Running title. Impact of exercise therapy on molecular biomarkers

Title. Impact of exercise therapy on molecular biomarkers related to cartilage and inflammation in people at risk of, or with established, knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials

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Abstract

Objective. To investigate the impact of exercise therapy on molecular biomarkers related to cartilage and inflammation in people at risk of, or with established, knee osteoarthritis by conducting a systematic review of randomized controlled trials (RCTs).

Methods. Literature search up to September 2017 in five major databases with no restriction on publication year or language. Data were extracted from the first available follow-up time point and we performed a narrative synthesis for the effect of exercise therapy on molecular biomarkers related to cartilage and inflammation. A subset of studies reporting sufficient data was combined in a meta-analysis, using an adjusted random effects model.

Results. Twelve RCTs, involving 57 study comparisons at 4 to 24 weeks following an exercise therapy intervention were included. Exercise therapy decreased molecular biomarkers in 17 (30%) study comparisons, had no effect in 36 (63%), and increased molecular biomarkers in four (7%) study comparisons. Meta-analyses of nine biomarkers showed that exercise therapy was associated with non-significant reductions of C-reactive protein, C-terminal crosslinking telopeptide of type II collagen, tumor necrosis factor alpha (TNF-α), soluble TNF-α receptor-1 and -2, C2C neoepitope of type II collagen and cartilage oligomeric matrix protein compared to non-exercising control groups and had no effect on interleukin-6 and soluble interleukin 6 receptor.

Conclusions. Exercise therapy is not harmful, as it does not increase the concentration of molecular biomarkers related to cartilage turnover and inflammation, implicated in osteoarthritis progression. The overall quality of evidence was downgraded to low because of the limited number of RCTs available.

Keywords: Exercise, cartilage, humans, knee, molecular biomarkers.

Significance and innovation

- This is the first study summarizing the effect of exercise therapy on molecular biomarkers related to cartilage turnover and inflammation in people at risk of, or with established, knee osteoarthritis.
- Based on the available evidence, people at risk of, or with established, knee osteoarthritis can be told that exercise therapy is not harmful to their knee joints.
- Future studies should preferably obtain synovial fluid from people at risk of, or at early stages of, OA and focus on a set of biomarkers, rather than single biomarkers.

Osteoarthritis (OA) is the most common joint disease and its prevalence in the western world has doubled since the mid-20th Century (1). OA represents one of the main reasons for disability, where the knee accounts for more than 80% of the disease burden (2). It is broadly agreed that OA is driven by a combination of biomechanical and pro-inflammatory factors, ultimately leading to osteochondral changes, with cartilage breakdown being one of the hallmarks of OA (3). Exercise is essential for the health of the knee joint with cartilage being able to adapt its structure, composition and metabolism to a wide range of activities (4-6). However, very high doses of exercise such as playing sports at elite level (7, 8) as well as the absence of exercise, in the forms of sedentary behavior (9) or immobilization (10), are associated with cartilage loss and OA development. Therapeutic exercise is a cornerstone in the management of OA, (11, 12). When prescribed for specific therapeutic goals, exercise has been shown to be clinically safe (13-15) and effective in reducing pain and improving function (16, 17). Yet, some patients with knee OA still believe that therapeutic exercise may be detrimental to their knee joints (18), constituting a barrier to exercise.

A moderate mechanical loading of the knee joint from exercise therapy is thought to slow down cartilage breakdown by balancing anabolic and catabolic reactions in the extracellular matrix (19). Molecular biomarkers in blood, urine and joint fluids are promising disease markers in predicting structural OA progression and assessing therapeutic response related to cartilage and inflammation (20, 21). People with knee OA have higher levels of circulating cartilage-derived biomarkers compared to healthy controls (22, 23). Systematic reviews, including overweight and normal weight youth (24) and adult (25) participants, with or without cardiovascular diseases (26), have shown a beneficial effect of exercise on reducing C-reactive protein (CRP),

a molecular biomarker related to systemic inflammation also involved in OA progression. In the OA population, individual studies indicate that single bouts of exercise therapy promote immediate changes to molecular biomarkers related to cartilage extracellular matrix (ECM) turnover (e.g. cartilage oligomeric matrix protein; COMP) and inflammation (e.g. interleukin 10; IL-10), that, in general, return to baseline levels after a short period of rest (6, 27-29). However, whether therapeutic exercise interventions have an impact on the molecular biomarker concentration has previously only been investigated in individual studies, and the effect has not been summarized in a systematic review and meta-analysis.

We aimed to investigate the impact of exercise therapy interventions on molecular biomarkers related to articular cartilage and inflammation, by systematically reviewing published randomized controlled trials in people at risk of, or with, established knee OA.

METHODS

Protocol

Study selection, eligibility criteria, data extraction and statistical analysis were performed according to the Cochrane Collaboration guidelines (30). The study has been reported according to the PRISMA guidelines and the study protocol was registered at PROSPERO (CRD42017055850).

Eligibility criteria

We included randomized controlled trials that investigated the impact of exercise therapy on molecular biomarkers related to cartilage and inflammation in people at risk of, or with established, knee OA. Studies were excluded when no-full text was

available, or when the active treatment arm involved other joint loading interventions besides exercise therapy.

Literature search

A systematic literature search was performed with no restriction on publication year and language in MEDLINE via PubMed, EMBASE via Ovid, CINAHL (including preCINAHL) via EBSCO, the Cochrane Central Register of Controlled Trials (CENTRAL) and Web of Science (WoS) up to January 2017. The search was re-run in these databases up to September 2017.

Search methods and study selection

The studies were identified by performing a customized search strategy (Supplementary file – MEDLINE search). All terms were searched, if possible, both as keywords [MeSH] and text words in titles and abstracts [TIAB]. In MEDLINE and EMBASE, animal studies were identified and removed before screening all the studies, using a validated animal filter (31, 32). At first, two of the authors (AB and CJ) independently screened titles and abstracts and all studies deemed eligible by at least one of the authors were checked independently in full text by the same reviewers. Disagreements regarding inclusion were discussed between the two reviewers until consensus was reached.

Data collection

Data were extracted by two of the authors (AB and CJ) from the manuscripts including tables and graphs of published manuscripts. A customized data extraction form was developed for each of the molecular biomarkers (33). The molecular biomarkers were grouped into synovial fluid, serum, plasma and urine. The following data extraction was mandatory: authors of the study, year of publication, design of

trial, intervention characteristics, location of the trial (in case of multi-center studies, the primary investigator's affiliation applied), number of patients allocated (to the exercise and control groups respectively), number of patients in the intention to treat (ITT) population (in the intervention and control groups respectively), the average patient age, average body mass index (BMI), number of females within the ITT population, duration of the study, intervention characteristics and site of collection of bio-fluid and analysis method of molecular biomarkers.

Molecular biomarkers classification

We classified molecular biomarkers based on their main function and grouped them into biomarkers of either inflammation or cartilage extracellular matrix (ECM) turnover. Molecular biomarkers related to inflammation were sub-grouped into: *markers of inflammation* (C-reactive protein [CRP], C-reactive protein degradation [CRPM], IL-6, tumor necrosis factor alpha [TNF-α] and transforming growth factor beta-1 [TGF-β1]), *cytokine receptors* (soluble interleukin 6 receptor [sIL-6r] and soluble TNF-α receptor-1 and -2 [TNFR1 and TNFR2]). Molecular biomarkers related to cartilage ECM turnover were sub-grouped into: *proteases* (matrix metalloprotease 3 [MMP-3]), *turnover of collagens* (type II collagen synthesis [type II collagen carboxy propeptide; CPII], type II collagen degradation [C2C neoepitope of type II collagen, C2M neoepitope of type II collagen, C-terminal crosslinking telopeptide of type II collagen; CTX-II] and total collagen [hydroxyproline; HP]); *glycoproteins* (cartilage oligomeric matrix protein [COMP]) and *glycosaminoglycans* (total GAG [DMMB], chondroitin sulfate [CS; 3B3 and 7D4], keratan sulfate [KS; 5D4] and hyaluronic acid [HA]).

Meta–analysis of a subset of molecular biomarkers

We performed meta-analyses when at least two study comparisons were available for

158 an outcome of interest. We estimated the standardized mean difference (SMD) as the
159 difference between mean change values (or post-intervention values when only post-
160 intervention data were available) in the intervention and control groups, divided by
161 the pooled standard deviation (SD), using a random effects model and Hedges'g
162 correction. The SD was extracted or estimated from the standard error (SE) of the
163 mean, the 95% confidence interval (95%CI), *P* value, or other methods recommended
164 by the Cochrane Collaboration (30). Between-study heterogeneity was calculated
165 using the I^2 statistic (34), measuring the proportion of variation (i.e., inconsistency) in
166 the combined estimates due to between-study variance (35). An I^2 value of 0%
167 indicates no inconsistency among the results of individual trials, while an I^2 value of
168 100% indicates maximum inconsistency. When several intervention groups were
169 compared to one control group, the number of participants in the control group was
170 divided by the number of intervention groups and each was analyzed as a separate
171 study comparison.

172 **Narrative synthesis of results**

173 For the effect of exercise therapy on molecular biomarkers, we reported a statistically
174 significant ($P < 0.05$) decrease or increase in molecular biomarker concentrations for
175 the exercise therapy group compared to the control group or no difference in (change
176 in) biomarker concentrations between the two groups. The effect estimates derived
177 from meta-analyses were included in the overall narrative synthesis of the results and
178 reported as a decrease if the SMD was less than -0.2; no difference if the SMD was
179 between -0.2 and 0.2; and as an increase if the SMD was higher than 0.2 (36).
180 We performed sub-group analyses on molecular biomarker localization (i.e. synovial
181 fluid, blood and urine), reporting the number of studies that reported a change in
182 concentration of the molecular biomarkers of interest.

When several intervention groups were included in a study, the between-group difference was reported for each possible comparison. For example, when a study had two intervention groups (A and B) and one control group (C), we compared A vs. C and B vs. C, and reported the results as two separate study comparisons. Although including multiple comparisons from the same study does not completely rule out dependence between estimates of effect in meta-analysis, this procedure is in accordance with the Cochrane handbook (30).

Sensitivity analysis

In addition, we performed a sensitivity analysis on the studies not included in the meta-analysis by calculating their effect size when sufficient data were available.

Quality of evidence

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) is a systematic approach to rate the overall quality of evidence, from high to very low. The presence of high-quality evidence indicates that 'future research is very unlikely to change the estimates of effect' while very low-quality evidence indicates that 'any estimate of effect is very uncertain'. The GRADE assessment involves the following domains: risk of bias (i.e. the methodological flaws of the studies); inconsistency (i.e. the heterogeneity of results across studies), indirectness (i.e. the generalizability of the findings to the target population), the precision of the estimates and the risk of publication bias (37).

Risk of bias and the overall quality of evidence was independently assessed by two authors (AB and CJ) using the GRADE approach (37). Disagreements in initial ratings of methodological quality assessment were discussed between two of the authors (AB, CJ) until consensus was reached. The risk of bias was assessed with

regard to the risk of selection bias, performance bias, detection bias, attrition bias, reporting bias and other sources of bias. Each of the following listed domains were assessed as adequate, unclear or inadequate: ‘sequence generation’, ‘allocation concealment’, 'blinding', ‘incomplete outcome data addressed’, ‘selective outcome reporting’ or ‘other bias’ (i.e. funding) (34).

Figure 1.

RESULTS

Study selection and characteristics

The literature search resulted in 4080 publications of which 42 individual studies were identified as potentially eligible and checked in full text. Ultimately, we included 12 papers involving 57 study comparisons (Figure 1). One study was reported in two different papers (38, 39). We included both papers and counted them as one study with two study comparisons, as suggested in the Cochrane guidelines. A subset of 31 study comparisons involving the molecular biomarkers of inflammation (CRP, IL-6 and TNF-a), cytokine receptors (sIL-6r, TNFR1 and TNFR2), type II collagen degradation (C2C and CTX-II) and glycoproteins (COMP) were included in the meta-analyses (Figure 2).

Participants

In the 12 papers, a total of 1114 participants were included of which 70% were women. Participant mean age was 65 (SD=5.7) years with a mean BMI of 29.7 (SD=3.2) kg/m^2. One study reported only the age range which was from 41 to 63 years (40) and one study included only those with BMI <35 (41). One study included

participants at risk of OA (i.e. no radiographic signs of OA, sedentary, age >60 and BMI >27) (42), and the remaining 11 studies included participants with or without pain but with radiographic knee OA ranging from Kellgren and Lawrence (KL) grade 1 to 4 (33, 39-41, 43-49) (Table 1).

Table 1.

Types of exercise therapy interventions and molecular biomarker outcomes

The types of exercise therapy interventions used were strengthening exercise in five studies (40, 41, 44-46), aerobic exercise in three studies (44, 48, 49) and a combination of strengthening and aerobic exercise in five studies (33, 39, 42, 43, 47) (Table 2).

Table 2.

Biomarker samples were obtained at 4 to 24 weeks following the exercise therapy intervention in all the studies. Additionally, three studies reported in four papers included one additional follow-up assessment at 18 months (38, 39, 42, 47), see supplementary Table S4 for a narrative synthesis of these results. However, we included the first available follow up time point in our analyses to allow for a more homogeneous time to follow-up ranging from 1 to 6 months across the included studies.

Of the 12 biomarker studies, five investigated markers of inflammation (39, 40, 42, 44, 47), two investigated cytokine receptors (42, 46), one investigated proteases (40), four investigated turnover of collagens (41, 43, 48), four investigated glycoproteins (33, 41, 45, 47) and five investigated glycosaminoglycans (41, 43, 47-49) (Table 3).

Some studies investigated more than one molecular biomarker. Detailed characteristics of molecular biomarkers are reported in Tables S1 and S2.

Overall narrative synthesis of results

Twelve studies included 57 study comparisons of which 63% (36 study comparisons) did not differ in molecular biomarker concentrations between the intervention and control groups. Thirty percent (17 study comparisons) reported a decrease and 7% (4 study comparisons) reported an increase in molecular biomarker concentration, all in favor of the exercise therapy intervention group. Results from individual studies are reported in Table 3.

Table 3.

Meta–analyses of a subset of molecular biomarkers

Meta-analyses showed statistically non-significant reductions of the molecular biomarkers CRP (SMD -0.78; 95% CI -2.01 to 0.44), CTX-II (SMD -0.84; 95% CI -2.65 to 0.97), TNF-a (SMD -0.28; 95% CI -0.85 to 0.29), TNFR1 (SMD -0.25; 95% CI -0.63 to 0.14), TNFR2 (SMD -0.18; 95% CI -0.57 to 0.20), C2C (SMD -0.29; 95% CI -1.05 to 0.47), and COMP (SMD -0.22 95% CI -0.63 to 0.18), all in favor of exercise therapy, and no effect for IL-6 (SMD 0.01; 95% CI -0.19 to 0.20) and sIL-6r (SMD 0.05; 95% CI -0.37 to 0.47) (Figure 2).

Figure 2.

Sub-group analysis on molecular biomarker collection site

For molecular biomarkers in synovial fluid, blood (serum and plasma) and urine, exercise therapy was associated with a change in biomarker concentrations in 50% (n=4), 36% (n=16) and 20% (n=1) of the study comparisons, respectively (calculated from Table 3). Further, 97% (n=28) of the studies on molecular biomarkers related to inflammation, and 89% (n=25) of the studies on molecular biomarkers of cartilage ECM turnover, were either unchanged or decreased after exercise (calculated from Table 3).

Sensitivity analysis for the effect of exercise on molecular biomarkers

Data were available to calculate an effect size for 51 out of 57 individual studies. Overall, the effect sizes for 44 out of 51 study comparisons supported our main analysis. The remaining seven study comparisons from three studies (41, 48, 49) changed from being classified as “no effect” to “decreased” for sCPII, uCTX-II, sHP, sf3B3, sf7D4, sf5D4 and sHA (Supp Table S3).

Quality of evidence

The majority of the studies applied proper randomization and allocation, although some studies failed to clearly report or adequately address dropouts of participants in the analyses (attrition bias) and failed to report whether outcome assessors (the people responsible for analyzing the samples) were blinded to the outcomes of interest (detection bias) (Figure S1). However, the high heterogeneity reported in some of the meta-analysis and the too few studies investigating the same outcomes, made us downgrade the quality of evidence for inconsistency (substantial heterogeneity) and imprecision (large 95% CI of the estimates).

DISCUSSION

We summarized the impact of exercise therapy on cartilage biomarkers in people at risk of, or with established, knee OA participating in randomized controlled trials. Our results suggest that exercise therapy is not harmful as it does not increase the concentration of molecular biomarkers related to inflammation and cartilage turnover, associated with cartilage breakdown. All in all, this finding was consistent in both the main and sensitivity analyses as the majority of studies point to either a decrease or an unchanged level. However, due to substantial heterogeneity and large confidence intervals in the meta-analysis estimates, the overall quality of evidence was downgraded to low.

Systematic reviews and meta-analyses have shown that exercise is a safe treatment associated with few and only minor adverse events, such as temporary flares in pain, in people with knee OA (13-17). Additionally, in a previous systematic review of exercise trials, we have shown that exercise is not harmful to cartilage when evaluated by MRI (50). The findings of the current study, evaluating the effect from exercise therapy on molecular biomarkers related to inflammation and cartilage extracellular matrix turnover, are in line with these previous findings and support exercise therapy being a safe treatment for knee joint cartilage in people at risk of, or with established, knee OA.

Molecular biomarkers obtained from the synovial fluid may be more sensitive for detecting changes from exercise therapy, due to the proximity of the synovial fluid to the joint tissues (51). In agreement, we found higher rates of biomarker concentration change in synovial fluid (50%) compared to that of blood (36%) or urine (20%).

Therefore, it is important to state the origin of the fluid when interpreting results from therapeutic studies.

As our meta-analyses indicate a reduction across the molecular biomarkers associated with inflammation (i.e. CRP and TNF-a) and cartilage breakdown or turnover (i.e. C2C, CTX-II and COMP) in favor of exercise therapy, it may be speculated that, if anything, exercise therapy is beneficial for cartilage assessed via molecular biomarkers; however, this hypothesis needs further investigation.

Limitations

This study has limitations. We could not perform meta-analyses of all the molecular biomarkers investigated due to the low number of studies reporting the same markers. Neither could we perform additional analysis, which is considered an important step in exploring relationships in evidence synthesis. Also, due to large heterogeneity and large confidence intervals, the overall quality of evidence was downgraded to low. To properly interpret this evaluation, it is important to note that the included studies followed the available guidelines in conducting and reporting the studies, and therefore, the low quality of evidence, rather than being related to methodological flaws of the studies, was caused by the limited number of randomized controlled trials in the literature.

Implication for researchers and clinicians

These results highlight the need for more high quality randomized controlled trials to further investigate the impact of knee joint loading exercise on cartilage and inflammation related to molecular biomarkers. Such studies should preferably include people at risk of, or at early stages of, OA, when the anabolic and catabolic reactions

in the cartilage extracellular matrix are better balanced and a therapeutic exercise intervention theoretically may have the ability to prevent or slow down the catabolic activities driving OA progression.

As no single biomarker has been shown to explain OA development and progression, defined by osteophyte formation or joint space narrowing, we recommend that future studies focus on a set of biomarkers, rather than single biomarkers, using established commercial biomarker assays such as those used in the OA Initiative (52).

The clinical implication of our findings is that people at risk of, or with established, knee OA can be told that exercise therapy is not harmful, and if anything, is positive for the turnover of articular cartilage and inflammation.

CONCLUSIONS

The therapeutic exercise commonly prescribed to prevent and treat symptomatic knee osteoarthritis appears safe for knee joint cartilage, as it does not increase the molecular biomarkers related to inflammation and cartilage turnover associated with osteoarthritis. However, due to the limited number of randomized studies, the overall quality of the evidence supporting this conclusion was downgraded to low.

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FIGURE LEGENDS

Figure 1. Flow chart of the included study.

Figure 2. Cumulative forest plot for the effect of exercise therapy on molecular biomarkers. S = serum; SF = synovial fluid; P = plasma; U = urine; n= Number; SMD= Standardized Mean Difference; CI= Confidence Interval; I^2= Statistical heterogeneity.