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

Britt Christensen, Sune Dandanell, Michael Kjaer, and Henning Langberg

Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, and Center of Healthy Aging, University of Copenhagen, Copenhagen, Denmark; Department of Endocrinology and Internal Medicine, NBI/THG, Aarhus University Hospital, Aarhus, Denmark; and Faculty of Health, Care and Rehabilitation, School of Physiotherapy, Metropolitan University College, Copenhagen, Denmark

Submitted 16 August 2010; accepted in final form 24 October 2010

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 PGE2 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 NH2-terminal propeptide of type I (PINP) and PGE2 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. PGE2 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 PGE2 and abolished the exercise-induced adaptive increase in collagen synthesis in human tendons.

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 PGE2) 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 PGE2 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 PGE2 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 PGE2 levels by monitoring the effect on collagen synthesis when PGE2 release is blocked by NSAID. Based on previous findings, it was hypothesized that the treatment with NSAID would lead to a decrease in PGE2 levels. Given that PGE2 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 PGE2. 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
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 $V_O_2$ was used as the peak oxygen consumption ($V_O_2peak$).

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 was monitored by weighing the veins 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 $\mu$L/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 $-80^\circ$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 $\pm$ SE. A student’s unpaired t-test was used to analyze 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 $V_O_2peak$) 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

<table>
<thead>
<tr>
<th>Table 1. –Anthropometric data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Group</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
</tr>
<tr>
<td>$V_O_2peak$, l/min</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index. There were no statistical significant differences between the 2 groups ($P > 0.05$).
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). PGE₂ 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 PGE₂ 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 PGE₂ and COX by the fibroblasts in a stretching frequency-dependent manner (4, 25, 39).

The consequences of these elevated levels of PGE₂ during exercise have been addressed in previous studies, showing that rabbit tendons injected with PGE₂ 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 PGE₂ decreased both the in vitro proliferation of human patellar tendon fibroblasts and the collagen production compared with the placebo group (7). Furthermore, PGE₂ 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 PGE₂ production seen in relation to exercise/stretching could play some role in tendon collagen degeneration. In support of PGE₂ being a growth factor for collagen synthesis, previous in vitro studies have shown that blockade of PGE₂ 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 PGE₂. Several studies have analyzed the effect of PGE₂ 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 PGE₂ 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 PGE₂...
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

This work was funded by the Danish Rheumatism Association, Danish Ministry of Health, Internal Affairs, Danish National Research Council, Danish Medical Research Council.

DISCLOSURES

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

REFERENCES