2013-06-14

The Effect of Anterior Knee Pain on Serum Cartilage Oligomeric Matrix Protein and Muscular Cocontraction During Running

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

Master of Science

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June 2013

ABSTRACT

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Knee pain can alter lower-extremity neuromechanics and often results in functional disability. The relationship between lower-extremity neuromechanical alterations, due to anterior knee pain, and articular cartilage condition is unclear. The purpose of this study was to determine the independent effect of anterior knee pain during running on articular cartilage condition, as reflected by serum cartilage oligomeric matrix protein concentrations and muscle cocontraction duration. Seven men and five women completed a 30-min run in three different sessions: control (no infusion), sham (isotonic saline infusion), and pain (hypertonic saline infusion). Saline was infused into the right infrapatellar fat pad for the duration of the run. Subject-perceived pain was recorded every 3 min on a 100-mm visual analog scale. During the run, bilateral electromyography was recorded for five leg muscles, and heel and toe markers were used to track foot position. During the 30-min run of the pain session average subject-perceived pain was 27.8 (SD = 2.3 mm) and 19.7 (SD = 1.9) mm greater than during the control (0.0 mm) and sham (8.1 mm) session, respectively ($p < 0.01$). Knee pain while running did not result in changes in muscular cocontraction duration ($p = 0.13$). Blood samples were drawn prior to the run, immediately following the run, and 60 min following the run. Samples were analyzed using enzyme-linked immunosortbent assay to determine serum cartilage oligomeric matrix protein concentration. Average serum cartilage oligomeric matrix protein concentration was 14% greater at immediate post run (132.19 ± 158.61 ng/ml; Range = 22.61-290.81 ng/ml) relative to pre run (116.02 ± 118.87 ng/ml; Range = 19.81-234.89 ng/ml) ($p < 0.01$), and 18% less at 60 min post run (108.45 ± 171.78 ng/ml; Range = 20.84-280.23 ng/ml) relative to immediate post run (Figure 4; $p < 0.01$). Serum cartilage oligomeric matrix protein did not significantly differ between baseline and 60 min post-exercise ($p = 0.29$). There was not a difference in cartilage oligomeric matrix protein concentration between sessions. Knee pain while running does not cause an increase in serum cartilage oligomeric matrix protein concentration ($p = 0.29$). There are two important findings from this study. First, anterior knee pain during a 30 min running session does not appear to independently affect cartilage oligomeric matrix protein concentrations. This implies other factors, aside from anterior knee pain alone, influence articular cartilage degradation during movement that occurs while individuals are experiencing anterior knee pain. Second, the present experimental anterior knee pain model can be used to evaluate the independent effects of anterior knee pain over an extended duration while subjects perform a dynamic activity like running.

Keywords: cartilage oligomeric matrix protein, experimental knee pain, electromyography, articular cartilage, exercise

ACKNOWLEDGEMENTS

First, I would like to acknowledge and give thanks to my supportive, encouraging, and loving family, especially my parents Tim and Sally. I acknowledge and am grateful for a loving Heavenly Father who has been supportive through my time at BYU and helped bless and direct my life through friends, teachers, and bishoprics. I would also like to thank my committee chair, Dr. Matthew K. Seeley, for his help, encouragement, and direction in this process along with committee members Dr. J. Ty Hopkins and Dr. Alan Parcell who have all helped refine my thesis into the document it is today. Thank you to my fellow students who offered support and help, specifically Matthew Denning for being a mentor from my initial proposal writing, through data collection, statistical analysis and to completion of this document; as well as Michael Leavitt for his help in the lab and in directing me through the thesis process. I appreciate the help I have received from our department secretaries Kim Poole and Maggie Shibla, as well as our graduate coordinator Dr. Brent Feland. I would also like to acknowledge Dr. Pat Vehrs for his help in coaching me through the IRB approval process. Finally, thank you to those who participated as subjects in my study

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Introduction

Knee pathology is common. Annual costs that are associated with knee pathology are approaching twenty billion dollars.[1] Although signs and symptoms of knee pathology vary and are numerous, pain is a chief symptom.[2] Knee pain has even been described as the latest musculoskeletal epidemic.[3] Forty two percent of running injuries affect the knee, with 62% of these knee injuries being attributed to anterior knee pain.[4] Knee pain alters lower-extremity neuromechanics[5] and often results in functional disability.[2] Anterior knee pain can independently alter lower-extremity neuromechanics in a way that likely results in abnormal loading of knee joint articular cartilage;[6] i.e., independent from all other knee pathology factors (e.g., joint inflammation or degradation), anterior knee pain alters lower-extremity neuromechanics in a way that abnormally loads knee joint articular cartilage.[2,5,6] With this said, the relationship between lower-extremity neuromechanical alterations, due to anterior knee pain, and articular cartilage condition is unclear.

Cartilage oligomeric matrix protein (COMP) is an important component of articular cartilage. It is a structural protein that is thought to help maintain the collagen matrix and contribute to mechanical properties of articular cartilage.[7] Researchers have hypothesized that serum COMP concentration in able-bodied individuals reflects (1) healthy articular cartilage remodeling, as a result of exercise for able-bodied individuals,[7-9] and/or (2) articular cartilage degradation associated with chronic knee pathology.[10,11] In addition to serum COMP concentration, researchers have linked leg muscle activation patterns during exercise to changes in knee articular cartilage volume; specifically, increases in cocontraction duration (i.e., the time that knee flexor and extensor muscles are simultaneously active) during the stance phase of running has been associated with knee joint articular cartilage volume decrement.[7]

Theoretically, increased muscle cocontraction during human movement would increase knee joint load and result in increased release of COMP into the blood serum.

The purpose of this study was to answer the following research question: does anterior knee pain during running, independent from other knee pathology factors, affect serum COMP concentration and muscle cocontraction duration? Related to this research question, we formulated two hypotheses: (1) running with anterior knee pain would result in asymmetrically increased lower-extremity muscle cocontraction duration for the involved (pain) leg, relative to the uninvolved (no pain) leg, and (2) running with anterior knee pain would cause a greater serum COMP concentration increase than running without anterior knee pain.

Methods

Subjects

A convenience sample of 12 able-bodied subjects (seven males and five females, age = 22 ± 4 years, mass = 72 ± 28 kg, height =1.79 ± 0.27 m, body mass index = 22.2 ± 3.7) volunteered to participate in this study. We required subjects to have no (1) history of lower-extremity injury within six months prior to participation or (2) any form of knee related surgery in their lifetime. Because physical activity level and body mass index (BMI) influence serum COMP levels before and following physical activity,[8] we recruited subjects with similar physical activity levels and BMI: subjects were each physically active (participate in moderate physical activity at least 3 times a week) and had a BMI between 18.5 and 25. We instructed subjects to refrain from exercising between data collection sessions.

Experimental Protocol

The subjects completed three different data collection sessions (control, sham, and pain) in a counterbalanced order, 48 h apart. For each session, subjects ran for 30 min on a treadmill.

During the control session, subjects ran on a treadmill in a normal fashion. During the sham session, subjects ran while receiving a continuous infusion of isotonic saline (0.9% NaCl) into the infrapatellar fat pad, at a rate of 0.216 mL · min^{-1} (total infused volume = 8 mL). During the pain session, subjects ran while receiving a continuous infusion of hypertonic saline (5.0% NaCl) into the infrapatellar fat pad, at the same rate and volume as the sham session. For each data collection session, subjects reported to the laboratory in the same athletic shoes and were given running shorts and a shirt to wear. Prior to all data collection sessions, subjects provided informed consent. All sessions were completed in the same biomechanics research laboratory. Data collection procedures were approved by the appropriate institutional review board before data collection.

For each data collection session, subjects first rested on a chair for 30 min to limit potential influence of preceding activity on serum COMP level.[8,14] After this 30-min rest, subjects stood for 10 min to allow for body fluids to be at a similar level across all blood draws. Then, a baseline blood sample was drawn (Blood Sample 1). Subjects then completed one of three running sessions: (1) control running (no infusion), (2) sham running (isotonic saline infusion), or (3) pain running (hypertonic saline infusion). A second blood sample was drawn immediately after the 30-min run (Blood Sample 2). Subjects then rested, seated, for 60 min, and a third blood sample was then drawn (Blood sample 3).

For each data collection session, after the initial 30-min rest and 12 min prior to the 30-min run, subjects warmed up by running on the treadmill at one of three speeds (3.0, 3.5, or 4.0 m/s) for 5 min. The average running speed for the present sample was 3.46 m/s. We instructed the subjects to select the greatest of three speeds they could run at for 30 continuous min. The same speed was used for each data collection session. After the aforementioned 5-min

warm-up, subjects lay supine on a table. For the sham and pain sessions, the isotonic and hypertonic saline infusions were initiated, respectively. For these infusions, the skin was shaved, if needed, and prepared with iodine and an alcohol wipe. Next, the catheter was inserted into the lateral superior portion of the infrapatellar fat pad of the right leg using previously described methods.[6] We used dynamic ultrasound in pilot testing to ensure correct placement of the catheter. After the catheter was placed and secured with medical and pro-flex tape, a plastic 30-inch connection tube was used to connect the catheter (B. Braun Medical Inc., Melsungen, Germany) to a portable syringe pump (Graseby Medical Ltd., Watford, England, UK). For the duration of the sham and pain running, this pump was held in a pocket that was sewn into a spandex shirt (Figure 1). Subjects were blinded regarding which saline had been prepared for infusion. After the infusions were initiated, subjects lay supine for 3 min, sat upright for 2 min, and stood for 2 min. We intended that these 7 min would help the subjects become familiar with the experimental knee pain (i.e., avoid passing out). Subjects then ran for 30 min at the selected speed while the saline was infused. The control session was identical to sham and pain sessions; however, no infusion was administered. For each session, we measured subject-perceived pain every 3 min, from the time of infusion to 60 min post-run, using a 100-mm visual analog scale. The general timeline for the three data collection sessions is described in Figure 2.

Serum COMP

All blood samples (6 ml) were drawn from an antecubital vein using a 20 gage shielded I.V. catheter (Becton Dickinson & Company, Franklin Lakes, NJ, USA). After placing the catheter, we flushed the catheter with 0.9% isotonic saline every 15 min to prevent clotting. After withdrawing the blood samples, we placed them in EDTA vacutainers (Becton Dickinson & Company, Franklin Lakes, NJ, USA) and then centrifuged them with an Eppendorf 5403

refrigerated centrifuge (Hamburg, Germany) for 15 min at 3,000 × gravity. The blood samples were then stored in a freezer at -20°C until analyzed. Serum COMP concentrations were determined using commercially available enzyme-linked immunosortbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN, USA). Assay procedures were conducted according to this specific human COMP immunoassay. Each sample was analyzed in triplicate across three ELISA plates. The present ELISA assay kits had inter- and intra-plate coefficients of variation of 13.66% and 2.61%, respectively, for a 273.3 ± 33.8 ng/ml sample.

Electromyography

Surface electrodes were applied bilaterally to five leg muscles in order to record electromyography (EMG) data (1000 Hz): vastus medialis (VM), vastus lateralis (VL), medial hamstring (HM), lateral hamstring (HL), and medial gastrocnemius (GM). Skin preparation procedures and electrode application locations followed previously recommended techniques.[12] The electrodes had 4 parallel bar contacts made of 99.9% silver that were 5 × 1 mm, and had a 1-cm interelectrode distance. The common mode rejection ratio was > 80 dB. These electrodes were applied for each session, after the subject's initial 30-min rest on the chair, and prior to the warm-up run. Thirty seconds of EMG data were recorded at four different parts of the 30-min run: 1, 10, 20, and 30 min into the run (Figure 2). The EMG data were treated using custom algorithms in MatLab (MathWorks, Natick, MA, USA): the DC offset was removed and a root mean square algorithm (moving window = 50 ms) was used to rectify and smooth the signal.[7] Each muscle was considered to be on when its amplitude was greater than 20% of the maximum reference amplitude. This reference amplitude was calculated as the average of ten amplitude peaks, from ten separate stance phases during the warm-up run.[7,13] Muscle cocontraction duration was determined to be the percentage of stance that at least one knee flexor and extensor

were on. The stance phase of running was determined using high-speed videography (200 Hz; VICON, Santa Rosa, CA, USA) and reflective markers that were placed bilaterally on the heel and toe. Five stance phases were manually identified during each of the four aforementioned 30-second trials (Times 1, 10, 20, and 30 min); muscle cocontraction duration was averaged across these five stance phases. Then, these averages were averaged across the four different times, representing the entire 30-min run. EMG data were recorded using Trigno Wireless sensors (Delsys, Boston, MA, USA).

Statistical Analysis

The independent variables for this study were session, time, and leg. The dependent variables were serum COMP concentration, lower-extremity muscle cocontraction duration, and subject-perceived pain. Serum COMP concentrations were compared between sessions across three times: Time -12 min (baseline), Time 30 min (immediately post-exercise), and Time 90 min (1 h post-exercise). Lower-extremity muscle cocontraction duration was compared between sessions, and between the involved and uninvolved legs. Subject-perceived pain was compared between sessions across 10 times (every 3 min between Times 0 and 30 min). The influence of session, time, and leg on the dependent variables was evaluated using repeated measures ANOVAs ($P < 0.05$). If a session × time interaction was detected, Tukey's post hoc comparisons were used to evaluate between time differences for each session ($P < 0.05$).

Results

We observed a significant condition × time interaction for subject-perceived pain ($p < 0.01$; Figure 3): pain increased across time for the pain session only. Across the entire 30-min run during the pain session, average subject-perceived pain was 27.8 (SD = 2.3 mm) and 19.7

(SD = 1.9) mm greater than average pain during the control (0.0 mm) and sham (8.1 mm) sessions, respectively ($p < 0.01$).

Although subject-perceived pain was greater during the pain session, we did not observe a session × time interaction for serum COMP ($p = 0.57$; Figure 4). Session did not have a significant main effect on serum COMP ($p = 0.48$). Time, however, did affect serum COMP ($p < 0.01$): pooling data from each session, average serum COMP concentration was 14% greater ($p < 0.01$) at immediate post run (132.19 ± 158.61 ng/ml; Range = 22.61-290.81 ng/ml), relative to pre run (116.02 ± 118.87 ng/ml; Range = 19.81-234.89 ng/ml), and 18% less ($p < 0.01$) at 60 min post run (108.45 ± 171.78 ng/ml; Range = 20.84-280.23 ng/ml), relative to immediate post run (Figure 4). Serum COMP concentration did not significantly differ between baseline and 60 min post-exercise ($p = 0.29$). Additionally, repeated measures ANOVAs were also used to determine that serum COMP concentration was not affected by running speed ($p = 0.79$), BMI ($p = 0.44$), gender ($p = 0.93$), age ($p = 0.51$), or activity level ($p = 0.62$).

Finally, lower-extremity muscle cocontraction duration was not influenced by session ($p = 0.13$; Figure 5), neither was there an asymmetrically increased muscle cocontraction duration for the involved leg, relative to the uninvolved leg in any session ($p = 0.28$). To summarize our results: (1) as expected, subject-perceived pain increased during the pain session, but not for the control and sham sessions; (2) serum COMP concentration increased as a result of running during each session, however, this increase did not differ between sessions (i.e., subject-perceived pain did not influence serum COMP concentration); and, (3) there were no differences in lower-extremity muscle cocontraction duration between sessions, or between the involved and uninvolved legs.

Discussion

We conducted this study to answer the following research question: Does anterior knee pain during running, independent from other knee pathology factors, affect serum COMP concentration and lower-extremity muscle cocontraction duration? We hypothesized that, because anterior knee pain influences lower-extremity neuromechanics[2,5,6] in a way that may abnormally load the knee joint, anterior knee pain would also increase: (1) lower-extremity muscle cocontraction durations and (2) serum COMP concentration. Our results show that, during running, anterior knee pain does not independently affect lower-extremity muscle cocontraction duration or serum COMP concentration (Figure 4 and 5).

Although influence of exercise on COMP has been studied in various samples, no one had measured the effect of anterior knee pain during exercise on COMP prior to this study. The present experimental knee pain model effectively and significantly induced anterior knee pain for the duration of the run (Figure 3). The experimental knee pain model used in this study supports the idea, previously described by Bennell[14] and Henriksen,[15] that hypertonic saline, injected into the infrapatellar fat pad, can be used to induce experimental anterior knee pain. Adapting the previously used injection models,[14,15] into an infusion model, is an effective method to induce experimental anterior knee pain for a minimum duration of 30 min, during dynamic activity, and further adds to research involving experimental anterior knee pain.

The present data suggest that anterior knee pain, alone, does not affect lower-extremity muscle cocontraction durations in the involved or uninvolved leg during running in able-bodied subjects; however, researchers have reported altered movement mechanics, as a result of pain.[6,16] Our measure of lower-extremity muscle cocontraction duration is an indirect indicator of lower-extremity mechanical changes; therefore, our subjects may have experienced mechanical

changes which we were unable to observe but otherwise would have with a more direct measurement such as joint angles or joint moments. Although we did not observe a bilateral statistical difference for lower-extremity muscle cocontraction duration, there did appear to be a trend indicating that during the painful condition, cocontraction durations in the involved leg may be less than for the uninvolved leg (Figure 5). In speculation, perhaps a statistical bilateral difference would have been observed in lower-extremity muscle cocontraction duration if (1) subject-perceived pain levels had been greater, or if (2) subjects had been required to run overground. While running on a treadmill subjects were required to maintain a consistent running speed between the three conditions, rather than potentially change their preferred speed between conditions; subjects may have been more likely to alter preferred running speed while running overground. A change in running speed, between conditions, would likely have resulted in altered gait neuromechanics, between conditions, including altered lower-extremity muscle cocontraction duration. The fact we did not observe between-condition changes for cocontraction duration or COMP concentration suggests, according to Kersting et al.,[7] no change in knee articular cartilage volume between the present conditions.

Our results showed a significant increase in serum COMP concentration due to exercise: serum COMP increased from baseline to immediately post-exercise in all conditions. This result is consistent with results from previous studies.[8,9,17-20] Also similar to previous research, our data demonstrate that serum COMP returns to baseline levels relatively quickly following exercise: serum COMP presently returned to baseline levels within an hour following exercise. This finding fits with the findings of Mundermann et al.[8,17] who showed a return of serum COMP to baseline within 30 min post activity. Kersting et al.,[7] Neidhart et al.,[9] and Kim et al.[18] also measured serum COMP levels following exercise; these authors showed elevated COMP levels

1-2 h,[7] 24 h,[9,18] and 6 days[18] after running, respectively. Unlike the present study, however, these studies involved subjects running for longer distances (previous distances ranged from 12.6 to 200 km)[7,9,18] and times (previous times ranged from 40 min to 33 h),[7,9,18] relative to the present study (5.4-7.2 km and 30 min). Collectively, all of the data discussed in this paragraph seem to indicate that serum COMP levels will remain elevated following exercise longer as running duration increases. Runs that are longer in duration result in more loading of the knee joint and higher amounts of COMP are released from articular cartilage. For example, Kim demonstrated a 60% increase in serum COMP levels after a marathon and 90% increase after a 200-km ultramarathon,[18] while Mundermann[8,17] reported increases of 6.3-9.7% after a 30-min walk. Our 30-min run resulted in a 14% increase in serum COMP. One study that is not completely congruent to this idea is Niehoff et al.,[19] who required subjects to run for 30 min, and observed serum COMP increases of 39% and a 1.5 h duration before COMP level returned to baseline. This elevated and prolonged COMP level may be the result of only using male subjects (Kersting,[7] Munndermann,[8,17] and the present study included males and females). Males have demonstrated higher serum COMP levels compared to females, pre, post, and 2.5 h post completion of a 60-min run.[7]

This study has some inherent limitations. We cannot be certain whether the observed COMP increases should be attributed to loading of articular cartilage in the involved or uninvolved leg, or from the knee joint or other joints that are also loaded during running. Similarly, we do not know if the increased COMP levels are associated with loading of articular cartilage or the loading of other tissues. These tissues could include tendons,[21] menisci,[22] or synovium, which each include fibroblasts, synovial fluid,[23] and fibrocartilage[24] all of which have been reported to release COMP. With this said, we believe the increase in serum COMP is at

least partially due to knee joint load, because the knee is the largest joint in the body and receives the greatest amount of stress during running.[7] Similarly, the present methods make it difficult to determine whether COMP was released from the involved or uninvolved knee. In the future, studies should be conducted in which synovial fluid is withdrawn directly from the knee joint capsule, in order to better define where COMP is released during human movement. Furthermore, the large variability in COMP concentrations between individuals limits the measure's sensitivity.[16] Additionally, our small sample size and associated limitations should be noted. Although an a priori power analysis indicated a sample size of 12 would achieve 80% power at a significance level of 0.05, our sample size does still increase the likelihood of committing a type II error.

There are two important findings from this study. First, anterior knee pain during a 30-min running session does not appear to independently affect serum COMP concentration or muscular cocontraction about the knee. This implies that other factors, aside from anterior knee pain alone for 30 min, are likely to influence articular cartilage degradation during movement that occurs while individuals are experiencing anterior knee pain. Second, the observed increase in subject-perceived pain throughout the infusion indicates that researchers can use the present experimental anterior knee pain model to evaluate the independent effects of anterior knee pain over an extended duration while subjects perform a dynamic activity like running. Researchers can use this model to evaluate independent effects of anterior knee pain during a variety of human movement, including running.

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Figure Captions

Figure 1. Knee saline infusion set-up: (A) syringe pump with syringe placed in shirt pocket, (B) tube connecting pump to catheter, and (C) catheter inserted into the infrapatellar fat pad and secured with tape.

Figure 2. Timeline for each data collection session, including blood sample times, times of infusion (if applicable), and EMG data collection times. Time (min) is shown on the horizontal axis.

Figure 3. Means and standard deviations for subject-perceived pain (vertical axis, measured in mm) across time (horizontal axis, measured in min). Subject-perceived pain significantly increased across time during the pain session only ($p < 0.01$). Asterisks indicate significant differences between the pain, and sham and control sessions. The plus symbols indicate significant differences between the pain and control sessions.

Figure 4. Means and standard deviations for serum COMP concentration at T-12, T30, and T90, for each session. When pooling data from each session, serum COMP protein concentration was significantly increased at T30 (immediately after the run) relative to T-12 and T90 (pre run and 60 min post run). COMP concentrations T-12 and T90 did not significantly differ from each other.

Figure 5. Means and standarad deviations for cocontraction duration, measured as a percent of stance phase, for each of the three sessions. There were no significant differences between conditions.

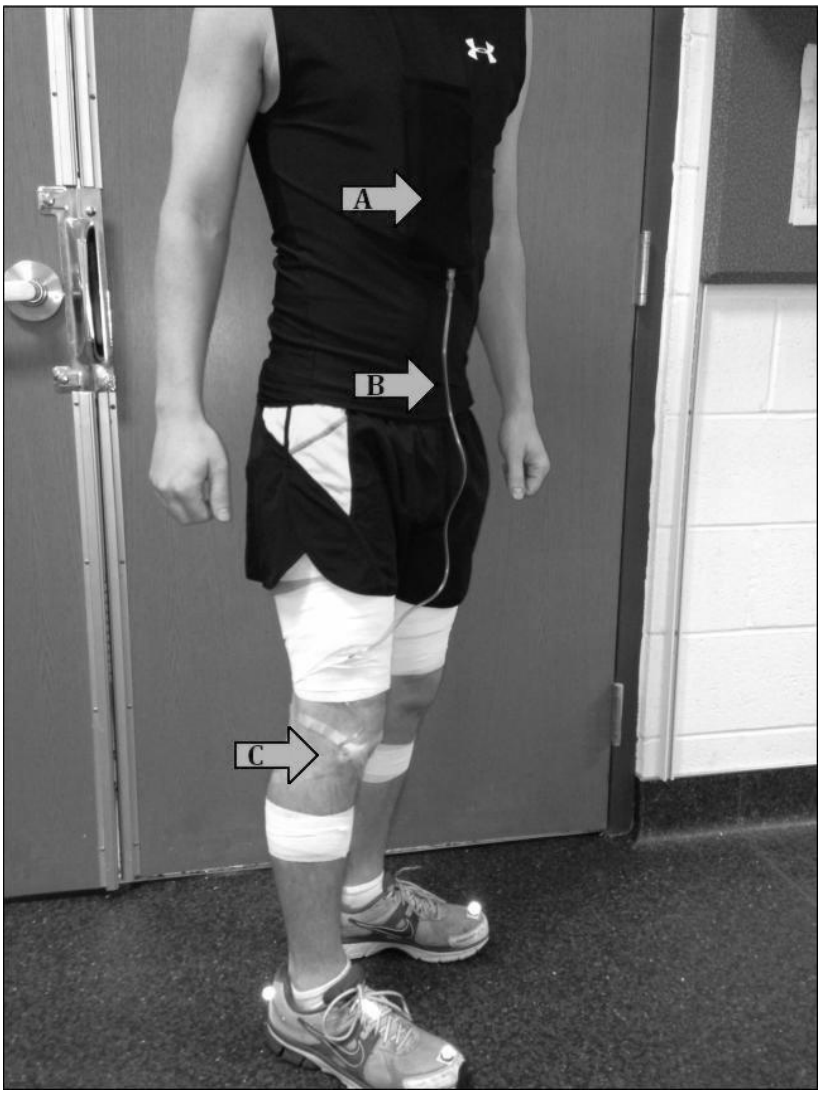

Figure 1.

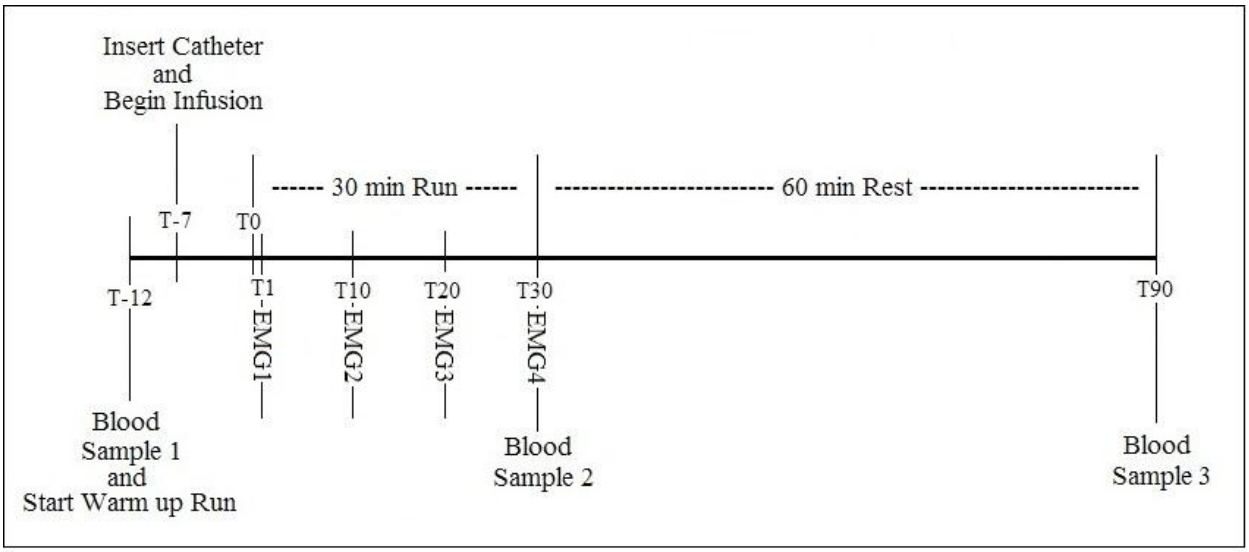

Figure 2.

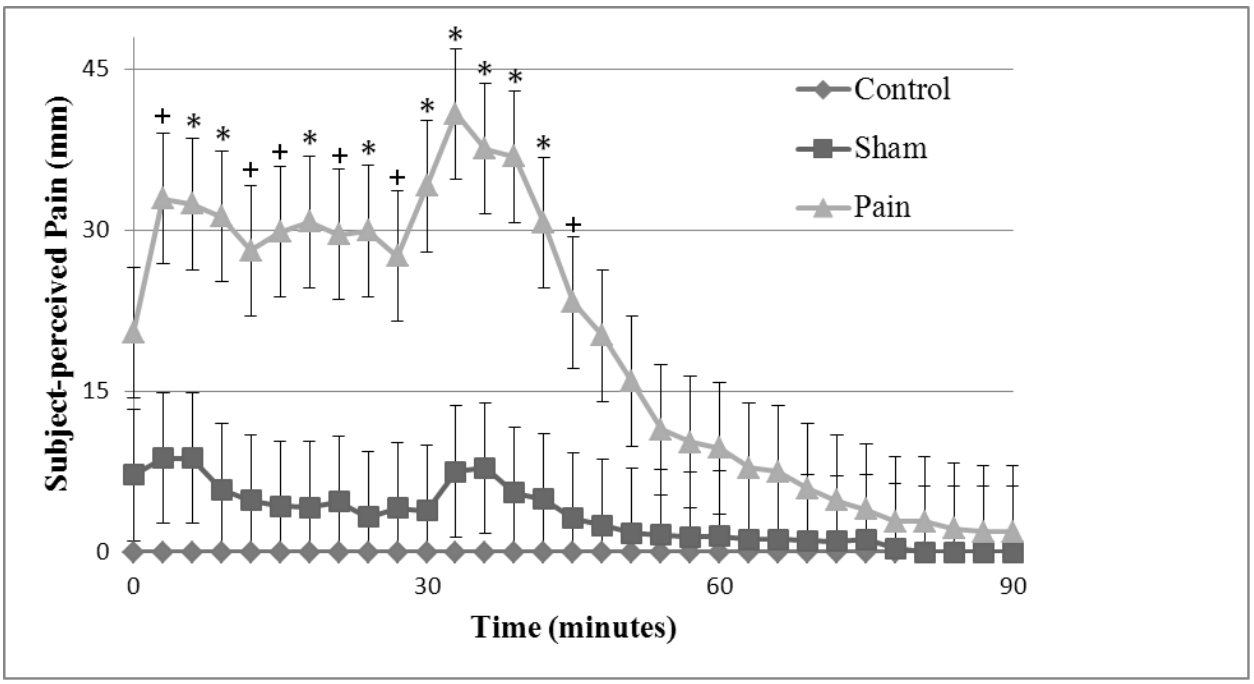

Figure 3.

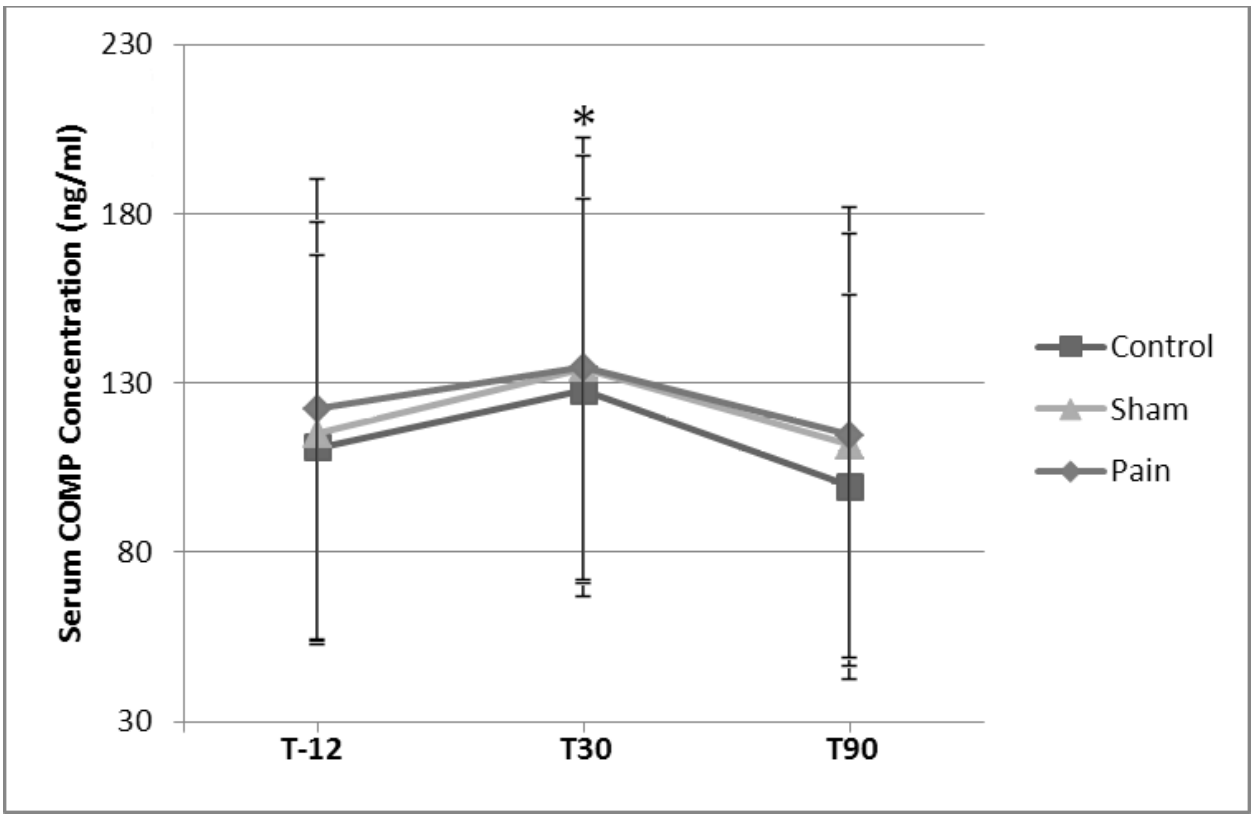

Figure 4.

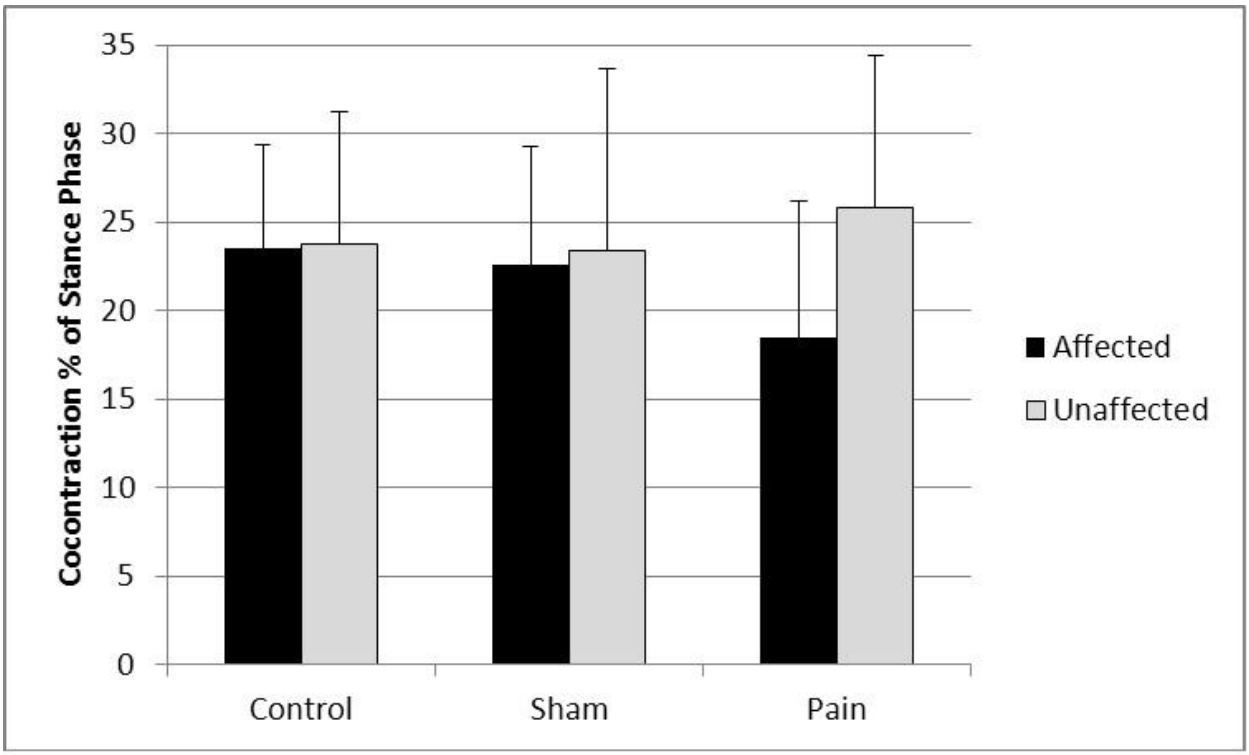

Figure 5.