Osteoarthritis and Cartilage

Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training

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Summary

Objective: To investigate changes in levels of serum cartilage oligomeric matrix protein (COMP) and urine c-telopeptide of type-2 collagen (CTX-II) as markers for cartilage turnover in patients with osteoarthritis (OA) of the knee, in response to muscle strength training in combination with treatment with glucosamine, ibuprofen or placebo.

Design: A 12-week double blind, placebo controlled, randomized study.

Method: Thirty-six elderly patients with bilateral tibiofemoral knee OA determined by radiography were randomly assigned to treatment with glucosamine (n = 12), ibuprofen (n = 12) or placebo (n = 12) during 12 weeks of strength training of both legs with focus on the quadriceps muscle. Strength tests (5 repetition maximum), blood and urine sampling were performed before and after the training period. Serum COMP and urinary CTX-II were measured by enzyme-linked immunoassay (ELISA).

Results: All three groups increased their muscle strength following 12 weeks of strength training (P < 0.001). Serum COMP levels were reduced in the glucosamine-treated group after the training period (P = 0.012), whereas they did not change in the two other groups. Glucosamine reduced COMP statistically significant compared to both placebo and ibuprofen; the mean reduction with glucosamine was 13% vs placebo (P = 0.0378) and 17% vs ibuprofen (P = 0.0122).

Urinary CTX-II levels did not change significantly in any of the three experimental groups.

Conclusion: Serum COMP decreased significantly over the 12-week training period when treatment with glucosamine was added to the training regimen. This suggests an effect by glucosamine on the response of the OA cartilage to a period of joint loading in humans with knee OA.

Key words: Glucosamine, Dietary supplement, NSAID, Ibuprofen, Exercise, Knee osteoarthritis, Cartilage oligomeric matrix protein, c-Telopeptide of type II collagen.

Introduction

Approximately 8–10% of all men and women have osteoarthritis (OA) in one or more of their joints, which causes disability, pain and reduced quality of life. The disease affects the cartilage, synovium, subchondral bone, tendons and muscles surrounding the joint. There are a number of established risk factors for OA in the knee such as age, increased body mass index (BMI), previous injury or surgery of the knee, and genetic factors. Studies also suggest that reduced quadriceps strength is a risk factor as well as a consequence of OA in the knee. It has repeatedly been shown, that exercise reduces pain and improves function in subjects with OA of the knee. These beneficial effects are seen in protocols including both strength and endurance training. To our knowledge, it is not known whether physical training affects cartilage homeostasis in these patients.

Cartilage oligomeric matrix protein (COMP) is a prominent component of cartilage matrix. This glycoprotein has a role in governing assembly of type II collagen fibres in cartilage, and in cooperation with other matrix proteins it stabilizes the collagen network. Patients with knee OA have increased serum concentrations of COMP. Furthermore, studies suggest that increased levels of serum COMP are related to progressive joint damage in knee OA measured by radiography over a 5-year period. COMP levels are also modified in response to acute exercise; an acute bout of exercise elevates serum COMP in
healthy adults\textsuperscript{30,31} as well as in patients with knee OA\textsuperscript{25}, whereas 6 weeks of chronic training did not influence the circulating levels of COMP\textsuperscript{35}. Urinary level of c-telopeptide of type-2 collagen (CTX-II) is a putative marker of collagen degradation, and found to be increased in OA patients\textsuperscript{32–35}. Moreover, in patients with established knee or advanced hip OA, serum CTX-II is associated with radiological progression over short-term periods\textsuperscript{34,36}. Also a long-term study over 5 years shows that progression of OA is associated with increased urinary CTX-II\textsuperscript{37}. Finally, a study of young, healthy athletes shows that sports activities such as running significantly increase urinary CTX-II levels, whereas activities like swimming do not affect levels of this marker\textsuperscript{38}.

OA is often treated with non-steroidal anti-inflammatory drugs (NSAIDs) or glucosamine\textsuperscript{15,39–41}. These treatments may relieve pain, but their effects on cartilage and synovium metabolism in patients with OA are controversial\textsuperscript{42,43}. In vitro studies have suggested that NSAIDs may reduce or increase\textsuperscript{44–47} the synthesis of cartilage proteoglycans.

Very few studies have examined the effects of NSAIDs or glucosamine on CTX-II levels in OA patients\textsuperscript{33,48–50}. It seems that only one pilot study has examined the effect of NSAID (Nimesulide) on serum COMP levels in OA patients\textsuperscript{51}. To our knowledge, no studies have yet investigated the effect of glucosamine on serum COMP levels in OA patients.

The purpose of this study was to investigate, whether 12 weeks of muscle strength training in combination with treatment with glucosamine or ibuprofen (an NSAID), affects levels of serum COMP (primary outcome) and urinary CTX-II (secondary outcome), as indicators of cartilage turnover, in patients with OA of the knee. A placebo-group was included for control.

Methods

DESIGN

A 12-week double blind, placebo controlled, randomized study of adults with OA of the knee. Staff personnel not involved in the project randomly assigned patients to the three groups using a random number table, and other personnel prepared the medication for each patient. All study personnel and participants were blinded to treatment assignment for the duration of the study.

PARTICIPANTS AND CRITERIA

To detect changes in levels of serum COMP of 10% or more at a significance level of 0.05 with a power of 0.8, and taking into account the inter- and intra-individual variation as well as variation in the methods, approximately 10 individuals were required in each group. In total 36 patients (20 women, 16 men); aged 50–70 years with bilateral tibiofemoral OA of the knee, based upon radiographs, were recruited in the period January 2005 to January 2006 in Copenhagen (Denmark) via advertisement in a local newspaper. Subjects were prescreened via telephone or face-to-face interview. Straight anterior–posterior radiographs of both knees were obtained, with the patient standing. An experienced reader classified each knee for severity of OA using the Kellgren and Lawrence (KL) grading system, with scores from 1 to 4\textsuperscript{52}. Inclusion criteria were age between 50 and 70 years with radiographic evidence of bilateral knee OA (KL-score of 1–4) who met the American College of Rheumatology (ACR) clinical\textsuperscript{53} and radiographic classification criteria.

At inclusion a medical doctor examined the patients and established a medical record for each patient. Normal kidney and liver function was verified by analyses of serum creatinine and alkaline phosphatase. All patients were negative for rheumatoid factor and their serum uric acid was normal. Patients were excluded if they had severe health problems such as cardiovascular disease, active cancer, diabetes, kidney or liver diseases, excess alcohol use (>21 alcoholic drinks per week) or severe overweight (BMI > 35). Furthermore none of the subjects had any history of injury or operation in the knee, planned knee operation in the study, all subjects provided written informed consent to participate in the study. The experimental protocol was approved by the local Ethical Committee for Copenhagen and Frederiksberg Communities (KF 01-189/04). All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

MEDIATION

Four patients from each group were previously treated with NSAID. Seven patients from the glucosamine-group and five from both the ibuprofen and placebo-group had previously taken glucosamine. They were instructed to stop the intake at least 1 month before the study began. Patients were randomly assigned to one of three medication groups. They were administered glucosamine (n = 12), NSAID (n = 12) or placebo (n = 12). The administration of placebo and glucosamine was started 4 weeks before the training, but active NSAID treatment was initiated only 1 week before (Fig. 1). We chose to initiate the administration of glucosamine 4 weeks before training in order to ensure that glucosamine levels were adequate. Furthermore, it has been shown that ibuprofen works more quickly than glucosamine, which has to be given at least 2 weeks before you can expect a potential effect on OA symptoms\textsuperscript{54,55}. Subjects in the glucosamine-group were given glucosamine sulphate tablets (Ferrosan) of 500 mg three times a day and subjects in the NSAID group were given tablets of 600 mg of ibuprofen (Nycor) twice a day. Placebo tablets were identically supplied and formulated as the glucosamine or ibuprofen tablets except that they contained no medicine.

The treatment was blinded to the patients, so that all subjects were supplied the same amount of pills (five a day) that had similar appearance. To ensure compliance, the subjects returned the empty packages every week to a lab technician not involved in other parts of the study. For further control, NSAID levels in the blood were traced in the beginning of the training period, as well as after 6 and 12 weeks. If any of the patients experienced severe pain during the training period, they were allowed to take "rescue medicine" 50 mg tramadol (synthetic, monoagamous opioid), which unlike NSAID does not influence prostaglandin synthesis. Alternatively the subjects were offered acupuncture.

TRAINING AND TESTING

All three groups of patients performed a strength training program with both legs for 12 weeks. The program consisted of bilateral progressive strength training of the leg muscles with focus on the quadriceps muscles. Training was carried out over three sessions per week, 36 sessions in total, with a minimum of 30 sessions required for all subjects (representing a minimum of 83.3% participation). After a 10 min warm up on a stationary bicycle, the subjects performed sitting knee extension and leg-press exercises with one leg at a time in adjustable leg-press and knee-extension machines (Technogym International). Physiotherapists or physical educated personnel attended every training session and instructed the subjects performing the exercises. Training intensity was changed from 15 to 8 repetition maximum (RM) (4 sets × 12–8 repetitions) from week 0 to 7 to avoid injuries, and was thereafter maintained at 8 RM (4–5 sets × 8 repetitions) for the remaining training period. (Definition: RM refers to the maximum load an individual can exert in an exercise no more or no less than eight times with his/her maximal effort.) The relative workload was 70–80% of 1 RM through all 12 weeks, and training load was adjusted on a weekly basis. Furthermore, 5 RM strength tests were performed in the first week of the study, after 6 weeks, and in the last week of the training study (Fig. 1). 5 RM tests were performed both in the knee extension and in the leg-press machine. After every strength training session, subjects were given a 200 ml chocolate milk drink (Matilde classic, Arla), which is known to ensure optimal muscle protein synthesis\textsuperscript{56}.

URINE AND BLOOD SAMPLING

Before and after the 12-week strength training blood and urine samples were collected from the subjects (Fig. 1), who were asked to limit their...
physical activity before sampling. No formal training was carried out for a minimum of 48 h prior to blood and urine sampling. On the day of the sampling the subjects met in the laboratory after having consumed a standardized, regular breakfast. Venous blood samples were drawn and serum prepared in a standard way and stored at $-80^\circ$C until analysis. The subjects themselves collected the urine in the morning (first morning void), before they met in the laboratory. The urine samples were stored at $-20^\circ$C until measurement.

COMP AND CTX-II ANALYSIS

Serum COMP was measured by a sandwich ELISA (COMP$^\text{R}$ELISA; AnaMar Medical AB, Lund, Sweden). Urinary CTX-II was measured by a competitive ELISA (Cartilaps, Nordic Bioscience, Herlev, Denmark)$^{22}$. Urinary CTX-II measurements were corrected for urinary creatinine measured by a standard colorimetric assay using a kit from Konelab (Espoo, Finland).

Biomarkers were analyzed in duplicate in ELISA by trained lab technicians.

STATISTICAL ANALYSIS

Statistical Analysis System (SAS, Version 9.2, SAS Institute, Cary, NC) was used to analyze the data. Analyses were performed on the log-transformed dependent variable (marker-level) to improve approximation of the normal distribution. To evaluate the combined effect of training and treatment over time within each group, paired $t$ tests were used. The change in log-transformed marker-level from pre to post treatment was compared between groups using an analysis of variance model with treatment as factor and log-transformed pre-treatment marker-level as a covariate. Treatment differences were estimated from the model and 95% confidence intervals were calculated. The results in the text are expressed as relative changes obtained by back-transforming the estimates from the model.

$P$ values $< 0.05$ were considered significant, and all tests were two tailed.

Results

The total number of persons prescreened via telephone or face-to-face interview during the recruitment period was 181 (Fig. 2). Of these, 36 were randomized, and 145 were

![Fig. 2. Progress of participants throughout the trial.](image)

**Table I**

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<th>Baseline characteristics of participants in the three groups</th>
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<td>CTX-II baseline (ng/mmol)</td>
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Data for continuous measures are mean ± SD. Data for biomarkers are backtransformed factor means x/± factor SD.

![Fig. 3. Leg-press 5 RM tests. All groups increased significantly in strength (***(P ≤ 0.001).](image)
either disqualified or not interested in participation. The most common reason for disqualification were lack of knee OA on X-ray or unilateral OA (28%), major health problems that met exclusion criteria (22%), previous knee-trauma or operation (19%), age under 50 or over 70 (10%), other rheumatologic diseases (5%), regular training (8%), previous gastric ulcer (4%) or intolerance/allergy towards contents of glucosamine or ibuprofen (4%). Of the 36 randomized participants, 35 subjects completed the study. The one subject belonging to the ibuprofen group withdrew for personal reasons. During the analysis for serum COMP, one blood sample (pre-treatment) was missing on another subject from the Ibuprofen group, but this was not the case when analyzing for CTX-II. There were no differences between the groups with respect to subject characteristics at the inclusion of the study (Table I).

All participants were physically active on a light to moderate level (bicycling or walking) but none of them trained regularly or performed strength training prior to inclusion in the present study.

Upon completion of the training period, there was no difference between the groups with regard to participation (95 ± 1%) in training sessions. No patient reported any side effects of the project drugs during the study. The blood samples that were analyzed for ibuprofen, revealed that all patients in the ibuprofen group took their medication as they were instructed, and none from the two other groups took any ibuprofen during the project. Three subjects (two from the placebo-group, and one from the ibuprofen group) used the rescue medicine tramadol for a short period (<5 days) during the study, two because of training related pain, and one because of tooth-pain. Furthermore, two persons were treated with acupuncture (both of them belonged to the ibuprofen group, one of them was also treated with tramadol).

**Discussion**

The present experiment demonstrated that in response to a period of muscle strength training (12 weeks) muscle...
strength improved to a similar degree (around 50%) whether individuals were treated with NSAID (ibuprofen), glucosamine or placebo (Figs. 2 and 3). Training per se did not seem to induce changes in circulating levels of markers for cartilage degradation, which is in line with previous findings\(^{23}\). Analyses of the specific medication-effect, revealed that glucosamine decreased COMP significantly compared to treatment with placebo or ibuprofen. Clearly, from these data it is not possible to elucidate whether this reduction in serum COMP levels transforms into a better clinical status in these patients. From earlier findings it is doubtful as to whether glucosamine per se has any beneficial effect on OA symptoms\(^{12}\). Whereas some studies show a beneficial clinical effect of glucosamine per se\(^{25,58 – 60}\) others have found no difference between glucosamine treatment and placebo groups\(^{61 – 63}\). A general critique of some of the previous studies has been that they were small, had poor design and flaws in the data analysis, and that there has been a potential for sponsor bias\(^{64 – 66}\). Furthermore, to our knowledge only one study has previously tried to combine physical training with glucosamine in knee OA patients\(^{62}\). In that study, no significant effect of the treatment was found after 6 months of combined treatment, either on function, pain or mobility\(^{62}\). Muscle strength in that study showed similar alterations, in that they did not find any major difference between glucosamine and placebo groups. Post hoc analysis from the study of Messier, did, however, indicate that more pill-compliant OA subjects had more improvements in pain and mobility than less compliant individuals. Taken together, our present findings suggest an effect by glucosamine on the response of the OA cartilage to a period of joint loading in humans with knee OA.

There is evidence in vitro that physiologic concentrations of glucosamine can induce a diminished proteolytic activity of matrix metalloproteinase and increased synthesis of aggrecan\(^{67}\). Furthermore, some animal studies have shown that glucosamine can stimulate the regeneration of cartilage\(^{68,69}\). It is thus possible that the altered COMP levels we now observe reflect an altered cartilage metabolism, and if sustained over a long time this might influence joint morphology and clinical presentation.

Regarding CTX-II levels in our study, no significant changes were observed in any of the three experimental groups. Interestingly, however, patients from the glucosamine-treated group with high baseline CTX-II levels responded with slightly reduced CTX-II levels after the training. This is in agreement with previous findings, where glucosamine treatment decreased se-CTX-II more in OA patients with high cartilage turnover than in patients with a lower cartilage turnover\(^{23}\). Thus, a higher turnover of cartilage components only be associated with a higher likelihood of response to treatment with glucosamine.

Acute exercise has been shown to influence circulating levels of COMP\(^{25,31}\), and even moderate amounts of dynamic exercise like walking or gymnastics have been shown to lead to 10–20% elevated circulating levels of COMP immediately after the event, both in healthy individuals\(^{31}\) and in OA patients\(^{35}\). It is, however, important to note that serum levels of COMP are normalized already 30 min after exercise\(^{25,31}\) implying that the effect was rather an effect of clearance than a metabolic effect. However, the patients in our study were investigated at least 24 h after the last training bout, and it is unlikely that acute exercise influenced our results. Only after very extreme exercise bouts like a marathon run in healthy individuals, was up to 24 h required for normalization of COMP levels\(^{30}\). Furthermore, our sampling of blood in the OA patients was performed at the same time of the day, to avoid any influence of diurnal variation in COMP concentrations\(^{70,71}\). In the present study only circulating levels of COMP were determined, and exact evaluation of changes in the joint is therefore not possible. However, as cartilage is a major contributor to circulating levels of COMP\(^{25,72}\), the reduction in COMP levels most likely represents reduced degradation of cartilage in the loaded joints. It could, however, also represent changes in extracartilage degradation of COMP, since COMP is not only found in cartilage, but also in tendon, ligaments, intervertebral discs, etc\(^{74}\). However, it is shown that the concentration of COMP in these tissues is very low in compare to

![Fig. 6. Urine CTX-II levels (on a logarithmic scale) pre and post 12-weeks strength training. Each line represents an individual patient. No significant alterations in CTX levels were observed in any of the groups.](image-url)
cartilage, so even though there might have been a small change in concentration of COMP in the extra-cartilage tissues, it constitutes a very small amount of the circulating COMP levels. Finally, an alternative, which cannot be ruled out is that our findings may indicate, that the synthesis and release of newly synthesized COMP-molecules is decreased with glucosamine treatment.

In conclusion, glucosamine modified the effect of physical strength training in elderly OA patients, in that serum COMP was significantly reduced over the 12-week training period when glucosamine was added to the training regimen. This suggests an effect of glucosamine on the response of the OA cartilage to a period of joint loading in humans with knee OA.

Conflict of interest

DH and TS are cofounders and minor shareholders in AnaMar Medical.

Acknowledgements

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.joca.2009.07.004.

References


