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

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Abstract

Non-Steroid Anti-inflammatory Drugs (NSAID) are widely used in the treatment of inflammatory diseases as well as of tendon diseases associated with pain in sports and labour. However, the effect of NSAID intake - and thus blockade of prostaglandin (PGE\textsubscript{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 2x100 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 (PINP) and PGE\textsubscript{2} concentrations were measured before and 72h 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\textsubscript{2} levels were significantly decreased 72h after the run compared to 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\textsubscript{2} and abolished the exercise induced adaptive increase in collagen synthesis in human tendons.

Keywords: Inflammation, NSAID, type I collagen
**Introduction**

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 insulin-like growth factor 1 (IGF-1) (1), transforming growth factor beta (TGF-β) (14; 36), platelet-derived growth factor (PDGF-bb) (5), interleukin 6 (IL-6) (Bisgaard et al, in revision), 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 (PG’s) 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 non-steroid anti-inflammatory drugs (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 plays 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 are needed
before it is possible to understand the pathology fully.

The purpose of the present study was to analyse 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 in order 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 hypothesised 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 2 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 post-exercise; 100 mg Confortid twice a day). Indomethacin is an NSAID which inhibit both 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 below 3 hours. 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. Minimum one week 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 which leg the collagen synthesis was determined. At least one week later, the subjects performed the 36 km of running, a route of 12 km was run a total of 3 times. 72 hours 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 (one 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 5-7 min. After the first 2 min of running the incline of the treadmill was adjusted to 2% and then increased by 2% every 1½ min until exhaustion. Respiratory variables were measured continuously (AMIS 2001 automated metabolic cart, Innovation, Odense, Denmark) and averaged for each 30s period. The mean of the three highest measurements of VO$_2$ was used as the peak oxygen consumption (VO$_{2peak}$).

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 anaesthesia (Lidocaine). The microdialysis catheters were positioned ventral and 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-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 fibre. Three nanomolar $^3$H-labelled human type IV collagen (130 kDa; specific activity: 7.0 TBq mg$^{-1}$; NEN, Boston, MA, USA) was added to
the perfusate in order to determine the relative loss. A high precision syringe pump (CMA100) insured a perfusion rate of 2 μl/min. Dialysate was collected for a total of 4 hours, of which the last 3½ hours were used for analysis minimising 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 PGE₂ were calculated using the internal reference method (37), as previously described (24).

**Measurements of PGE₂**

PGE₂ concentrations in dialysate from the peritendinous tissue of the patella tendons were measured with the Prostaglandin E₂ EIA kit (monoclonal; Cayman Chemical Company, USA, cat. no. 514010). Samples were diluted 1:5 before analysis, and all samples from the same subject were analysed in the same assay. Intra-assay variation (coefficient of variation) was 3.9 % and inter-assay variation 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 analysed 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 $\pm$ SEM. A student’s unpaired t-test was used to analyse for differences in anthropometric data between the two groups. Differences between the placebo and NSAID group in regard of PINP and PGE$_2$ levels, respectively, were analyzed by a 2-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, BMI, and VO$_{2\text{peak}}$) 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 immediately 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) (Figure 1a+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 post running (p<0.001) was found between the groups (Figure 2a+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 the 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 (Figure 2). This is in accordance with previous studies showing that Achilles tendon tissue respond upon an acute bout of different types of 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 one-hour 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-$\beta$, 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 showed that a regime 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 fibre 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 analysed 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 to 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. it was found that Indomethacin treatment resulted in a significantly reduced cross sectional area of the tendon regenerate, but failure load was unchanged (12). On the other side, protein synthesis (measured as an increase in $^3$H-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 (Figure 2). Unfortunately no measurements were performed immediately after exercise in the present study, but a significant lowering of the PGE$_2$ concentrations 72h after exercise following NSAID intake was demonstrated (Figure 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 non-specific 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 hypothesised 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 sourness, 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 hypothesised 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 has 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\textsubscript{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\textsubscript{2} production in the tissue.


Legends to tables and figures

Table 1 – Anthropometric data

Anthropometric data on the subjects in the two groups (Placebo and NSAID), respectively. Values are means ± SEM, there were no statistical significant differences between the two groups (p>0.05).

Figure 1 – PGE$_2$ blockade in the patella tendon

Measurements of patella tendon peritendinous concentrations of PGE$_2$ before and 72 hours after a 36 km run after (a) placebo treatment (n=5) and (b) NSAID treatment (n=6). Bars represent means ± SEM, * p<0.05.

Figure 2 - Effects of PGE$_2$ blockade on collagen synthesis in the patella tendon

Measurements of peritendinous concentrations of PINP before and 72 hours after a 36 km run and (a) placebo treatment (n=6) or (b) NSAID treatment (n=7). Bars represent means ± SEM, * p<0.05.
### Table 1 – Anthropometric data

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<td>185 ± 2</td>
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