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Jeffrey Ray Tolley
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Are Cardiovascular Disease Inflammatory Markers Elevated in Those with Nonspecific Chronic Musculoskeletal Pain Compared to Nonpain Case Controls?

Jeffrey Ray Tolley

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

Ron Hager, Chair
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Department of Exercise Sciences
Brigham Young University

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ABSTRACT

Are Cardiovascular Disease Inflammatory Markers Elevated in Those with Nonspecific Chronic Musculoskeletal Pain Compared to Nonpain Case Controls?

Jeffrey Ray Tolley
Department of Exercise Sciences, BYU
Master of Science

CONTEXT: Recent studies have considered the role of inflammation in the development of both cardiovascular disease (CVD) and musculoskeletal conditions, such as rheumatoid arthritis. Studies suggest that inflammation plays a significant role in the development of cardiovascular disease. In conditions of chronic pain, as with rheumatoid arthritis, inflammation has also been noted through elevated levels of inflammatory markers. There are currently no studies that examine the possible connection between inflammatory markers related to increased risk of cardiovascular disease and nonspecific chronic musculoskeletal pain (NCMP).

OBJECTIVE: The purpose of this study was to determine whether urinary levels of microalbumin (MA) and F2-isoprostanes (F2-isoPs), inflammatory biomarkers associated with increased CVD risk, are elevated in persons with NCMP compared to nonpain case controls. NCMP refers to pain present for more than 3 days per week and for more than 12 weeks. This type of pain is not due to injury but is associated with interference of normal function.

DESIGN: Nonrandomized observational study.

METHODS: A cross-sectional study with 120 participants (60 pain subjects, 60 nonpain case-controls). A single first-morning void urine sample was collected from each subject. Urine specific gravity and total volume were measured and then a sample was sent to a lab for analysis of MA and F2-isoPs. Inflammatory biomarker levels in the pain and nonpain groups were compared.

RESULTS: There were no significant differences in F2-isoPs levels between the chronic pain group (0.65ng/mg ± 0.05) and the nonpain group (0.80ng/mg ± 0.07) (95% CI (-0.32, 0.03)). However, MA levels were significantly higher in the chronic pain group (2.41mg/g ± 0.24) compared to the nonpain group (1.88mg/g ± 0.14) (95% CI (0.34, 1.68)). MACR levels were also significantly higher in the chronic pain group (2.07mg/g ± 0.31) compared to the nonpain group (1.14mg/g ± 0.14) (95% CI (0.32, 1.64)).

CONCLUSION: These findings suggest a possible link between at least one inflammatory marker (microalbumin) and NCMP. This in turn allows for a limited but reasonable inference that NCMP may be a risk factor for cardiovascular disease, mediated through the MA inflammatory biomarker. Further research is needed to more fully understand the possible connection between NCMP and CVD.

Keywords: chronic pain, inflammation, cardiovascular disease, inflammatory biomarkers, microalbumin, F2-isoprostanes
AKNOWLEDGEMENTS

I would first like to express my gratitude to Dr. Ron Hager for his consistent support and dedication assisting me in the process of completing my thesis. I have gained much valuable knowledge and experience from Dr. Hager, not just academically but also in many aspects of my life. Without his encouragement, I would not be where I am today. I would also like to thank Dr. Pat Vehrs, Dr. Lance Davidson, and Dr. Gil Fellingham for their support and contributions to this project.

Additionally, I would like to express my appreciation to friends and peers who have supported me throughout this entire process. I am grateful for their confidence in me and in my abilities to challenge myself. Last but certainly not least, I would like to thank my parents who have always supported and encouraged me in everything I do. They taught me to work hard and to always believe in myself. Their love and support helped me keep going. I am truly grateful for everyone who helped me complete this endeavor.
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INTRODUCTION

Chronic musculoskeletal pain is a main cause of disability in the aging population and is increasingly a worldwide problem.\(^1\) Nonspecific chronic musculoskeletal pain (NCMP) refers to pain present more than three days per week and generally for more than 12 weeks. This type of pain is not due to injury but is associated with and often results in interference of normal function.\(^2\) Prevalence of NCMP in the US is projected to increase from 47.8 million people in 2005 to nearly 67 million people by 2030 (about 25% of the adult population).\(^1\)

Cardiovascular disease (CVD) is the leading cause of death in the US with nearly 610,000 heart disease related deaths every year.\(^3\) Approximately 1 in every 4 deaths in the US is from CVD. Additionally, every year about 735,000 Americans have a heart attack, and of those about 120,000 are fatal.\(^4\)

Research indicates that chronic inflammation is an underlying root cause of both pain and cardiovascular disease. Local or acute inflammation, usually restricted to the site of tissue damage or infection, begins when pro-inflammatory hormones signal an increase in white blood cell production to help fight infection and tissue damage.\(^5\) However, chronic or systemic inflammation harms rather than heals.\(^6\) Excessive inflammation on previously inflamed tissue can create a vicious cycle of injury often resulting in pain, damage to tissues and organs\(^7\) and loss of motor function.\(^8\)

Recently, researchers have linked inflammation, often present at the site of pain,\(^8\) to the development of cardiovascular disease.\(^6,9-12\) In conditions resulting in inflammation, elevated levels of certain biomarkers are often found in many biological fluids (e.g., synovial fluid, blood serum, urine, etc.). Some biomarkers have been indicative of triple the risk of a cardiovascular event (e.g., stroke, heart attack, atherosclerosis, etc.).\(^6\) The inflammatory markers of interest in
this study are microalbumin (MA) and F2-isoprostanones (F2-isoPs), which are two of many inflammatory markers known to be associated with the development of cardiovascular disease.\textsuperscript{6} MA and F2-isoPs have both been linked to a tripling of risk for stroke, myocardial infarction, and other cardiovascular disease conditions.\textsuperscript{6,13}

Both MA and F2-isoPs (a marker of oxidative stress), have been associated with severe cases of inflammation such as rheumatoid arthritis,\textsuperscript{14,15} fibromyalgia,\textsuperscript{16} and other conditions such as chronic fatigue syndrome,\textsuperscript{17} muscle wasting,\textsuperscript{18} and delayed onset muscle soreness.\textsuperscript{19} However, there is limited if any research that has considered these CVD biomarkers in connection with NCMP. These two urinary biomarkers, MA and F2-isoPs, were selected for this study because of their simple and noninvasive method of measurement.

The purpose of this study was to identify any connection between MA and F2-isoPs and NCMP. It was hypothesized that there would be elevated urinary levels of MA and F2-isoPs in those with NCMP compared to nonpain case-controls.

METHODS

Research Design

This study is a nonrandomized cross-sectional study. Participants suffering from NCMP of various kinds (e.g., low back, hip, knee, etc.) were selected for the pain group. Participants who were pain-free and matched demographically were used as nonpain controls. Chronic musculoskeletal pain was assessed using the Visual Analog Scale (VAS) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire. A first-morning void urine sample was taken to measure levels of MA and F2-isoPs for each study participant. The levels of inflammatory markers in the pain group and the nonpain control group were compared.
Subjects

Participants for this study included 60 sedentary to moderately active adults 18 years of age and older, who experience moderate to severe (> 3 on the VAS) chronic musculoskeletal pain and 60 nonpain, case-control participants. A power analysis using a power level of 80% and alpha set at 0.05 indicated a sample size of 120 participants. Participants were eligible to participate in this study if they met the following inclusion criteria described below.

Participants were recruited from the local community. Flyers, advertisements, and word-of-mouth announcements were sent to nearby physical therapy, orthopedic, and chiropractic clinics. Interested participants contacted the researcher and the initial telephone/email contact with a prospective participant included self-reporting height and body mass and verbal responses to the preparticipation questionnaire to screen for eligibility to participate in this study. If the prospective participant met the inclusion criteria, an appointment was made to visit the lab; otherwise their participation was politely declined. Qualifying individuals were instructed to not take any pain-relieving anti-inflammatory medications during the 12 hours prior to providing a first-morning void urine sample. The time the participant was sleeping prior to providing the first-morning void sample acted as a standard fasting period. Pain group participants were informed that they could take their pain medication after the urine sample was provided.

 Procedures

All data collection occurred in the Human Performance Research Center in the Department of Exercise Sciences. This study was reviewed and approved by the Institutional Review Board for the Use of Human Subjects prior to the collection of any data.

During their scheduled appointment, all prospective participants completed a written version of the same preparticipation questionnaire that was verbally given during the initial
phone contact or email. The body mass and height of each participant were measured to the nearest 0.1 kg and the nearest 0.1 cm using a digital scale (Oahu’s Model CD-33, Ohaus Corporation, Pine Brook, NJ) and a digital wall-mounted stadiometer scale (SECA Model 264; SECA, Chino, CA), respectively. Body mass index was calculated from measured height and body mass values. This was done to confirm their eligibility to participate in the study.

The preparticipation questionnaire included questions about age, gender, physical activity, previous injuries, musculoskeletal pain, pain medication use, and previous or planned surgeries. The qualifying variable of chronic musculoskeletal pain was defined as pain not associated with an injury, that interferes with or requires modification of normal function on more than 3 days per week for at least 12 weeks.2,20

Individuals were invited to participate in this study if they (a) reported an initial pain level that is greater than 3 cm on the VAS (pain group) or (b) reported an initial pain level of zero on the VAS (nonpain group). To be eligible to participate in this study, all prospective participants met all of the following inclusion criteria: (a) have a BMI < 30 kg·m⁻², (b) does not currently consume alcohol, smoke, or use other tobacco products, (c) not having had any surgeries designed to alleviate musculoskeletal pain in the past 12 months, (d) not currently receiving pain treatment from intra-articular injections, (e) not currently experiencing signs of sickness or infection of any kind,21 (d) not pregnant or menstruating, (f) have not previously been diagnosed by a doctor as having any form of CVD (e.g., hypertension, atherosclerosis, myocardial infarction, stroke, etc.), or diabetes, (g) have not previously been diagnosed with conditions of rheumatoid arthritis, osteoarthritis, or fibromyalgia. Fibromyalgia has symptoms that resemble some rheumatic illnesses, including rheumatoid arthritis and lupus22,23 and therefore, subjects with fibromyalgia were excluded from the study.
If qualified to participate, participants completed two versions of the VAS for average daily pain: one for pain at rest and one for pain during motion. If pain in multiple areas of the body occurred in the participant, they were instructed to record their overall level of pain. Participants were instructed to indicate their “usual pain” over the past 7 days and were asked the duration for which they have been experiencing pain. Additionally, participants also completed the WOMAC at the time of the first visit.

Each consenting participant, whether in the nonpain group or the pain group was given a large 700mL urine collection container with incremental measurements (mL) on the side, and a smaller leak-resistant standard urine sample container. They were instructed to take both containers home and upon first waking in the morning, complete a full first-morning void sample of urine into the large container. Each subject was then instructed to place the large container on flat surface and record total urine volume in mL. After the measurement was recorded, the subject was to transfer a sample of urine into the small leak-resistant standard urine sample container. The subject was also instructed to refrigerate the sample until they could transfer the sample to the lab. Upon delivery of the sample, the subject was then dismissed and no further involvement was required.

Total urine volume was noted and urine specific gravity (USG) was assessed, using a refractometer. Total urine volume was recorded to adjust for any diluted biomarker concentration within the urine sample. The researcher then transferred a sample of urine from the collection container to the urinalysis test tubes (yellow-top) via a vacutainer transfer device to send to the lab. The specimen sample was then labeled and immediately refrigerated at 2°C to 8°C for no more than 7 days before shipment. All remaining urine was stored at -80°C in a storage freezer in case of potential need for additional sample at the onsite lab or at Cleveland Heart Lab.
(Cleveland, OH) where urine was analyzed. The prepared specimens were then shipped weekly to the Cleveland Heart Lab for analysis according to protocol.

Measurements

For each subject, the variables of interest included F2-isoPs and MA values, self-reported pain using the VAS scale and WOMAC questionnaire and general physical activity levels. Each subject was assessed only once for each variable.

Visual Analog Scale

Pain level was assessed using the VAS which is a scale comprised of a horizontal line, 10 cm in length, anchored by 2 verbal descriptors, one for each symptom extreme (“no pain” and “worst possible pain”). The participant was asked to draw a line perpendicular to the VAS line at the point that represents their pain intensity.\textsuperscript{24–26} The VAS is a simple and frequently used method for the assessment of intensity of pain.\textsuperscript{24} Test-retest reliability has been shown to be very good (r = 0.94, \( p < 0.001 \)) in regards to chronic pain and physical dysfunction.\textsuperscript{27}

WOMAC Questionnaire

Functional ability and stiffness were assessed using the WOMAC which is a self-administered, multidimensional questionnaire designed for osteoarthritis patients that assesses pain, stiffness, and physical function. Of 24 questions, 5 questions deal with pain at rest and during activity, 2 questions deal with stiffness, and 17 questions deal with the degree of function in common daily activities. Each question is answered using a 5-point Likert scale. The WOMAC has been found to be both reliable and valid in a variety of chronic pain related conditions.\textsuperscript{28,29}
**F2-Isoprostanes**

F2-isoprostanes analysis was conducted by the Cleveland Heart Lab using a mass-spectrometry analysis process, the preferred method and gold standard of measuring oxidative stress and F2-isopPs. When dealing with markers of oxidative stress, it is important to avoid any auto-oxidation, which can easily occur in blood samples if not stored immediately at the required temperatures. Auto-oxidation is not a problem with urine samples.

**Microalbumin**

Microalbumin was also analyzed by the Cleveland Heart Lab using an immunoturbidimetric assay method. Although there are many methods of measuring MA, immunoturbidimetric assays are considered a precise, accurate, and economical assay, in terms of cost and efficient running time. The test is typically used in conjunction with a creatinine test to provide microalbumin-to-creatinine ratio (MACR). Creatinine is a waste product in the blood that the kidneys should remove. When kidney damage occurs, creatinine levels in the urine decrease while albumin levels may increase.

**First-Morning Void**

The gold standard to assess microalbuminuria is 24-hour urinary albumin excretion (UAE). A 24-hour UAE is where all urine excreted from an individual is collected and measured during a 24-hour period. However, despite the measurement of microalbumin excretion in a 24-hour urine collection being the gold standard, collection errors due to improper timing and missed samples may lead to significant overestimation or underestimation of microalbuminuria. Also, because a 24-hour urine collection is cumbersome for each participant, a second, but validated approach was taken for urine collection. Guidelines suggest measuring albuminuria in a first-morning void (immediately upon first waking in the morning).
either as urinary albumin concentration (UAC) or adjusted for creatinine concentration, and reported as the microalbumin-to-creatinine ratio (MACR).\textsuperscript{35} First-morning void samples are also shown to be more accurate predictors of microalbuminuria than random spot samples.\textsuperscript{37} Due to the long sleep period, first-morning void samples tend to resemble a 24-hour urine collection sample rather than a random-spot sample.\textsuperscript{36,37}

Statistical Analysis

Some of the variables of interest in this study (F2-isoPs, MA, MACR, and WOMAC) were generally right skewed, thus standard methodologies associated with normal variability were inappropriate. Bayesian methodology offered some advantages\textsuperscript{38,39} with skewed data. A Bayesian approach produces posterior distributions of the parameters of interest. In the Bayesian framework, the model consists of the scaled product of the likelihood of the data given the parameters and prior probability densities for each of the parameters.\textsuperscript{38,39} Current practice to analyze such a model is to implement a Markov Chain Monte Carlo (MCMC) procedure to produce samples from the posterior distributions of interest.\textsuperscript{40,41} The program JAGS\textsuperscript{42} was used to generate the samples from the posterior distributions using MCMC. The sampling chains were then analyzed using the program R.\textsuperscript{43}

The posterior distributions were used to determine 95% credible intervals (CI) for the differences in F2-isoP and MACR values and WOMAC scores between the pain and nonpain groups. The 95% CI defines the range within which there is a 95% probability that the true value of the parameter lies. Since a difference of zero (0) indicates no difference, if zero (0) fell within the 95% CI of the variable, we concluded that there were no differences between the pain and nonpain groups. If zero (0) fell outside of the CI, we concluded that there were significant differences in the parameters of the pain and nonpain groups.
Comparison of means for demographic data including age, sex, height, body mass, and VAS scores in the chronic pain and the nonpain case-controls were assessed with analysis of variance (ANOVA).

VAS scores were analyzed using analysis of covariance (ANCOVA). Age, gender, BMI, physical activity, location of pain, history of joint surgery, arthritis, likelihood of future surgery, pain at baseline, and pain medication use were used as covariates in the analysis. Participant demographics were analyzed to determine means and standard deviations in order to describe the sample. Statistical significance was set at $p < 0.05$.

RESULTS

Subject demographic information and mean comparison results are presented in Table 1. There was no statistical difference in subject age (y), height (cm), or body mass (kg) between the two groups. Additionally, BMI was $\leq 25$ in both groups. Analysis did show that there was a significant difference ($p < 0.0001$) in pain level between the groups as determined by the VAS (Table 1).

The WOMAC scores that assessed overall pain & stiffness levels within the 2 groups were initially analyzed using a zero-inflated gamma likelihood. The mean WOMAC score among the nonpain group was $3.85 \pm 0.60$, and the mean WOMAC score among the pain group was $33.22 \pm 2.20$. The 95% CI for the mean difference was (25.1, 34.0). The mean WOMAC score was elevated approximately 10-fold in the chronic pain group (Table 2).

Analysis of F2-isoPs levels revealed that there were no significant differences between the pain group and the nonpain group. In addition, the trend in the F2-isoPs values was unanticipated in that the nonpain group ($0.80\text{ng/mg} \pm 0.07$) had a slightly higher mean than the chronic pain group ($0.65\text{ng/mg} \pm 0.05$). Because the 95% CI (-0.32, 0.03) for the difference of
the means between the pain group and the nonpain group included zero, we are unwilling to conclude that the means are statistically different (Table 2).

For comparisons of MA, there were some instances in both groups (nonpain and pain), where MA results were undetectable by lab analysis and recorded as zero. These zero values were due to the microalbumin in the subject’s urine being below assay detection ability and therefore a MA test could not be calculated. The probability of having a nonzero response was essentially the same for both groups (n = 30 for pain group, n = 28 for nonpain group). The 95% CI for the proportion of zeros in the pain group minus the proportion of zeros in the no pain group is (-0.71, 1.23). The analysis for the MA variable was done by first taking the log of the nonzero data, and then analyzing the result using a zero-inflated gamma likelihood. The likelihood was constructed using a mixture of a point mass at zero, and a gamma likelihood for the nonzero log corrected data. The analysis showed that among those with nonzero responses, both the mean and standard deviation of the chronic pain group (2.41 mg/g ± 0.24) were significantly elevated compared to the nonpain group (1.88 mg/g ± 0.14). The 95% CI for the mean of the chronic pain group minus the mean of the nonpain group was (0.34, 1.68). Since this interval does not include zero, we are willing to conclude that the means are statistically different. Conditional on the measurement being detectable, the mean is significantly elevated in the pain group. The mean MA level in the chronic pain group nonzero responses was roughly twice that of the nonpain group (Table 2).

In further analysis of MA, a MACR test was performed in order to adjust for concentration levels. The analysis showed that among those with nonzero responses, the mean of the chronic pain group (2.07 mg/g ± 0.31) was significantly elevated compared to the nonpain group (1.14 mg/g ± 0.14). The 95% CI for the mean of the chronic pain group minus the mean of
the nonpain group was (0.32, 1.64). Since this interval does not include zero, we are willing to conclude that the means are also statistically different.

Further analyses were done to see if there was an effect due to gender. These analyses revealed the same differences for groups as seen previously, but no effect due to gender was seen, and no interaction of gender with the presence of chronic pain was observed.

DISCUSSION

The fundamental purpose of this study was to evaluate differences in known cardiovascular inflammatory markers in subjects with chronic pain compared to subjects with no history of pain. If differences are seen, a reasonable inference for NCMP as a risk factor for CVD can be made. Differences in biomarker levels between pain and nonpain groups may help establish a connection between inflammation in conditions of NCMP and CVD risk. This is especially pertinent because of the prevalence of both CVD and chronic musculoskeletal pain worldwide.

F2-isopPs and MA are two of several known inflammatory markers that are commonly associated with increased cardiovascular disease risk.\textsuperscript{6,9,44,45} Results of this study indicate that MA levels are higher in those with NCMP compared to those without chronic pain and therefore suggest a possible connection between CVD risk and NCMP. This relationship is also noted in significantly elevated MACR levels in those in the pain group (2.07mg/g) as compared to those in the nonpain group (1.14mg/g). While this observation does not prove that those with chronic pain are at a greater risk for developing CVD, this preliminary study provides the basis for further research that explores the connection between biomarkers for CVD and musculoskeletal pain.
During the past two decades, it has been documented that inflammation plays a pivotal role in the development of both cardiovascular disease and musculoskeletal ailments. According to Han et al. and Sattar et al., these biomarkers have been shown to accelerate the aging process of the arterial walls, ultimately leading to plaque build-up and atherosclerosis. Furthermore, those with higher inflammatory marker levels had three times the risk of myocardial infarction (relative risk, 2.9; \( p < 0.001 \)) and nearly two times the risk of ischemic stroke (relative risk, 1.9; \( p = 0.02 \)).

Every year about 735,000 Americans have heart attacks. Of these, 525,000 are a first heart attack and 210,000 occur in people who have already had a heart attack. Heart attacks and most strokes are triggered when a diseased artery becomes so inflamed that it can no longer contain the plaque, leading to a breach in the vessel wall, and consequently the creation of a clot that obstructs blood flow.

Similarly, it has also been recognized that various inflammatory biomarkers can be elevated in cases of severe and persistent pain such as low back pain, arthritis and fibromyalgia. The literature suggests that inflammatory biomarkers of various kinds play a role in the initiation and perpetuation of the osteoarthritis process by contributing to cartilage degradation. Musculoskeletal pain is one of the most common causes of severe chronic pain and physical disability that affects millions of people worldwide. By 2030, 25 million people (9.3% of the adult population) are projected to report musculoskeletal pain activity limitations, with one-third of those cases including working-age adults (45-64 years). Previous studies have shown that many different cardiovascular inflammatory markers have been detected in patients with rheumatoid arthritis (RA), who by definition manifest persistent high levels of inflammation, and are at greater risk of developing cardiovascular disease. Recent studies
have shown surprising similarities between the inflammatory response in atherosclerosis to that of RA.\textsuperscript{55} Cardiovascular disease has also been reported as the leading cause of mortality in RA.\textsuperscript{56}

With the findings from this study, it is reasonable to suggest that there might to be a connection between the inflammation associated with conditions of NCMP and the inflammation associated with cardiovascular disease. Although there are correlations with CVD and RA, this study shows that the connection might go beyond arthritic conditions and include those with NCMP.

The results of this study have some practical clinical implications\textsuperscript{9} for counseling with patients with chronic musculoskeletal pain and cardiovascular health. Since there were significantly elevated levels of MA found in those with NCMP, an effort to reduce this inflammation/pain to prevent the potential development of an inflammation-mediated cardiovascular disease is warranted. While this study is preliminary and further research is needed, significantly elevated levels of MA, a CVD\textsuperscript{6} biomarker, suggest that there is a link between NCMP and CVD. This information could be valuable for physicians treating patients presenting with NCMP and consideration might be given to assessing CVD inflammatory markers in such patients. Similarly, cardiologists might seek to alleviate NCMP in those who have recently experienced a cardiovascular episode or those at elevated risk for CVD development.

\textit{Limitations}

Only one of the two inflammatory markers assessed in this study was found to be statistically different between groups. As there are other markers to consider, further research may help to establish the relationship between NCMP and CVD. According to Bale et al.,\textsuperscript{6} there are at least six inflammatory markers in what the author calls the “Fire Panel” and all six have
been shown to be associated with increased CVD risk. Some research has indicated that elevated levels of these markers increase risk of suffering a stroke by nearly 7 times.\textsuperscript{57-59} It is possible that other “Fire Panel” markers and other inflammatory markers could be elevated in those with NCMP.

Although efforts to control for other factors that could influence the inflammatory response were considered in the present study (Table 1), there are still other possible explanations for why MA was elevated in the pain group. A case-control design helped to account for differences between groups that might affect inflammatory marker levels. Inclusion and exclusion criteria considered were: activity level, medication intake, smoking and/or alcohol consumption, previous diagnosed cardiovascular disease, illness, and current pregnancy or menstruating status. However, possible limitations to this study include other factors that can effect oxidative stress and inflammation in the body such as dietary intake choices, increased stress and other emotions, weight gain, and quality of sleep that could influence the levels of inflammatory markers studied.\textsuperscript{7,60} Even dental health and hygiene habits have been linked to systemic inflammatory responses in the body.\textsuperscript{6} Perhaps, these factors are also possible explanations of why F2-isoPs were not elevated. Any of these factors could have masked the potential inflammation associated with NCMP in the participant.

Additionally, although the mean BMI for both the nonpain and pain groups was ≤ 25, the location of adipose tissue could possibly contribute to the increased levels of MA in the pain group. Mounting evidence highlights the role of adipose tissue, specifically abdominal adipose tissue, in the development of a systemic inflammatory state that contributes to obesity-associated cardiovascular risk.\textsuperscript{61} Even though mean BMI in study participants was ≤ 25, the location of adipose tissue could be a factor related to an inflammatory response.
CONCLUSION

Differences between groups for WOMAC results indicate that participants in the chronic pain group experienced significantly greater functional limitations and debilitating conditions than those participants in the nonpain group. Additionally, F2-isoPs levels were found to not be significantly different between groups. However, both MA and MACR values were significantly greater among the chronic pain group compared to their nonpain case controls. The findings of this study indicate a possible connection between NCMP and MA levels.
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Table 1. Descriptive Demographics of Subjects

<table>
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<tr>
<th></th>
<th>Nonpain Group</th>
<th>Pain Group</th>
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<th>p-value</th>
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<td></td>
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<tr>
<td>Number of Males</td>
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<td></td>
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<tr>
<td>Number of Females</td>
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<tr>
<td>Age (y)</td>
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<td>40.63 ± 14.79</td>
<td>0.004</td>
<td>0.950</td>
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<td>Height (cm)</td>
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<td>172.14 ± 9.56</td>
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<td>Body mass (kg)</td>
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<td>0.192</td>
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<td>VAS</td>
<td>0.13 ± 0.36</td>
<td>5.97 ± 1.64</td>
<td>726.651</td>
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</tbody>
</table>

* Indicates significant difference between group means (p < .05)
Table 2. Between group comparison of WOMAC, F2-isoPs, MA, and MACR

<table>
<thead>
<tr>
<th></th>
<th>Nonpain Group</th>
<th>Pain Group</th>
<th>95% Credible Interval</th>
</tr>
</thead>
<tbody>
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<td>WOMAC</td>
<td>3.85 ± 0.60</td>
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<td>(25.1, 34.0)*</td>
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<td>0.65 ± 0.05</td>
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<td>MA</td>
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<td>2.41 ± 0.24</td>
<td>(0.34, 1.68)*‡</td>
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<td>MACR</td>
<td>1.14 ± 0.14</td>
<td>2.07 ± 0.31</td>
<td>(0.32, 1.64)*‡</td>
</tr>
</tbody>
</table>

* Indicates significant difference between group means (CI does not cross 0)

‡ Conditional on the measurement being detectable.