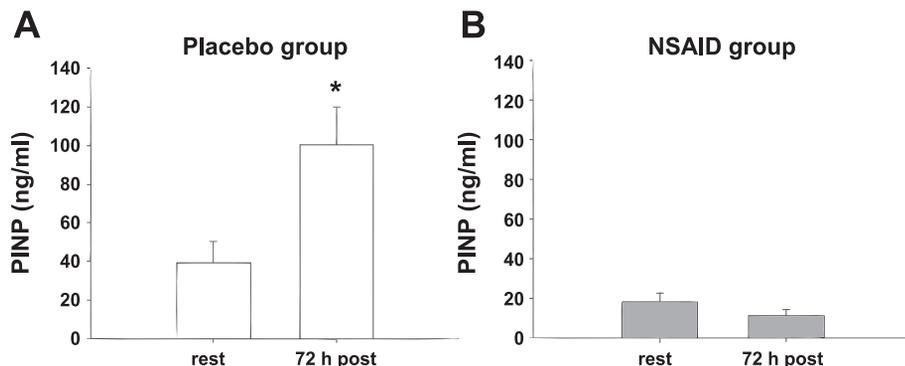

Fig. 2. Effects of PGE_2 blockade on collagen synthesis in the patella tendon. Measurements of peritendinous concentrations of NH_2-terminal propeptide of type I (PINP) before and 72 h after a 36-km run and placebo treatment ($n = 6$) (*A*) or NSAID treatment ($n = 7$) (*B*). Bars represent means $\pm$ SE, $*P < 0.05$.

concentrations 72 h after exercise following NSAID intake was demonstrated (Fig. 1). A number of in vivo studies, have shown that it is possible to block the prostaglandin release through a blockade of COX by indomethacin, a nonspecific NSAID, leading to a decrease in the cellular production of PGE_2 in response to stretching of fibroblasts from human patellar tendons in culture (25) as well as in cultures of fibroblasts from hand tendons (4). Similar results have been found in a human study showing that the interstitial concentrations of PGE_2 could be blocked by both specific and unspecific NSAIDs (20).

The present data show that intake of NSAID has a pronounced effect on collagen synthesis. It is well known that repetitive mechanical loading can lead to pathologic changes in the tendon tissue, occurring in both occupational and athletic settings. However, at present the pathology behind these tendon disorders are poorly understood (17, 34, 38). Bearing in mind the wide use of NSAID in the treatment of overuse injuries, this may represent a paradox, as the intake may compromise the adaptation of the tendons to loading. Thus, it could be hypothesized that using NSAID in the treatment of patients with chronic tendinopathy, where no sign of inflammation can be verified (2), or even using NSAID in healthy athletes to prevent delayed onset of muscle soreness, may be detrimental to the adaptive response of the tissue to exercise. This should be mediated through an inhibition of the exercise-induced release of PGE_2 by NSAID and thus lead to a reduced collagen synthesis as demonstrated in the present study. However, it could also be hypothesized that in situations with high concentrations of PGE_2, such as during acute inflammation, it may be important to reduce the PGE_2 levels, as high PGE_2 concentrations have been demonstrated to lead to increased collagen degradation (7, 8, 17). The appropriate clinical application of NSAID both during exercise and to treat tendinopathy merits further research.

Conclusion. In the present study, it was found that intake of NSAID results in a diminishing of the exercise-induced increase in collagen synthesis in human patella tendons. The effect of the NSAID intake reduced the release of PGE_2 in the tissue in the days following the loading. This may indicate that the reduction in collagen synthesis by the intake of NSAID is mediated through a blockade of the PGE_2 production in the tissue.

GRANTS

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **Abrahamsson SO, Lohmander S.** Differential effects of insulin-like growth factor-I on matrix and DNA synthesis in various regions and types of rabbit tendons. *J Orthop Res* 14: 370–376, 1996.
2. **Alfredson H.** The chronic painful Achilles and patellar tendon: research on basic biology and treatment. *Scand J Med Sci Sports* 15: 252–259, 2005.
3. **Almekinders LC.** The efficacy of nonsteroidal anti-inflammatory drugs in the treatment of ligament injuries. *Sports Med* 9: 137–142, 1990.
4. **Almekinders LC, Baynes AJ, Bracey LW.** An in vitro investigation into the effects of repetitive motion and nonsteroidal anti-inflammatory medication on human tendon fibroblasts. *Am J Sports Med* 23: 119–123, 1995.
5. **Banes AJ, Tsuzaki M, Hu P, Brigman B, Brown T, Almekinders L, Lawrence WT, Fischer T.** PDGF-BB, IGF-I and mechanical load stimulate DNA synthesis in avian tendon fibroblasts in vitro. *J Biomech* 28: 1505–1513, 1995.
6. **Carlstedt CA, Madsen K, Wredmark T.** The influence of indomethacin on collagen synthesis during tendon healing in the rabbit. *Prostaglandins* 32: 353–358, 1986.
7. **Cilli F, Khan M, Fu F, Wang JH.** Prostaglandin E2 affects proliferation and collagen synthesis by human patellar tendon fibroblasts. *Clin J Sport Med* 14: 232–236, 2004.
8. **Devkota AC, Tsuzaki M, Almekinders LC, Banes AJ, Weinhold PS.** Distributing a fixed amount of cyclic loading to tendon explants over longer periods induces greater cellular and mechanical responses. *J Orthop Res* 25: 1078–1086, 2007.
9. **Ferry ST, Dahners LE, Afshari HM, Weinhold PS.** The effects of common anti-inflammatory drugs on the healing rat patellar tendon. *Am J Sports Med* 35: 1326–1333, 2007.
10. **Fini ME, Strissel KJ, Girard MT, Mays JW, Rinehart WB.** Interleukin 1α mediates collagenase synthesis stimulated by phorbol 12-myristate 13-acetate. *J Biol Chem* 269: 11291–11298, 1994.
11. **Fischer CP.** Interleukin-6 in acute exercise and training: what is the biological relevance? *Exerc Immunol Rev* 12: 6–33, 2006.
12. **Forslund C, Bylander B, Aspenberg P.** Indomethacin and celecoxib improve tendon healing in rats. *Acta Orthop Scand* 74: 465–469, 2003.
13. **Goodwin JS, Ceuppens JL.** Effect of nonsteroidal antiinflammatory drugs on immune function. *Semin Arthritis Rheum* 13: 134–143, 1983.
14. **Heinemeier K, Langberg H, Olesen JL, Kjaer M.** Role of TGF-β1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *J Appl Physiol* 95: 2390–2397, 2003.
15. **Huang H, Kamm RD, Lee RT.** Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 287: C1–C11, 2004.
16. **Jensen K, Jorgensen S, Johansen L.** A metabolic cart for measurement of oxygen uptake during human exercise using inspiratory flow rate. *Eur J Appl Physiol* 87: 202–206, 2002.
17. **Khan MH, Li Z, Wang JH.** Repeated exposure of tendon to prostaglandin-E2 leads to localized tendon degeneration. *Clin J Sport Med* 15: 27–33, 2005.
18. **Kjaer M.** Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev* 84: 649–698, 2004.
19. **Langberg H, Bjorn C, Boushel R, Hellsten Y, Kjaer M.** Exercise-induced increase in interstitial bradykinin and adenosine concentrations in skeletal muscle and peritendinous tissue in humans. *J Physiol* 542: 977–983, 2002.
20. **Langberg H, Boushel R, Skovgaard D, Risum N, Kjaer M.** Cyclooxygenase-2 mediated prostaglandin release regulates blood flow in connective tissue during mechanical loading in humans. *J Physiol* 551: 683–689, 2003.
21. **Langberg H, Olesen JL, Gemmer C, Kjaer M.** Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *J Physiol* 542: 985–990, 2002.
22. **Langberg H, Rosendal L, Kjaer M.** Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. *J Physiol* 534: 297–302, 2001.
23. **Langberg H, Skovgaard D, Karamouzis M, Bulow J, Kjaer M.** Metabolism and inflammatory mediators in the peritendinous space measured by microdialysis during intermittent isometric exercise in humans. *J Physiol* 515: 919–927, 1999.
24. **Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjaer M.** Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *J Physiol* 521: 299–306, 1999.
25. **Li Z, Yang G, Khan M, Stone D, Woo SL, Wang JH.** Inflammatory response of human tendon fibroblasts to cyclic mechanical stretching. *Am J Sports Med* 32: 435–440, 2004.
26. **Lonnroth P, Jansson PA, Smith U.** A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol Endocrinol Metab* 253: E228–E231, 1987.
27. **Mackey AL, Kjaer M, Dandanell S, Mikkelsen KH, Holm L, Dossing S, Kadi F, Koskinen SO, Jensen CH, Schroder HD, Langberg H.** The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans. *J Appl Physiol* 103: 425–431, 2007.
28. **Magnusson SP, Langberg H, Kjaer M.** The pathogenesis of tendinopathy: balancing the response to loading. *Nat Rev Rheumatol* 6: 262–268, 2010.

29. **Matoba H, Gollnick PD.** Response of skeletal muscle to training. *Sports Med* 1: 240–251, 1984.

30. **Mikkelsen UR, Helmark IC, Kjaer M, Langberg H.** Prostaglandin synthesis can be inhibited locally by infusion of NSAIDS through microdialysis catheters in human skeletal muscle. *J Appl Physiol* 104: 534–537, 2008.

31. **Miller BF, Olesen JL, Hansen M, Dossing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, Babraj JA, Smith K, Rennie MJ.** Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol* 567: 1021–1033, 2005.

32. **Olesen JL, Heinemeier KM, Gemmer C, Kjaer M, Flyvbjerg A, Langberg H.** Exercise dependent IGF-I, IGFBPs, and type-I collagen changes in human peritendinous connective tissue determined by microdialysis. *J Appl Physiol* 102: 214–220, 2007.

33. **Orum O, Hansen M, Jensen CH, Sorensen HA, Jensen LB, Horslev-Petersen K, Teisner B.** Procollagen type I N-terminal propeptide (PINP) as an indicator of type I collagen metabolism: ELISA development, reference interval, and hypovitaminosis D induced hyperparathyroidism. *Bone* 19: 157–163, 1996.

34. **Riley G.** The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford)* 43: 131–142, 2004.

35. **Riley GP, Cox M, Harrall RL, Clements S, Hazleman BL.** Inhibition of tendon cell proliferation and matrix glycosaminoglycan synthesis by non-steroidal anti-inflammatory drugs in vitro. *J Hand Surg [Br]* 26: 224–228, 2001.

36. **Robbins JR, Evanko SP, Vogel KG.** Mechanical loading and TGF-β regulate proteoglycan synthesis in tendon. *Arch Biochem Biophys* 342: 203–211, 1997.

37. **Scheller D, Kolb J.** The internal reference technique in microdialysis: a practical approach to monitoring dialysis efficiency and to calculating tissue concentration from dialysate samples. *J Neurosci Methods* 40: 31–38, 1991.

38. **Thampatty BP, Li H, Im HJ, Wang JH.** EP4 receptor regulates collagen type-I, MMP-1, and MMP-3 gene expression in human tendon fibroblasts in response to IL-1β treatment. *Gene* 386: 154–161, 2007.

39. **Wang JH, Li Z, Yang G, Khan M.** Repetitively stretched tendon fibroblasts produce inflammatory mediators. *Clin Orthop Relat Res* 243–250, 2004.