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# The Effect of Mechanical Load on Biomarkers of Knee Joint Inflammation for Individuals Who Are Predisposed to Knee Cartilage Degeneration: An Exploratory Study

Alyssa Evans  
*Brigham Young University*

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The Effect of Mechanical Load on Biomarkers of Knee Joint Inflammation  
for Individuals Who Are Predisposed to Knee Cartilage Degeneration:

An Exploratory Study

Alyssa Evans

A thesis submitted to the faculty of  
Brigham Young University  
in partial fulfillment of the requirements for the degree of

Master of Science

Matthew K. Seeley, Chair  
Robert D. Hyldahl  
J. Ty Hopkins

Department of Exercise Sciences

Brigham Young University

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## ABSTRACT

### The Effect of Mechanical Load on Biomarkers of Knee Joint Inflammation for Individuals Who Are Predisposed to Knee Cartilage Degeneration: An Exploratory Study

Alyssa Evans  
Department of Exercise Sciences, BYU  
Master of Science

#### *Objective*

Physical exercise decreases disability and pain associated with chronic articular cartilage degradation. However, understanding of the pathology is lacking. In this study, the levels of 17 biomarkers of inflammation and cartilage degradation were measured in synovial fluid (SF) before and after a 30-minute run in able-bodied and previously-injured individuals.

#### *Materials & Methods*

Four able-bodied recreational runners (3 men and 1 woman:  $24 \pm 2$  years,  $68 \pm 7$  kg, and  $173 \pm 9$  cm) and 4 recreational runners who had undergone a unilateral anterior cruciate ligament reconstruction (ACLR) (2 men and 2 women:  $23 \pm 1$  years,  $71 \pm 6$  kg, and  $175 \pm 4$  cm) were recruited to participate in this study. Using a saline-assisted method, SF was aspirated before and after both a 30-minute unloading and 30-minute exercise session. Samples were corrected for blood contamination and analyzed for 15 cytokines and 2 matrix metalloproteinases (MMPs). Mixed model analyses were used to determine the main effects of session, case/control status, pre/post aspirations, and the interactions between case/control status and pre/post aspirations.

#### *Results*

Blood protein contamination was calculated and accounted for in 15 of 32 synovial fluid samples. Granulocyte colony stimulating factor (GCSF) was the only detectable cytokine of the 15 analyzed. No statistical differences were found in GCSF concentrations between pretreatment and posttreatment aspirations ( $p = 0.45$ ), ACLR and able-bodied control groups ( $p = 0.60$ ), or unloading and exercise sessions ( $p = 0.96$ ). MMP-13 was undetectable. No statistical differences were found in MMP-3 between pretreatment and posttreatment aspirations ( $p = 0.15$ ), ACLR and able-bodied control groups ( $p = 0.85$ ), or unloading and exercise sessions ( $p = 0.14$ ).

#### *Conclusions*

Two (GCSF and MMP-3) of the 17 measured biomarkers were detectable. There were no significant differences in either GCSF or MMP-3 due to a 30-minute run or 30-minute unloading period in either the able-bodied or ACLR participants. Further, there were no significant differences between biomarker concentrations and case-control status. A novel method of controlling for blood contamination in synovial fluid samples was implemented.

Keywords: inflammation, articular cartilage degeneration, load

## TABLE OF CONTENTS

Title Page .....	i
ABSTRACT.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES .....	v
LIST OF FIGURES .....	vi
INTRODUCTION .....	1
METHODS .....	3
Subjects.....	3
Experimental Design.....	4
Synovial Fluid Sampling.....	5
Protein Concentration Standardization .....	5
MMP Analysis .....	7
Statistical Analysis.....	7
RESULTS .....	7
Cytokine Concentrations.....	7
MMP Concentrations .....	8
DISCUSSION.....	8
Cytokines: GCSF .....	9
Matrix Metalloproteinases: MMP-3 .....	10
Methodological Limitations.....	11
Correcting for Blood Contamination .....	12
CONCLUSION.....	13

CONFLICT OF INTEREST .....	13
REFERENCES .....	14

## LIST OF TABLES

Table 1 .....	22
Table 2 .....	23

LIST OF FIGURES

Figure 1 ..... 24

Figure 2 ..... 25

## INTRODUCTION

Chronic degradation of healthy knee articular cartilage is a common problem that leads to osteoarthritis (OA), the most common joint disease worldwide. There is currently no cure for chronic articular cartilage degeneration (ACD).<sup>1</sup> ACD can be painful and debilitating and it affects 10% of men and 18% of women over the age of 60.<sup>2</sup> Factors that influence chronic ACD progression are numerous and unclear. However, accumulating evidence suggests that the onset and progression of chronic ACD are partly influenced by knee joint load.<sup>3-5</sup> Joint biochemistry is another factor that is thought to influence ACD onset and progression. Increased, low-grade synovial inflammation is believed to precede structural degeneration<sup>6-8</sup>. Further, chronic intra-articular inflammation is a reliable predictive measure of ACD progression in early-stage patients<sup>9</sup>. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are examples of cytokines specific to inflammation. Both cytokines have been used to reflect ACD prevalence and progression.<sup>10,11</sup>

Stimulated by inflammatory cytokines, matrix metalloproteinases (MMPs) are partially responsible for chronic articular cartilage degradation and can be used as pathogenic biomarkers. MMP-3 and MMP-13 are 2 highly active MMP subtypes that play a role in ACD and have been targeted for therapeutic intervention.<sup>12,13</sup> MMP-3, also known as stromelysin-1, is one of the most abundantly expressed MMPs. It is responsible for the activation of MMP-1 and MMP-13, 2 types of collagenases with the unique ability to cleave the triple helix of collagen, allowing other proteinases to cause further degradation.<sup>38,39</sup> Increased levels of MMP-3 and MMP-13 have been found in synovial fluid of patients with chronic articular cartilage degradation when compared with healthy controls.<sup>15,16</sup>

Physical exercise is the most commonly recommended nonpharmacological intervention for patients with chronic articular cartilage degradation.<sup>17</sup> It is known to decrease disability and pain associated with chronic ACD.<sup>18–20</sup> Despite the prevalence of data that supports physical exercise as a successful symptomatic treatment for degenerative joint disorders like ACD and OA, the mechanism(s) underlying potential chondroprotective effects of exercise is(are) unclear. Physical exercise appears to be capable of attenuating systemic low-grade inflammation associated with a number of pathologic conditions including cardiovascular disease, diabetes, and obesity.<sup>21</sup> Additionally, we recently observed that a 30-minute running protocol decreased knee intra-articular inflammation for a small pilot sample of young, able-bodied subjects, indicating that running might protect articular cartilage for this demographic.<sup>22</sup> Further, studies show a significant change of serum MMP-3 concentration with running.<sup>23,24</sup> However, it remains unclear whether running can decrease knee-joint synovial fluid concentrations of inflammatory cytokines MMP-3, and MMP-13 for all individuals—including those who are elderly, are obese, or have suffered traumatic joint injury, such as an anterior cruciate ligament injury.

Anterior cruciate ligament injury reconstruction (ACLR) induces structural,<sup>25,26</sup> biochemical,<sup>26,27</sup> and biomechanical<sup>28,29</sup> changes that increase the risk of articular cartilage degradation up to 10 to 20 fold.<sup>30</sup> For example, decreased collagen synthesis, diluted synovial fluid, and increased (a) hemarthrosis, (b) cell necrosis, and (c) joint inflammation occur up to a year after traumatic knee injury.<sup>27,31,32</sup> Although some symptoms eventually subside, other effects appear to be permanent (eg, diluted synovial fluid).<sup>33</sup> The effect(s) that any, or all, of the aforementioned changes might have on the influence of exercise on joint biochemistry and corresponding/subsequent joint health is unclear. Evidence suggests that different loading protocols contribute to unique changes of biomarkers of cartilage turnover and inflammation in

the knee.<sup>34,35</sup> This may suggest that populations with different loading patterns would similarly have distinct biochemical changes in the knee. However, recent research has also shown that moderate acute exercise stimulated a similar biochemical (inflammatory) response in both injured and matched able-bodied controls. More research is needed to better understand the effects of load on biochemistry of normal and abnormal (injured) cartilage so that more supported conclusions can be made about the effects of load on articular cartilage health.

The purpose of this study was to evaluate how physical activity (an acute bout of exercise: a 30-minute running protocol) affects knee-joint inflammation and degradation for subjects with and without a history of ACLr. We hypothesized that running would decrease inflammatory cytokines, MMP-3, and MMP-13 in knees of able-bodied subjects, but either not change or increase inflammation in knees of ACLr subjects.

## METHODS

### *Subjects*

Four able-bodied recreational runners (3 men and 1 woman:  $24 \pm 2$  years,  $68 \pm 7$  kg, and  $173 \pm 9$  cm) and 4 recreational runners who had undergone a unilateral ACL reconstruction (2 men and 2 women:  $23 \pm 1$  years,  $71 \pm 6$  kg, and  $175 \pm 4$  cm) were recruited to participate in this study. All subjects provided informed consent in accordance with the appropriate Institutional Review Board and the Declaration of Helsinki.

ACLR participants were required to: (1) be between the ages of 18 and 30, (2) have a BMI between 18.0 and 24.9, (3) be able to run at 3.0 m/s for 30 continuous minutes, (4) be engaging in a minimum of 20 minutes of moderate physical activity 3 times a week, (5) have had a unilateral ACL reconstruction surgery between 2 and 6 years before data collection, and (6) have

physician approval to return to unrestricted participation in physical activity. Each able-bodied participant matched ACLr requirements 1 to 4.

The following criteria excluded participation from the ACLr group: (1) a lower extremity injury or pain limiting physical activity for more than 2 months in the past 6 months, (2) competing in athletic activities at the collegiate level, (3) balance or neuromuscular disorders, (4) regular daily intake of any anti-inflammatory medication, (5) a previous knee injury prior to the ACL tear that required surgery or limited physical activity for more than 3 months, or (6) previous revisions of a previous ACL reconstruction. The following criteria excluded participation from the able-bodied group: (1) ACLr group exclusion criteria 1 to 4 and (2) any previous lower extremity surgery.

### *Experimental Design*

Each participant completed 2 experimental sessions (exercise and unloading) in a counterbalanced order, 1 week apart. For both experimental conditions, baseline synovial fluid samples were collected at a local healthcare facility. Participants were then immediately transported to a nearby university biomechanics laboratory. Participants remained in an unloaded (seated) position during this transport. Approximately 15 minutes after the baseline samples were collected, participants ran for 30 minutes at a self-selected speed of 3.0, 3.5, or 4.0 m/s (all participants chose 3.0 m/s) on an AMTI (Watertown, MA, USA) treadmill. Immediately following the run, subjects were seated in a wheelchair. Participants were then transported back to the local health care facility, in an unloaded position (wheelchair), where a second synovial fluid sample was taken approximately 30 minutes after the completion of the run. The control session was identical to the run session except that the participants rested in an unloaded, seated position in the laboratory for 30 minutes rather than running for 30 minutes.

### *Synovial Fluid Sampling*

Aspirations were taken from the lateral suprapatellar space of the participant's affected knee using a 10-mL syringe and 18-gauge, 1.5" hypodermic needle. Initially we attempted (n = 2) to use an "incremental technique" as described by Driban et al,<sup>33</sup> whereby  $\leq 14$  mL of sterile saline was added to the joint in 2 mL increments until enough synovial fluid was aspirated. We found that the time required to inject a sufficient amount of saline into the joint was past the point of comfort for the participant. Instead, we standardized the protocol across the remaining subjects (n = 6). The physician first attempted a traditional, ultrasound-guided aspiration.<sup>33</sup> If unsuccessful, a saline-assisted aspiration<sup>33</sup> method was used by injecting 12 mL of saline into the joint, and again attempting the aspiration.<sup>36</sup> Once aspirated, protease inhibitor was added and samples were stored on ice until the end of the session and stored at  $-80^{\circ}\text{C}$  until analysis.

### *Protein Concentration Standardization*

In order to account for different amounts of saline in each sample, protein concentrations were quantified and normalized. Total protein concentrations (all synovial fluid and, if present, blood proteins) were measured using a Pierce Bicinchoninic Acid (BCA) Protein Assay Kit in compliance with manufacturer's parameters (Thermo Fisher Scientific, Waltham, MA, USA).

Many (15/32) of the synovial fluid samples were contaminated with blood. In an attempt to estimate the level of blood contamination, hemoglobin concentrations were measured using a Hemoglobin Colorimetric Detection Kit (Arbor Assays, Ann Arbor, MI, USA) following manufacturer's parameters. Blood volume was then estimated using Equation 1, under the assumption that 150 mg of hemoglobin are in 1 mL of blood.<sup>37</sup>

$$\text{Equation 1: Blood Volume (mL)} = \frac{\text{Hemoglobin Concentration } \left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Sample Volume (mL)}}{150 \left(\frac{\text{mg}}{\text{mL}}\right)}$$

Protein contamination from the blood was then calculated using Equation 2, under the assumption that, on average, 1 mL of blood contains 70 mg of protein.<sup>38</sup>

$$\text{Equation 2: Blood Protein Contamination (mg)} = \text{Blood Volume (mL)} \times 70 \left(\frac{\text{mg}}{\text{mL}}\right)$$

Blood protein contamination was then subtracted from total synovial fluid protein concentrations as measured using the aforementioned BCA assay.

### *Cytokine Analysis*

A Magpix multiplexing platform (Luminex, Austin, TX, USA) was used for multianalyte profiling of synovial fluid samples. Cytokines were measured using a 15-plex cytokine kit in compliance with manufacturer's parameters (EDM Millipore, Burlington, MA, USA). However, instead of adding 25  $\mu\text{L}$  of both sample and assay buffer into each respective well, we used the aforementioned corrected total synovial fluid protein concentrations to calculate the needed sample-to-assay buffer ratio to obtain 5  $\mu\text{g}$  of protein from each sample in the 50  $\mu\text{L}$  solution. The cytokines we analyzed included the following: GCSF, GM-CSF, IFN $\gamma$ , IL-10, IL-12p40, IL-13, IL-15, IL-1RA, IL-1 $\beta$ , IL-1 $\alpha$ , IL-4, IL-6, IL-8, MIP-1 $\beta$ , and TNF $\alpha$ . The 5  $\mu\text{g}$  of synovial fluid sample was incubated overnight at 4°C with antibody-conjugated magnetic beads. The bead complex was then washed and followed by a 1-hour incubation at room temperature on a plate shaker in biotinylated detection antibody. Streptavidin–phycoerythrin was subsequently added and samples were incubated for an additional 30 minutes on a plate shaker at room temperature. A Magpix system was used to quantify bead complexes. All samples were run in duplicate. Two quality controls were used to ensure the assay was working correctly. Cytokines that measured below the lower limit of quantification in greater than half of the samples among the 8 subjects were considered undetectable and not included in the analysis.

### *MMP Analysis*

MMP-3 and MMP-13 were measured using a 2-plex MMP kit in compliance with manufacturer's parameters (EDM Millipore, Burlington, MA, USA). However, instead of adding 25  $\mu$ L of both sample and assay buffer into each respective well, we used the aforementioned corrected total synovial fluid protein concentrations to calculate the needed sample-to-assay buffer ratio to obtain 5  $\mu$ g of protein from each sample in the 50  $\mu$ L solution. The 5  $\mu$ g of synovial fluid sample was incubated for 2 hours at room temperature with antibody-conjugated magnetic beads. The bead-complex was then washed, followed by a 1-hour incubation at room temperature on a plate shaker in biotinylated detection antibody. Like the cytokine analysis, streptavidin–phycoerythrin was subsequently added and samples were incubated for an additional 30 minutes on a plate shaker at room temperature. A Magpix system was used to quantify bead-complexes. MMP-13 measured below the lower limit of quantification in greater than half of the samples among the 8 subjects and was not included in the analysis.

### *Statistical Analysis*

Both protein and cytokine concentration residuals were randomly scattered around zero and therefore considered to be normally distributed. Mixed model analyses were used to determine the main effects of session, case/control status, pre/post aspirations, and the interactions between case/control status and pre/post aspirations. All statistical analyses were conducted using JMP 13 Statistical Software (SAS Institute, Cary, NC, USA).

## RESULTS

### *Cytokine Concentrations*

Fourteen of 15 cytokines were undetectable across  $\geq 75\%$  of our samples. Granulocyte-colony stimulating factor (GCSF) was detectable in each of the 32 samples. One of the samples

was thrown out of the analysis, as it was 13 times higher than the second highest concentration of GCSF (Table 1). We suspect that this outlier is a result of a pipetting error.

Changes in GCSF concentrations were highly variable in each of the unloading and exercise sessions (Figure 1) and in both ACLr and able-bodied controls. We observed no significant differences in the main effects of pretreatment and posttreatment aspirations ( $p = 0.45$ ), case/ control status ( $p = 0.60$ ) or session ( $p = 0.96$ ).

### *MMP Concentrations*

MMP-13 was undetectable across  $\geq 75\%$  of our samples. MMP-3 was detectable in 31 of the 32 samples. The sample that measured below the detectable limit was indicated as the value of the lowest point on the calibration curve (74 pg/mL) divided by 2 (37 pg/mL). A list of MMP-3 concentrations is shown in Table 2.

Changes of MMP-3 concentration in both the unloading and exercise sessions are shown in Figure 2. We observed no significant difference in synovial fluid protein concentrations between pretreatment and posttreatment aspirations ( $p = 0.15$ ), ACL and able-bodied control groups ( $p = 0.85$ ) or unloading and exercise sessions ( $p = 0.14$ ).

## DISCUSSION

The purpose of this project was to evaluate how a 30-minute run affects concentrations of biomarkers of knee-joint articular cartilage degradation (specifically inflammatory cytokines and MMPs) for subjects with and without a history of ACLr. We hypothesized that running would decrease concentrations of these biomarkers in knees of able-bodied subjects and would either not change or increase in subjects with a history of ACLr. We also hypothesized that these biomarker concentrations would be higher at baseline in knees with a history of ACLr than knees of able-bodied subjects. Our results suggest that there is no difference in inflammatory cytokine

or MMP concentrations between normal, healthy and previously reconstructed knees following either loading or unloading. However, due to the difficulty of extracting synovial fluid samples from healthy joints, and our chosen saline dilution methodology, our assessment has major limitations. In this study we also developed and implemented a novel approach to correct for blood contamination of synovial fluid samples.

*Cytokines: GCSF*

Inflammation is known to be a predecessor of cartilage degeneration and a potential indicator of joint health. In this study, granulocyte colony stimulating factor (GCSF) was the only inflammatory cytokine from our panel that was detectable in all of our samples. It is an anabolic cytokine and is activated by proinflammatory cytokines IL-1, IL-6, and TNF $\alpha$ .<sup>39</sup> The role of GCSF in articular cartilage health remains inconclusive; some evidence suggest that it plays a positive role in articular cartilage health.

It has been reported that GCSF concentrations are decreased in OA synovial fluid when compared to healthy controls ( $p < 0.05$ ),<sup>40</sup> and that injecting GCSF subcutaneously in rats promoted faster cartilage repair.<sup>41</sup> Further, an increase in blood serum GCSF concentration corresponded with a significant ( $p = 0.04$ ) decrease in pain for those with total knee arthroplasty when compared to those that did not have a change in pain.<sup>42</sup> This negative correlation between synovial fluid concentration and serum concentration of biomarkers of ACD has been demonstrated.<sup>22</sup> It is speculated to be caused by the potential transfer of the biomarkers from the synovial fluid into the blood serum.

In counterpoint, GCSF may instead have a negative effect on articular cartilage health as it has been shown to act similarly to granulocyte macrophage colony stimulating factor (GMCSF),<sup>43</sup> a proinflammatory cytokine. GMCSF in synovial fluid is known to be significantly

elevated in cases of knee OA<sup>44,45</sup> and in cases of knee injury that precede knee OA.<sup>46</sup> Some also show that neutralization of GMCSF might effectively control OA pain and progression.<sup>47</sup> In our recent study we saw that a 30-minute run significantly ( $p = 0.03$ ) decreased synovial fluid GMCSF concentration,<sup>22</sup> supporting the belief that physical activity reduces risk for OA.<sup>17</sup>

However, the results from this study demonstrated that there were no significant differences in GCSF concentrations between ACL injured and uninjured subjects. Unfortunately, our assessment of joint inflammation was limited to GCSF, as our chosen methodology (saline dilution of synovial fluid) led to the unexpected finding that 15 of 16 biomarkers were undetectable.

#### *Matrix Metalloproteinases: MMP-3*

MMPs are stimulated by proinflammatory cytokines and contribute to the degradation of cartilage in both normal and pathologic conditions.<sup>48,14</sup> MMP-3 was the one detectable of two measured MMPs in our analysis. Studies have shown that serum MMP-3 concentrations increase in the serum with running,<sup>23,24</sup> with a positive correlation between speed and MMP-3 concentration.<sup>23</sup> Accordingly, we expected to see (1) a significant decrease of synovial fluid MMP-3 concentration after 30 minutes of running and either no change or a significant increase after 30 minutes of unloading and (2) generally higher concentrations in ACLr participants compared to able-bodied participants. The results from this study do not support our hypothesis, as there were no statistically significant differences between sessions, pre/post aspirations, or case/control status.

Possible explanations as to why our results differ from expectations are (a) our sample size was too small to identify statistically significant results, (b) the 30-minute unloading and exercise periods were not long and/or intense enough to elicit a statistically significant result, (c)

the change of MMP-3 synovial fluid concentration does not occur within the 15-minute period between the 30-minute session and aspiration, (d) a change of MMP-3 concentration occurs and subsequently reverts to base level concentrations, or (e) joint health post-ACLR may greatly vary between subjects due to an individualized reconstruction and/or rehabilitation causing individualized responses to joint loading. These factors must be addressed before a strong conclusion about the effects of joint loading (eg, running) on concentrations of MMP-3 can be drawn.

### *Methodological Limitations*

Synovial fluid cytokine concentrations have been successfully measured during a state of high effusion with an associated increased volume of synovial fluid (eg, after injury or surgery).<sup>49-51</sup> Aspirations of noneffused, but pathological knees is still a novel approach, successfully completed by only a few laboratory groups.<sup>11,52</sup> Furthermore, the aspiration of healthy (no symptomatic or radiographic evidence of OA), noneffused knees is even more uncommon, with successful reports, as far as we have identified, coming from only two laboratories.<sup>36,53</sup> As determined by a previous study performed by our lab, it is very difficult to obtain sufficient sample volume using the traditional aspiration method on healthy, noneffused knees.<sup>22</sup> Accordingly, we attempted a saline-assisted aspiration protocol based on the study by Driban et al.<sup>36</sup> They reported that a saline-assisted aspiration protocol may be a viable way to measure biomarkers of OA in a noneffused, healthy knee.

In their study, Driban et al<sup>36</sup> reported detectable levels of MMP-3, IL-13, IL-10, and IL-1 $\beta$ . In our study, of these 4 biomarkers we measured detectable concentrations for only MMP-3. Reasons vary for why the other 14 biomarkers (including IL-13, IL-10, and IL-1 $\beta$ ) that we measured were undetectable. It is possible that (1) the studied populations (ACLR and normal,

healthy knees) have lower concentrations than the OA population Driban et al measured and/or (2) some cytokines that we analyzed are, in actuality, not present. Driban did use a slightly more sensitive assay (SearchLight Proteome Multiplex Protein Array (Aushon BioSystems, Billerica, MA, USA)), but we think that using the same assay would not have greatly affected our final results.

This saline-assisted process is contrasted by the traditional technique used by our lab group previously,<sup>22</sup> which obtained straight, undiluted synovial fluid samples. The resulting pure samples of synovial fluid allowed for complete analysis of 11 OA biomarkers. The difficulty with this technique is that 1 in 5 aspirations were not successful, making it very difficult to obtain enough complete sets of samples. Future studies might consider (a) a larger sample size, (b) a longer/more intense exercise session, and (c) immediate and additional aspirations after the 30-minute session. Additionally, new approaches to concentrate saline-diluted synovial fluid samples to ensure adequate protein concentration for analysis should be explored.

#### *Correcting for Blood Contamination*

A novel component of the preset study was the development of a method for controlling for blood contamination in synovial fluid samples. Due to high protein concentration in blood, it is difficult to measure synovial fluid dilution when there is blood in the sample. In previous literature, samples of synovial fluid have been excluded from the study due to blood contamination.<sup>36,54</sup> Had we collected blood samples from each participant, we could have measured individualized hemoglobin and total protein concentrations (as found in Equations 1 and 2) without making any assumptions. Yet, even without the addition of the blood draw, our approach may be viable to control for blood contamination in synovial fluid samples.

## CONCLUSION

Two (GCSF and MMP-3) of the 17 measured biomarkers were detectable. There were no significant differences in either GCSF or MMP-3 due to a 30-minute run or 30-minute unloading period in either the able-bodied or ACLr participants. Further, there were no significant differences between biomarker concentrations and case-control status. It is difficult to draw additional strong conclusions from our study as we were not able to comprehensively test our hypothesis (many cytokines were undetectable). A novel method of controlling for blood contamination in synovial fluid samples was implemented. Future research aimed at identifying ways to successfully obtain and measure synovial fluid samples from non-effused knees should be conducted.

## CONFLICT OF INTEREST

The authors report no conflict of interest.

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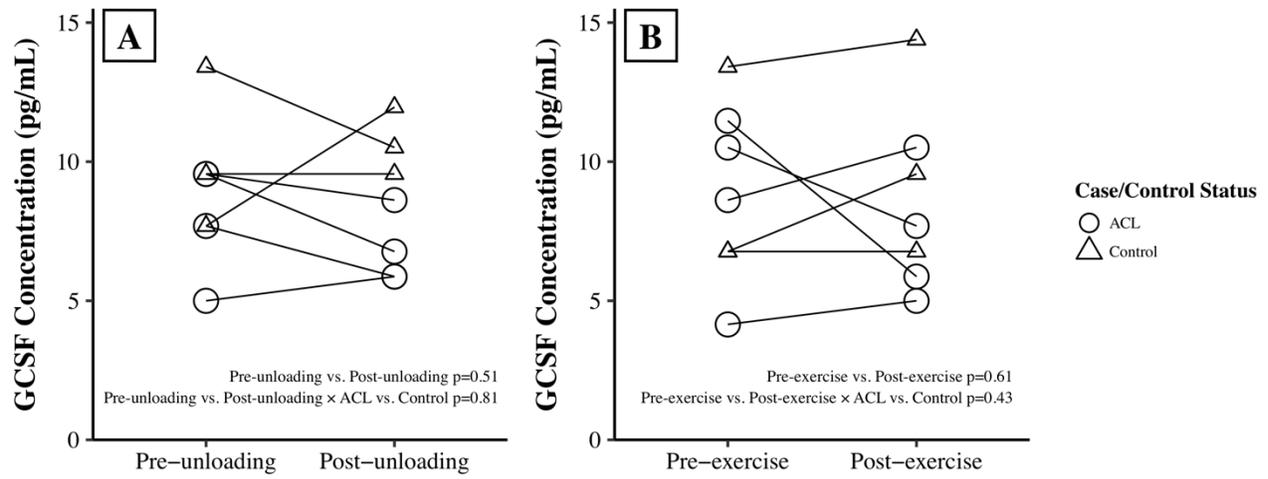
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**Table 1:** Concentrations of Granulocyte-colony stimulating factor (GCSF) before and after each unloading and exercise session. \*This sample was thrown out.

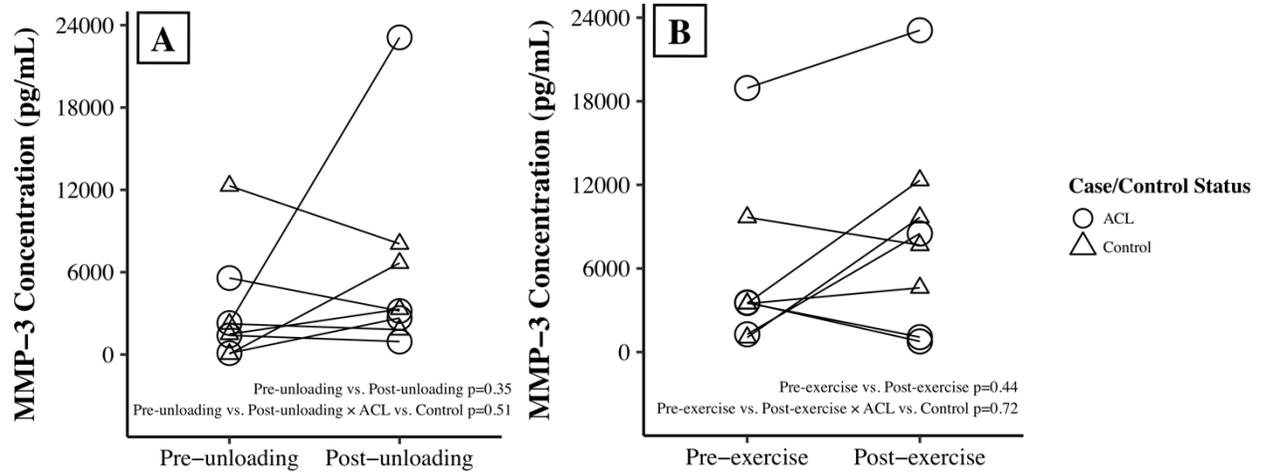
Participant Number	Case/Control Status	GCSF Concentration (pg/mL)			
		Unloading		Exercise	
		Pre	Post	Pre	Post
1	Control	186.92*	3.34	5.87	3.34
2	Control	9.56	9.56	6.77	6.77
3	Control	13.41	10.51	6.77	9.56
4	Control	7.69	11.96	13.41	14.39
Mean (1-4)	Control	10.22	8.84	8.21	8.52
5	ACL	9.56	6.77	8.62	10.51
6	ACL	7.69	5.87	11.47	5.87
7	ACL	9.56	8.62	4.15	5.00
8	ACL	5.00	5.87	10.51	7.69
Mean (5-8)	ACL	7.95	6.78	8.69	7.27
Mean (1-8)	Control/ACL	8.92	7.81	8.45	7.89

**Table 2 :** Concentrations of MMP-3 before and after each unloading and exercise session.

Participant Number	Case/Control Status	MMP-3 Concentration (pg/mL)			
		Unloading		Exercise	
		Pre	Post	Pre	Post
1	Control	37.36	6673	9676	7678
2	Control	102.68	2661	1278	8511
3	Control	2301	23114	18951	23096
4	Control	1397	945.19	3526	1074
Mean (1-4)	Control	959.51	8348.30	8357.75	10089.75
5	ACL	12294	8067	3498	12328
6	ACL	2237	1814	3478	4617
7	ACL	1487	3310	1051	9670.00
8	ACL	5577.00	3178	3565	765.28
Mean (5-8)	ACL	5398.75	4092.25	2898.00	6845.07
Mean (1-8)	Control/ACL	3179.13	6220.27	5627.88	8467.41



**Figure 1:** GCSF concentrations do not significantly change following a 30-minute (A) unloading or (B) exercise session in either the ACLr or control group.



**Figure 2:** MMP-3 concentrations do not significantly change following a 30-minute (A) unloading or (B) exercise session in either the ACLr or control group.