

SEX RELATED INFLUENCES ON PAIN SENSITIVITY AND INFLAMMATION IN
TEMPOROMANDIBULAR JOINT DISORDER

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2010

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To my husband and Mom for all their support and understanding and unconditional love

ACKNOWLEDGMENTS

I thank Dr. Roger Fillingim for his incomparable mentorship, for all his availability and willingness to teach and to help throughout my academic life. I thank all professors of the Advanced Postgraduate Program in Clinical Investigation for their support and teaching, especially, Dr. Wei Hou for his dedication and hard work. I thank Eve Johnson for her dedication and hard work. I thank Dr. Shannon Wallet and Nadia Calderon for their teaching, patience and help with the inflammatory assay. I thank the Clinical and Translational Science Institute for financial support. I thank all of Dr. Fillingim's lab for their friendship and tireless help. I thank Lori Levin for all her assistance. I thank all my friends and family who support and believed in me for all these years. Especially, I thank all participants in this research project, without whom this work would not have been possible.

TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	7
LIST OF FIGURES.....	8
LIST OF ABBREVIATIONS.....	9
ABSTRACT.....	11
CHAPTER	
1 INTRODUCTION.....	13
Clinical Relevance.....	13
Sex Difference in Clinical and Experimental Pain.....	13
Gonadal Hormone Influences on Sex Difference in Pain Perception.....	14
Estrogen and Temporomandibular Muscle and Joint Disorders.....	16
Estrogen and Inflammation.....	17
2 MATERIALS AND METHODS.....	19
Research Design.....	19
Subject Selection.....	19
General Experimental Procedures.....	19
Menstrual Phase Staging.....	19
Medical History Questionnaire.....	20
Physical Examination.....	20
Sensory Testing Session: Assessment of Basal Pain Sensitivity.....	20
Pressure Pain Procedures.....	20
Thermal Testing Procedures.....	21
Heat Pain Threshold and Heat Pain Tolerance.....	21
Temporal Summation of Thermal Pain.....	22
Ischemic Pain Procedure.....	23
Immunologic Assays.....	24
Data Analysis.....	25
3 RESULTS.....	27
Subjects.....	27
Clinical Pain Results.....	27
Evoked Palpation Sensitivity - Research Diagnose Criteria for Temporomandibular Disorder Results.....	28
Basal Pain Sensitivity Results.....	28

Pressure Pain Procedure	28
Thermal Pain Procedure.....	28
Ischemic Pain Procedure and Associations Between Clinical and Experimental Pain	29
Immunological Response.....	29
Estrogen Stimulation and Inflammatory Response.....	29
Thermal Pain Procedure.....	30
4 DISCUSSION	37
Clinical Pain Response	37
Experimental Pain Responses.....	38
Immunologic Assays.....	38
Study Limitations	39
Future Directions	40
LIST OF REFERENCES	41
BIOGRAPHICAL SKETCH.....	48

LIST OF TABLES

<u>Table</u>		<u>page</u>
2-1	Study exclusion criteria.....	25
3-1	Demographics	31
3-3	Thermal pain measures, Mean (SD).....	32
3-4	Ischemic pain measures, Mean (SD).....	32

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1 Subject recruitment and study design.....	26
2-2 Timeline used during the sensory test.....	26
3-1 Correlation of clinical pain with sensory tests and research diagnose criteria (RDC) for temporomandibular disorder palpation sensitivity scores.	33
3-2 Sum of palpation scores for the right and left side, and the total number of positive palpation sites (i.e. scores greater than zero) for each side using the RDC by gender.....	34
3-3 Sum of palpation scores for the right and left side, and the total number of positive palpation sites (i.e. scores greater than zero) for each side using the RDC by group.....	34
3-4 Pressure pain threshold procedure by gender.....	35
3-5 Pressure pain threshold procedure by group.....	35
3-6 Cytokine responses at baseline and after stimulation with low and high estrogen across groups.	36
3-7 IL6 and chemokines responses at baseline and after stimulation with low and high estrogen across groups.	36

LIST OF ABBREVIATIONS

avg46°C	Average heat pain rating at 46 degrees Celsius
avg48°C	Average heat pain rating at 48 degrees Celsius
avg50°C	Average heat pain rating at 50 degrees Celsius
IL	Interleukin
kPa	Kilopascals
L_nonzero	Left average of grades different from zero during evoked pain
l_sum	Left sum of positive painful sites - RDC
LlatPter	Left lateral pterigoid
Lmass	Left masseter
Lmedmas	Left medial masseter
Ltemp	Left temporalis
Ltentem	Left tendon temporalis
LTMJ	Left temporomandibular joint
LTrap	Left trapezium
Lulna	Left ulna
r_nonzero	Right average of grades different from zero during evoked pain
r_sum	Right sum of positive painful sites - RDC
RDC	Research Diagnose Criteria for Temporomandibular Disorder
Rmass	Right masseter
Rmedmas	Right medial masseter
Rtemp	Right temporalis
RTMJ	Right temporomandibular joint
RTMJDLp	Right temporomandibular joint lateral pole
RTMJp	Right temporomandibular joint posterior

RTrap	Right trapezium
Rulna	Right ulna
TMJ	Temporomandibular joint
TMJD	Temporomandibular muscle and joint disorders

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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May, 2010

Chair: Julie Johnson

Major: Medical Sciences – Clinical and Translational Science

Painful temporomandibular muscle and joint disorders (TMJD) represent one of the most common pain conditions, affecting approximately 12% the United States population. Women are at increased risk for TMJD compared to men. The purpose of this study was to evaluate sex-related influences on pain sensitivity and inflammation in individuals with TMJD. A total of 84 participants (32 TMJD, 52 controls) were enrolled in the study. All participants were screened over the phone. Eligible participants completed a clinical visit, which included questionnaires, clinical examination and sensory tests (pressure, thermal and ischemic pain procedures). Also, blood samples were taken for immunologic testing. The blood sample was stimulated with low and high levels of estrogen corresponding to the normal range of the menstrual cycle. The statistical analyses included t- tests, Chi-square test, and linear and logistic regression using SAS v9.1.

Results indicated that women had greater sensitivity to manual pressure palpation than men (summed palpation score right and left sides $p=0.03$; number of sites different from zero, right side $p= 0.04$ and left side $p=0.01$). Also, TMJD

participants had greater palpation sensitivity than controls (sum of score right and left sides $p < 0.001$; number of sites different from zero, right and left sides $p < 0.001$).

Regarding the experimental pain procedures, compared to men, women showed lower pain threshold and tolerance for pressure and heat ($p < 0.05$). Also, women showed lower ischemic pain thresholds than men ($p = 0.04$). TMJD participants reported higher heat pain ratings at 46°C ($p = 0.001$) and 48°C ($p = 0.04$) than controls. In the TMJD group, self reported clinical pain was strongly correlated with palpation sensitivity ($p = 0.02$, $r = 0.42$) and marginally correlated with heat pain ratings at 46°C ($p = 0.008$, $r = 0.33$).

Our findings indicate that women showed greater sensitivity to most pain procedures and TMJD patients were more sensitive for pressure pain, and heat pain ratings may be associated with clinical pain. Exploratory analyses of immunologic data suggest that estrogen may exert anti-inflammatory effects for some cytokines in TMJD patients.

CHAPTER 1 INTRODUCTION

Clinical Relevance

Approximately 33% of the United States population demonstrates signs and symptoms of musculoskeletal disease.¹ As a result, the United States currently spends \$118.5 billion/year on the care of musculoskeletal conditions. Musculoskeletal disorders are the second leading cause of work disability, following heart disease.

Temporomandibular disorders (TMJD) are complex musculoskeletal disorders characterized by pain, jaw locking or limited jaw opening, and abnormal popping/clicking noises in the temporomandibular joint (TMJ). The prevalence of at least one sign of TMJD in community-based adult populations ranges from 40% to 50%^{2,3} and women are twice as likely to be affected as men.^{4,5}

Although TMJD pain is associated with considerable morbidity, societal costs and reduced quality of life, TMJD etiopathogenesis remains poorly understood. Additionally, the increased prevalence of these disorders in females and the low prevalence in childhood indicate that sex hormones, such as estrogen, may play an important but complex role in these disorders' pathophysiology.

Sex Difference in Clinical and Experimental Pain

Sex differences in clinical and experimental pain have garnered significant scientific attention in the last decade. Women are at increased risk for many painful disorders such as migraine headache, temporomandibular disorder, fibromyalgia, irritable bowel syndrome, and osteoarthritis.⁶ Also, women reported more severe back pain⁷ and greater post-operative pain than men.⁸ Moreover, women with

musculoskeletal pain report higher pain intensity, greater pain-related interference with function, and more disability days due to pain than men.⁹

In experimental pain, many studies have reported sex difference in healthy and chronic pain patients. In the majority of these studies women show lower pain threshold and tolerance for pressure pain,^{10, 11, 12, 13} ischemic pain¹⁴ and heat pain.^{11, 15, 16} The exact mechanisms to explain this sex difference remain a matter of debate, with one possible explanation being the influence of gonadal hormones.

Gonadal Hormone Influences on Sex Difference in Pain Perception

The role of sex hormones extends beyond their primary role in the reproductive function. Studies have shown that women have a higher prevalence of certain pain conditions after puberty.¹⁷ Moreover, gonadal hormone receptors, especially estrogen, are widely distributed in brain regions implicated in pain processing (thalamus, hypothalamus, nucleus accumbens, and amygdala).¹⁸

Sex hormones are produced primarily by the ovaries (progesterone and estrogen) and gonads (testosterone and dihydrotestosterone). Also, in both sexes, androgens can be converted into estrogens by an enzymatic process, called aromatization, which is catalyzed by the enzyme aromatase (CYP19 or estrogen synthase); therefore, an extra source of estrogen for women may be produced locally in the brain by aromatization of androgens which freely enter the central nervous system.

The mechanisms by which hormones contribute to gender differences in pain conditions are complex and poorly understood. This complexity is partly driven by the fact that hormones can have multiple effects on pain. For example, estrogen can exacerbate or minimize pain conditions according to menstrual cycle, the type of pain (acute or chronic) and type of experimental procedure.¹⁹ In contrast, androgens appear

to protect against the development of chronic pain in humans, and testosterone was found to have analgesic effects on experimental pain.^{20, 21, 22} An important point to emphasize is that the temporal characteristics of estrogen, progesterone and testosterone levels²³ are different between genders. For women the hormone levels change during and after pregnancy, after menopause and monthly throughout most of the female's reproductive lifetime (menstrual cycle). While in men the overall hormone levels are more stable, exhibiting a chronological change, particularly with aging.

Hormonal influences are indirectly evidenced by epidemiologic patterns of some clinical pain conditions. For example, prepubertal girls and boys have an approximately equal (4%) prevalence of migraine.²⁴ After puberty, however, the lifetime prevalence of migraine increases to 18% for women and 6% for men, suggesting a hormonal link between female sex and migraine.^{25, 26} Similar prevalence patterns have been reported for TMJD, with no difference between boys and girls in childhood and higher prevalence in women after puberty.²⁷ Estrogen levels in pregnant women increase throughout each trimester and sharply decline postpartum. These hormone fluctuations correlate with the incidence of migraine attacks, with nearly 80% of 47 women with migraine without aura in one study reporting no migraine attacks in the third trimester.¹⁷ These data were confirmed in prospective analyses,²⁸ and similar findings have been reported among TMJD patients.²⁹

Interestingly, a study done with transsexual participants who underwent to treatment with cross sex hormones to change or maintain somatic characteristic of the opposite sex revealed a change in response to pain.³⁰ In this study about one-third of the male to female subjects developed chronic pain concomitantly with estrogen/anti-

androgen treatment, while about half of the female to male subjects treated with testosterone reported a significant improvement of the chronic pain (headache) already present before the start of treatment. These findings support experimental data in animals and clinical data in humans suggesting that sex steroid hormones play an important role in pain perception.

One mechanism whereby sex hormones may exert their effects on pain involves their impact on multiple central nervous system pathways influenced by neuromodulators such as opioid, dopamine, serotonin and other endogenous components involved in nociceptive processing. Zubieta et al.¹⁸ demonstrated that women in low estrogen states showed decreased brain μ -opioid receptor availability compared to men, and that a high estradiol, low progesterone state, increased pain-related brain mu-opioid receptor binding among women.³¹ Similar findings were reported in studies with mice, such that intact and estradiol treated ovariectomized female rats had significantly fewer brain opioid-binding sites than their ovariectomized counterparts.³²

Estrogen and Temporomandibular Muscle and Joint Disorders

In addition to these effects on pain modulation, estrogen influences the tissue of the extracellular matrix. Indeed, estrogen is involved in pathophysiological changes in several tissues including bone and cartilage. Estrogen is an important regulator of cartilage homeostasis, by acting directly or indirectly on chondrocyte proliferation, differentiation and matrix protein synthesis.³³

Moreover, as cited previously, estrogen can be synthesized locally from androgen via aromatase, suggesting that an intracrine pathway contributes, in addition to the

known endocrine pathway, to the estrogen production what may influence cartilage physiology. Also shown was that estrogen may enhance glycosaminoglycan synthesis (GAG) in chondrocytes via up-regulation of the UDP-glucose dehydrogenase (UGDH) gene expression and enzyme activity providing insights into the molecular mechanisms involved in the regulation of the UGDH gene.³⁴

Estrogen and Inflammation

Estrogen can also act to regulate the release of peripheral cytokines, such as interferon, interleukins (IL) and tumor necrosis factor alpha (TNF α).³⁵ Apparently, estrogen, depending on its level, can have an anti-inflammatory or proinflammatory action regulating TNF α . Studies have shown that 17 β -estradiol (E2), the main endogenous estrogen, regulates several immune responses in mouse models.^{36,37,38} Most, if not all, of the effects of estrogens are mediated by two members of the nuclear receptor superfamily, estrogen receptor (ER)³⁹ α and β . Macrophages are crucial cells of the innate immune system that not only contribute to the first line of defense against pathogens, but also play an important role in directing adaptive immune responses. One of the most potent inflammatory agents able to activate macrophages is bacterial lipopolysaccharides (LPS), a component of the outer membrane of Gram-negative bacteria that binds to CD14, a membrane anchored protein, and stimulates the Toll-Like Receptor 4 (TLR4)/MD2 complex in macrophages. TLR4 signaling results in a rapid and strong production of inflammatory mediators and cytokines by macrophages, such as IL-1 α , IL-1 β , IL-6, IL-12p40, and TNF- α ,⁴⁰ mainly through the activation of the NF- κ B and MAPK signaling pathway.⁴¹ Previous reports, most of them based on *in vitro* studies, suggested that E2 exerts anti-inflammatory effects on LPS-activated monocyte/macrophage cell lines or microglial cells, macrophage cells of the CNS^{42,43,44}

For instance, recent experiments have shown that short-term exposure to E2 prevented NF- κ B nuclear translocation in response to LPS, leading to the inhibition of inducible NO synthase (iNOS) expression in the macrophage cell line RAW 264.7 and in microglial cells. This anti-inflammatory effect was mediated by ER α , leading to the subsequent activation of the PI3K pathway before TLR4 stimulation with LPS.⁴²

Estrogen deficiency has been also shown to upregulate TNF α levels in aged animals. In addition, estrogen supplementation reduces TNF α levels in monocytes/macrophages.⁴⁵ TNF α is a major proinflammatory cytokine that acts through its receptor and mediates inflammation that has effects on a wide range of tissues. This cytokine is elevated in plasma and synovial fluid of patients with chronic inflammatory diseases, like rheumatoid arthritis.⁴⁶ TNF α has not only direct modulatory effects on pain and tissue degradation but also indirect effects by inducing production of other proinflammatory cytokines like interleukin 1 (IL1), IL6 and IL8.⁴⁷ Nordahl et al.⁴⁸ showed that patients with temporomandibular joint pain have elevated TNF α levels in the synovial fluid compared with those without pain.

The role of estrogen in the inflammatory process remains a matter of debate.^{49, 50, 51} The estrogen concentration can determine its action in inflammation, such that low estrogen levels can be pro-inflammatory and high levels anti-inflammatory (as in pregnancy).⁵² Therefore, estrogen can affect pain bidirectionally, depending on its plasma level, which may help explain the interindividual differences in pain during the menstrual cycle among patients with chronic inflammatory disease. More human studies regarding hormonal effects on inflammatory responses are needed to elucidate their influence on clinical pain.

CHAPTER 2 MATERIALS AND METHODS

Research Design

Subject Selection

Subjects were selected through posted advertisement and by word of mouth. Potential participants underwent a telephone screening to determine their eligibility for the study (Figure 2-1). The exclusion criteria are on Table 2-1.

General Experimental Procedures

All subjects participated in a single testing session. For women, all sessions were conducted during the follicular phase of the menstrual cycle. The session was divided into three parts: 1) consent and questionnaires: all subjects provided verbal and written informed consent and completed a series of health and psychological questionnaires; 2) clinical examination: a baseline blood draw sample was collected and a clinical examination was performed using the Research Diagnostic Criteria (RDC)⁵³ for TMJD for both controls and TMJD patients; 3) experimental pain testing: experimental pain testing was performed, including thermal pain, pressure pain, and ischemic pain (described in detail below). All procedures were approved by the University of Florida's Institutional Review Board.

Menstrual Phase Staging

After the screening, for all women, follicular phase timing was determined by having participants call at onset of menses, and the session was scheduled within the next 4-10 days. For oral contraceptive women, the follicular phase cycle was based on predicted menses onset according to the contraceptive pill packet. Menses onset was determined by self-report.

Medical History Questionnaire

All patients completed a medical history, which assessed the self-reported duration of TMJD and grades of their pain using a scale of 0 to 100, current and past treatments for TMJD, comorbidity conditions, and current medication use. This medical history information was reviewed with the patient by the clinical research staff to ensure all items were completed accurately.

Physical Examination

Each patient underwent a clinical examination to diagnose TMJD or healthy normal participants according to RDC. In our study, to be classified as TMJD, participants had to report pain in the medical history questionnaire (clinical pain) and during the exam they had to report pain in at least one of the facial and/or head muscles and/or joint for 5 or more days in the last 30 days. Also, during the clinical palpation exam they had to report pain on palpation in at least 3 muscles and/or 1 joint.

Sensory Testing Session: Assessment of Basal Pain Sensitivity

Following the RDC exam, all participants underwent assessment of basal responses in a laboratory session. Assessment included responses to pressure, thermal, and ischemic pain. A timeline for this session is provided in Figure 2-2.

Pressure Pain Procedures

A digital, handheld, clinical grade pressure algometer was used for the pressure procedures (Somedic, Sollentuna, Sweden) to determine sensitivity bilaterally at the temporalis, TMJ, masseter, trapezius and ulna. These sites were chosen because they are widely used in clinical settings,^{54,55,56,57} the first four sites are related to clinical symptoms of TMJD, good inter-examiner reliability at these sites has been reported,⁵⁸ and normative values are available.^{59,60} An application rate of 30 kilopascals (kPa) per

second were used, as this relatively slow application rate reduces artifact associated with reaction time.⁶¹ To assess pressure pain threshold, the examiner applied a constant rate of pressure and the participant was instructed to press a button when the sensation first became painful, at which time the device recorded the pressure in kPa. Additionally, all pressure pain threshold measurements, an initial practice trial was performed, then three trials were conducted in succession and the average of the three was computed for data analysis.

Thermal Testing Procedures

The following three testing procedures were conducted in the following order, with a five minute rest period between procedures: threshold, tolerance, temporal summation. All thermal stimuli were delivered using a computer-controlled Medoc Pathway Thermal Sensory Analyzer (Ramat Yishai, Israel), which includes a peltier-element-based stimulator with a 1.6 cm X 1.6 cm contact area, as well as a combined heat-foil/peltier-thermode (2.5 cm in diameter), which provides rapid heating rates of up to 70 °C per second. Due to its rapid rise time and the high sampling rate, this thermode is particularly useful for producing temporal summation of heat pain.⁶² The threshold and tolerance were delivered to 6 areas separated 2 inches from each other on the right forearm, and the temporal summation were delivered in 3 different areas on the left forearm. Alternating stimulation sites were used to prevent carryover effects due to local sensitization.

Heat Pain Threshold and Heat Pain Tolerance

Heat pain thresholds and heat pain tolerances were assessed using an ascending method of limits. From a baseline of 32 °C, probe temperature increased at a rate of .5 °C/sec until the subject responded by pressing a button on a handheld device. This

slow rate of rise preferentially activates C-fibers⁶³ and diminishes artifact associated with reaction time. For heat pain threshold, subjects were instructed to press the button when the sensation “first becomes painful,” and for tolerance the instruction was to press the button when they “no longer feel able to tolerate the pain.” Six trials of heat pain threshold were delivered in the 6 spots in the first line of 6 dots separated 2 inches from each other in the right forearm, followed by a 5-minute rest period. Then, six trials of heat pain tolerance were delivered in the other 6 areas in the second line of 6 dots separated 2 inches from each other in the right forearm. The average of the trials was computed for heat pain threshold and heat pain tolerance. The position of the thermode was altered slightly between trials in order to avoid either sensitization or habituation of cutaneous receptors. In addition, interstimulus intervals of at least 30 seconds were maintained between successive stimuli.

Temporal Summation of Thermal Pain

This thermal procedure involved brief repetitive suprathreshold thermal stimuli to assess temporal summation of pain. During this procedure, the thermal stimulus was applied to three test sites in the ventral forearm. The stimulus parameters were based on extensive experience in our laboratory and our previously published work.⁶³ In order to account for individual differences in sensitivity to heat stimuli, we assessed temporal summation at multiple stimulus intensities. Four series of 10 heat pulses were delivered in ascending order. For all series, the baseline temperature was 35 °C, and the target temperatures were 46, 48 and 50 °C. For most individuals, these temperatures range from mild to intense pain. The target temperature was delivered for a 700-millisecond duration, with a 2-second interpulse interval at the baseline temperature. Subjects were

asked to rate the intensity of every stimulus using a 0-100 numerical scale (NRS). The procedure continued for up to 10 trials, or until the subject provided a rating of 100 or requested to stop. We have used similar stimulus intensities and rating scales in previous testing, and subjects are able to rate the pulses consistently. Moreover, we provided training for the participant using nonpainful stimuli, which acclimated them to the timing of the ratings. Two measures were derived from this procedure for each stimulus intensity. The average rating was computed for the pain ratings across 10 trials, with the last value carried forward for individuals who discontinued before completing 10 trials, and this measure reflects overall sensitivity to the heat pain stimuli. The other measure was the slope of the increase in pain ratings from the first trial to the maximum rating obtained, and this measure is an index of temporal summation of heat pain.

Ischemic Pain Procedure

A modified submaximal effort tourniquet procedure was used to evoke ischemic pain. The subject's right arm was elevated and supported in vertical position for 30 sec to promote venous drainage. Then, an arm cuff positioned above the elbow was inflated to 240 mm Hg, a stopwatch started, and the subject's arm was lowered to a horizontal position. The subject squeezed a handgrip dynamometer at 50% of maximum force of grip 20 times. The duration of each squeeze was 2 sec with an inter-squeeze interval of 2 sec. Subjects were instructed to verbally report when they first felt pain in their hand or forearm and when they could no longer endure or tolerate any more arm pain. The tourniquet was maintained for 15 minutes or until pain tolerance was achieved. This procedure has proven to be very effective in demonstrating differences between pain-free subjects and TMJD patients.⁶⁴

Immunologic Assays

For the determination of a “hyper-responsive” phenotype, monocytes were isolated from heparinized peripheral blood samples by ficoll gradient after which 1×10^4 cells were plated in a 96 well tissue culture plate. Cultures were stimulated with LPS (*E. coli* 1ug/ml). These assays were performed in the absence and/or presence of human recombinant estrogen. Culture supernatants from all stimulation assays were collected 24 hours post-stimulation. The resulting supernatants were used to perform Milliplex® assays (Millipore) to simultaneously detect and quantitative 12 cyto/chemokines (IL1 α , IL1 β , IL4, IL5, IL6, IL8, IL10, IL12, TNF alpha, IP10, MCP1, MIP1. To correlate inflammatory response to experimental pain induction, serum from heparinized peripheral blood samples was diluted 1:5 and 1:10 in Milliplex® Assay Buffer (Millipore), after which Milliplex® assays described above were performed to detect and quantities systemic cytokine and chemokines levels. All assays were performed on samples collected at baseline. In order to determine presence or absence of a “hyper-reactive” trait, comparisons of cytokine expression were done between TMJD and healthy controls at each collection point. In order to determine if this trait is affected by estrogen levels, comparisons of cytokine expression were done between estrogen stimulated and unstimulated cultures for all experimental groups. The level of estrogen to be used was 36ng to replicate early follicular phase, 380ng to replicate pre-ovulatory phase and 250 ng to replicate luteal phase of the menstrual cycle.⁶⁵ To determine if systemic cytokine levels are altered by pain sensitivity, cytokine levels in serum detected at each point of collection were compared within and among experimental groups.

Data Analysis

Descriptive statistics, specifically measures of central tendency (mean, median) and dispersion (variance, interquartile range), were calculated for all continuous outcome measures (WOMAC scores, performance on the SPPB), and continuous potential mediator variables (pain sensitivity, pain inhibition, biologic factors: measures of estrogen level and inflammatory markers). Analyses of covariance controlled for possible confounders in comparisons between TMJD and controls. All statistical tests were two-tailed. Technical obstacles prevented collection of some samples, and those instances are identified in figure legends. SAS v 9.1 (SAS Institute, Cary, NC) was used to perform the statistical analyses. All statistical analyses were overseen by Dr. Wei Hou, a statistical geneticist who has collaborated with our group for several months.

Table 2-1. Study exclusion criteria

Exclusion	Condition
1	Ongoing medical problems
2	Uncontrolled chronic diseases (e.g. diabetes, asthma, arthritis)
3	Use of centrally acting medication (e.g. analgesics, antidepressants)
4	History of pain-related disorders
5	Hypertension
6	Irregular menstrual cycles (exclusively for women)
7	Pregnancy, lactation, or attempting to become pregnant (exclusively for women)
8	Menstrual cycle disorders (e.g. pre-menstrual syndrome, dysmenorrhea) (exclusively for women)

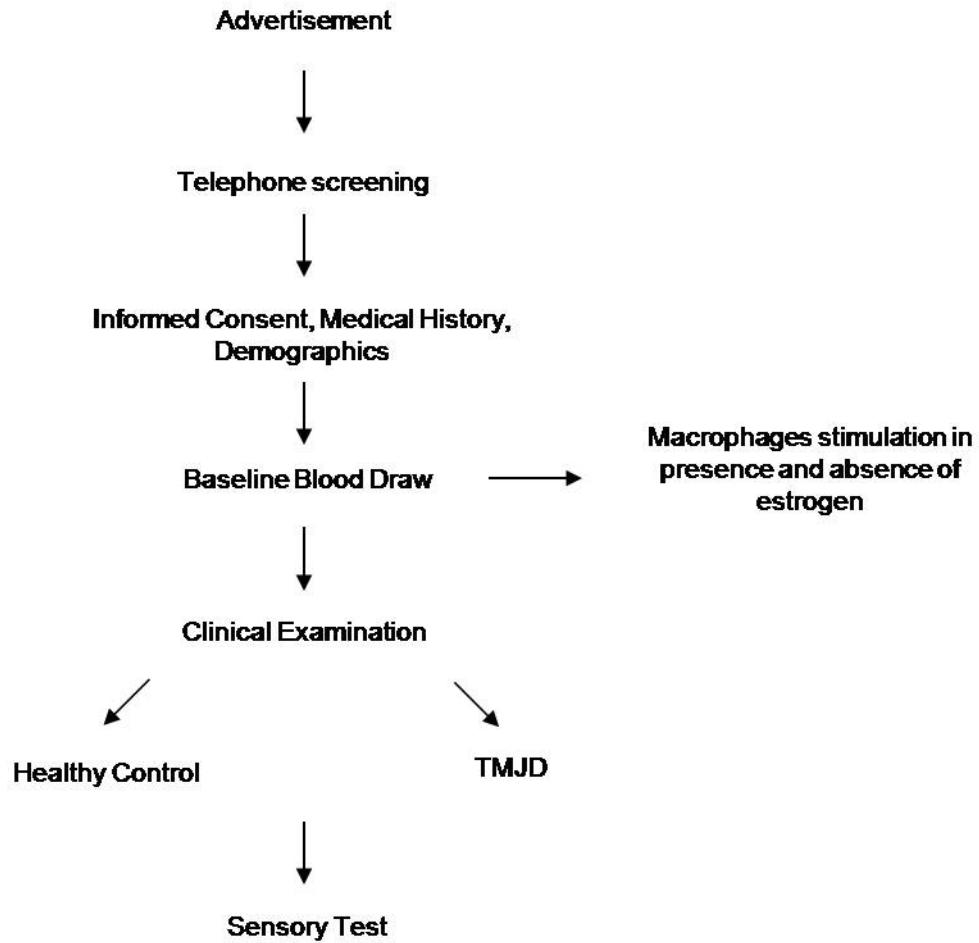


Figure 2-1. Subject recruitment and study design.

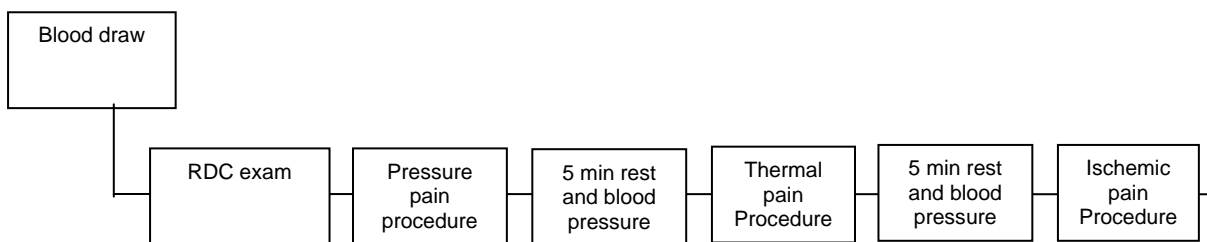


Figure 2-2. Timeline used during the sensory test.

CHAPTER 3 RESULTS

Subjects

A total of 45 women and 39 men were enrolled. The demographic composition of each of the subject groups is shown in Table 3-1. Thirty-eight percent of the participants met research diagnostic criteria (RDC) for TMJD and the other sixty two percent were age-matched controls without TMJD. Based upon the prevalence of TMJD in the U.S., we recruited 75% women and 25% men with TMJD. There were proportionately more males among the control group; therefore, sex was controlled for in all group comparisons.

Clinical Pain Results

The overall clinical characterization of the TMJD population studied, based on the medical questionnaire, were classified as mild to moderate cases, such that the total mean of the current pain at the time of the exam was 1.5, the total mean of the worst pain in the last 6 months was 6.5 and the interference of the pain with the lifestyle was graded between 1-2, all on a scale of 0 to 10. TMJD women reported the higher worst pain ($p=0.006$), marginally higher average pain in the last 6 months ($p=0.05$) and more interference with daily activities ($p=0.09$) than men (Table 3-2).

An overall index of clinical pain severity was computed by averaging the ratings of the present pain, worst pain and average pain in the last 6 months. Women showed higher values on this index than men ($p=0.01$). Moreover, this index was used to examine correlations between clinical pain and experimental pain procedures and evoked palpation pain findings from RDC exam.

Evoked Palpation Sensitivity - Research Diagnose Criteria for Temporomandibular Disorder Results

Two indices were derived from the palpation data of the RDC exam: the sum of palpation scores for the right and left side (right sum and left sum), and the total number of positive palpation sites (i.e. scores greater than zero) for each side. The total sum of palpation scores and the number of positive palpation sites were significantly higher in women than men ($p < 0.05$), and both indices were significantly higher in TMJD participants than controls ($p < 0.0001$) (Figures 3-2 and 3-3).

Basal Pain Sensitivity Results

Pressure Pain Procedure

Basal pain sensitivity scores were calculated for each pain assay and compared between groups (Control and TMJD) and between gender (Female and Male). The TMJD group showed higher sensitivity to pressure pain than controls for the joint, trapezius and ulna sites, and the group difference was marginally significant in the right temporalis ($p = 0.05$) and right masseter ($p = 0.09$). All the sites tested showed significant sex differences, with women being more sensitive to pressure pain than men (Figures 3-3 and 3-4).

Thermal Pain Procedure

There were sex differences for heat pain threshold ($p = 0.01$) and heat pain tolerance ($p = 0.005$), with women reporting lower pain threshold and tolerance than men. However, there was not a group effect (control and TMJD) for heat pain threshold or tolerance ($p > 0.05$ for each). For heat pain ratings, there was a group effect at 46°C ($p = 0.001$) and 48°C ($p = 0.04$), with TMJD participants giving higher pain score than

control participants. But, there was no main effect of gender for these heat pain ratings (Table 3-3).

Ischemic Pain Procedure and Associations Between Clinical and Experimental Pain

Women displayed significantly lower ischemic pain threshold ($p=0.04$) compared to men but did not show difference for pain tolerance. There was no group effect for either ischemic pain measure (Table 3-4). Clinical pain was strongly correlated with left-sided palpation summary scores: RDC-left side sum ($p=0.02$, $r=0.42$) and RDC-nonzero left scores ($p=0.04$, $r=0.37$). For the experimental pain findings, no significant correlations emerged for pressure, thermal or ischemic pain; however, clinical pain was marginally correlated ($p=0.08$) with average ratings of thermal pain at 46°C (Figure 3-1).

Immunological Response

Estrogen Stimulation and Inflammatory Response

To obtain preliminary data regarding the influence of estrogen on inflammatory responses, we analyzed samples of those participants that were more chronic cases for TMJD and Control samples from participants that reported no palpation pain during the clinical exam. Only 12 samples met these criteria, 8 female (4 TMJD and 4 Controls) and 4 were from male (3 controls and 1 TMJD). Therefore, to facilitate the analysis due to the small sample and unequal gender distribution, we only included female samples in the analysis.

Due to the sample size and high inter-individual variability, none of the findings achieved statistical significance. However, examination of the means revealed a trend for TMJD participants to show a higher basal serum concentration of IL1 alpha, IL1 beta, IL12, TNF alpha and IP10. Also, for TMJD participants, the trend suggests that

estrogen had an anti-inflammatory effect on IL6, IL8 and IL10 independent of the amount of estrogen used during the stimulation; both low and high stimulation decreased the inflammatory response. Estrogen in low dose also showed anti-inflammatory effects on IL1alpha, IL1 beta, IL12, TNF alpha, IP10 and M1P1. High stimulation of estrogen had an inverse effect, being proinflammatory for IL1 alpha and beta and for IL12, TNF alpha and IP10 when compared with low stimulation (Figures 3-6 and 3-7). Additional exploratory analyses sought to examine associations between inflammatory responses and experimental pain perception. Regression analysis showed that some cytokines were significantly associated with pain responses.

Pressure Pain Procedure

TNF alpha was associated with pressure pain threshold in the masseter in blood stimulated with low estrogen for TMJD ($p=0.01$, $\beta = -2.38$). It was marginally significant in the control group ($p=0.08$, $\beta = -1.20$). Also, pressure pain in the temporomandibular joint was a significant predictor for TNF alpha in blood stimulated with low estrogen for controls ($p=0.01$, $\beta = -2.32$) and TMJD patients ($p=0.0003$, $\beta = -4.49$).

Thermal Pain Procedure

In the TMJD group thermal pain tolerance was significantly associated with IL1 Beta in blood stimulated with high estrogen ($p=0.03$, $\beta = -24.51$). Thermal pain threshold was associated with IL1 Beta in blood stimulated with low estrogen for both controls ($p=0.02$, $\beta = -8.28$) and TMJD participants ($p=0.03$, $\beta = -16.36$). Also, thermal pain threshold was associated with IL6 in blood stimulated with low estrogen for the control group ($p=0.03$, $\beta = -51.18$) and TMJD group ($p=0.03$, $\beta = -106.03$). We also found that thermal pain threshold was marginally related to IL6 in blood stimulated with high estrogen for control ($p=0.08$, $\beta = -42.10$) and TMJD ($p=0.05$, $\beta = -93.15$) groups.

Table 3-1. Demographics

	TMJD n=32	CONTROL n=52
Mean age years (SD)	24 (5)	24 (6)
Male (%)	8 (25)	31 (60)
Female (%)	24 (75)	21 (40)
Caucasian (%)	19 (59)	26 (50)
Others (%)	13 (41)	26(50)

SD, standard deviation.

Table 3-2. Self reported pain and pain interference by gender, Mean (SD)

	Female	Male	Total
Mean of "present pain" rating (0-10)	2 (1.22)	1 (1.34)	1.5 (1.8)
Mean of "average of pain in the past 6 months" ratings (0-10)**	4 (1.77)	2.5 (0.78)	4 (1.6)
Mean of "worst pain in the last 6 months" (0-10) *	7(1.8)	5(2.3)	6.5 (2.2)
Number of days in the least 6 months lost because the pain (days)	1 (2.17)	1 (1.03)	1 (1.9)
Interference with daily activities in the last 6 months (0-10)**	2.5 (1)	2 (1)	2 (2)
Change in social life (0-10)	2 (1.72)	1 (0.75)	1.5(1.8)
Change in work activity (0-10)	2 (1.24)	1 (1.06)	1.5(1.8)

SD, standard deviation,

* Gender difference $p < 0.05$

** Marginal gender significance $0.05 < p < 0.10$

Table 3-3. Thermal pain measures, Mean (SD)

	TMJD n=32	Control n=52	Female n=45	Male n=39
Heat pain threshold (°C)*	41.08 (2.71)	42.35 (3.35)	40.79 (3.45)	43.10(3.00)
Heat pain tolerance (°C)*	45.11 (2.71)	46.31 (2.20)	44.91 (2.39)	46.94 (2.10)
Average heat pain rating at 46 °C** (0-100)	64.21 (3.5)	41.76 (21.97)	57.27 (28.72)	42.28 (19.54)
Average heat pain rating at 48 °C** (0-100)	75.09 (10.6)	60.91 (24.82)	70.67 (27.80)	61.29 (21.88)
Average heat pain rating at 50 °C (0-100)	85.20 (23.9)	75.47 (23.25)	80.83 (26.36)	77.27 (20.71)

(SD) standard deviation, * Gender Difference $p < 0.05$, ** Group difference $p < 0.05$

Table 3-4. Ischemic pain measures, Mean (SD)

	TMJD n=31	Control n=49	Female n=43	Male n=39
Ischemic pain Threshold*	247.84 (197.98)	271.2 (201.47)	199.12 (175.04)	333.71 (202.87)
Ischemic pain tolerance	549.03 (266.42)	614.24 (259.93)	530.93 (276.25)	654.28 (233.56)
Final intensity ratings	15.90 (5.67))	16.47 (6.29)	16.53 (5.47)	15.92 (6.68)
Final unpleasantness ratings	16.26 (5.44)	16.78(4.93)	17.23 (4.76)	15.84 (5.43)

(SD) standard deviation,

* Gender difference $p < 0.05$

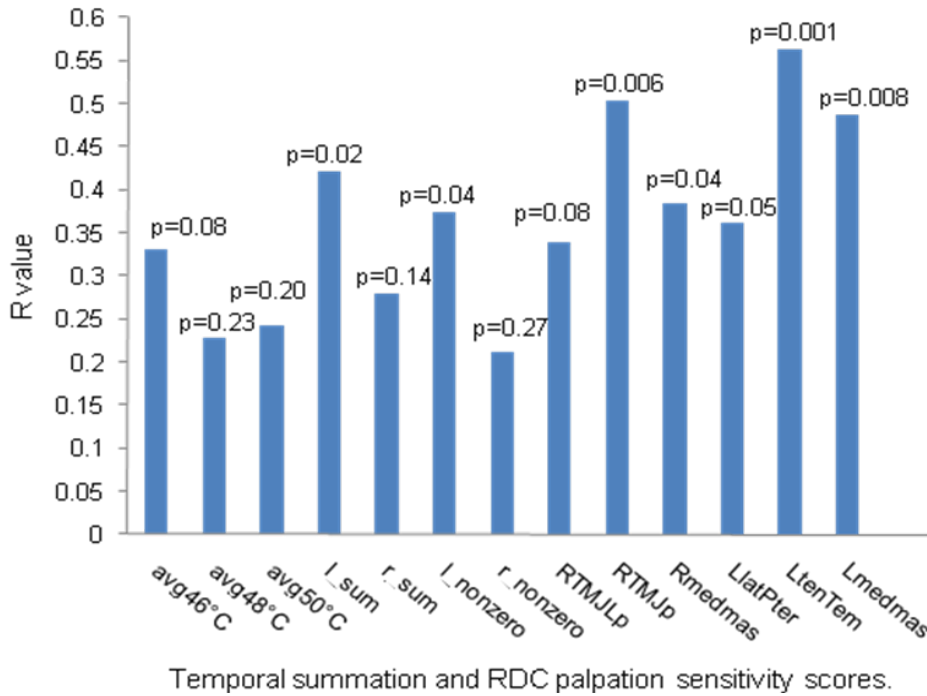


Figure 3-1. Correlation of clinical pain with sensory tests and research diagnose criteria (RDC) for temporomandibular disorder palpation sensitivity scores.

Abbreviations: avg46°C (average heat pain rating at 46 degrees Celsius); avg48°C (average heat pain rating at 48 degrees Celsius); avg50°C (average heat pain rating at 50 degrees Celsius); I_sum (left sum of positive painful sites – RDC); r_sum (right sum of positive painful sites – RDC); L_nonzero (left number of sites greater than zero during evoked pain); r_nonzero (right number of sites greater than zero during evoked pain); RTMJLp (palpation pain grade at the right temporomandibular joint lateral pole); RTMJp (palpation pain grade at the right temporomandibular joint posterior); Rmedmas (palpation pain grade at the right medial masseter); LlatPter (palpation pain grade at the left lateral pterigoid); LtenTem (palpation pain grade at the left tendon temporalis); Lmedmas (palpation pain grade at the left medial masseter)

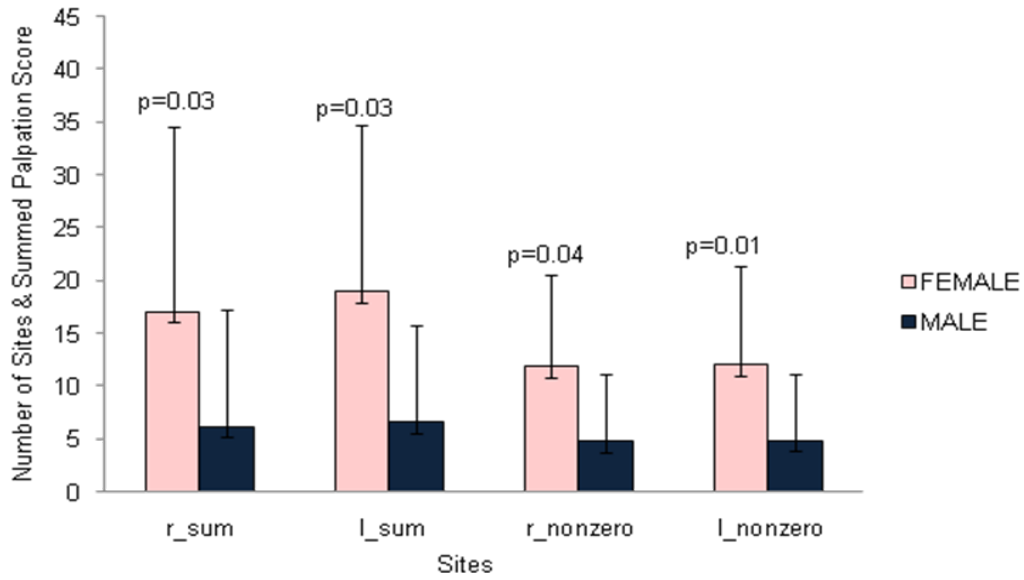


Figure 3-2. Sum of palpation scores for the right and left side, and the total number of positive palpation sites (i.e. scores greater than zero) for each side using the RDC by gender.

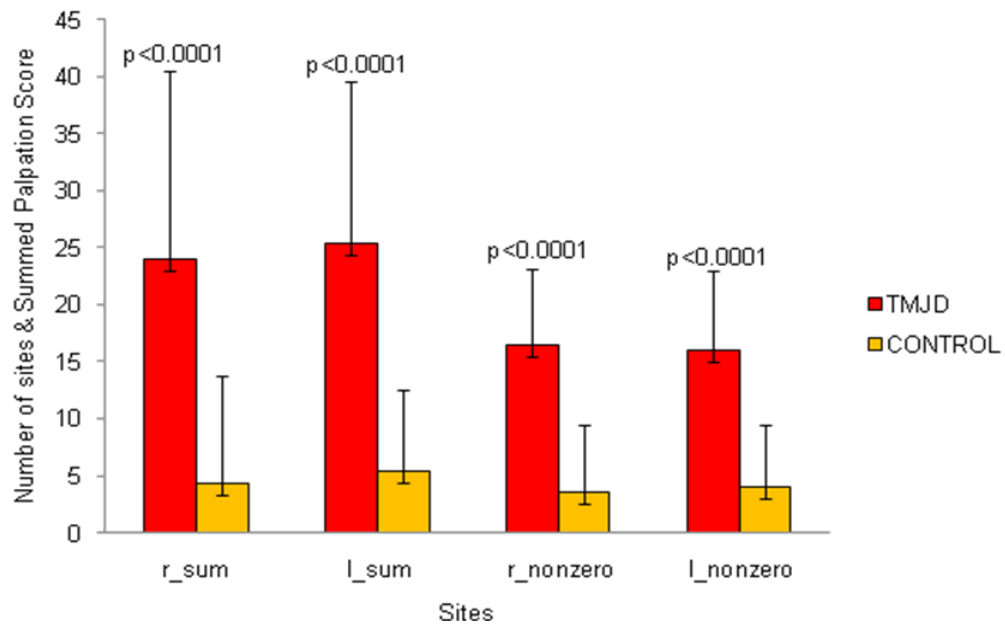


Figure 3-3. Sum of palpation scores for the right and left side, and the total number of positive palpation sites (i.e. scores greater than zero) for each side using the RDC by group.

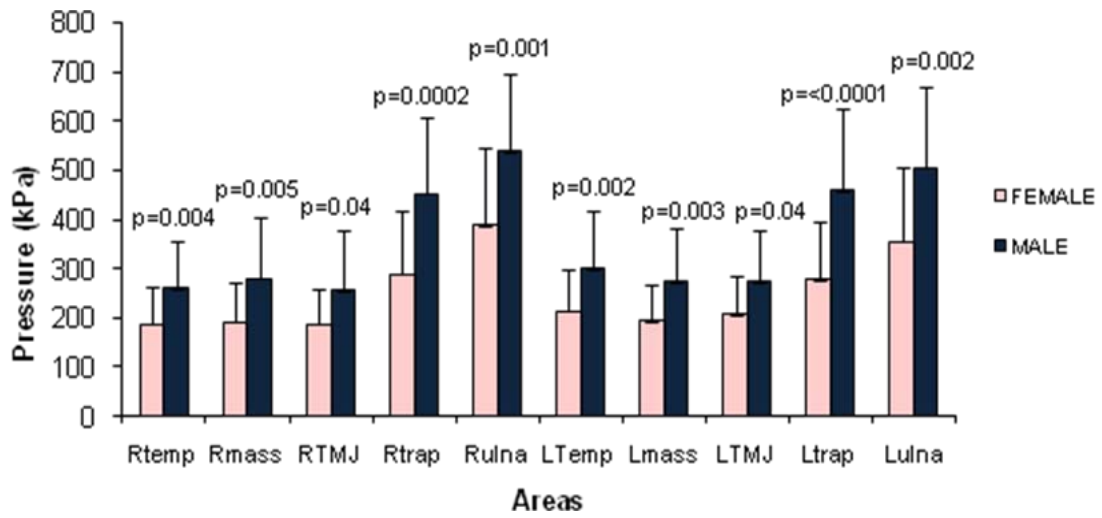


Figure 3-4. Pressure pain threshold procedure by gender.

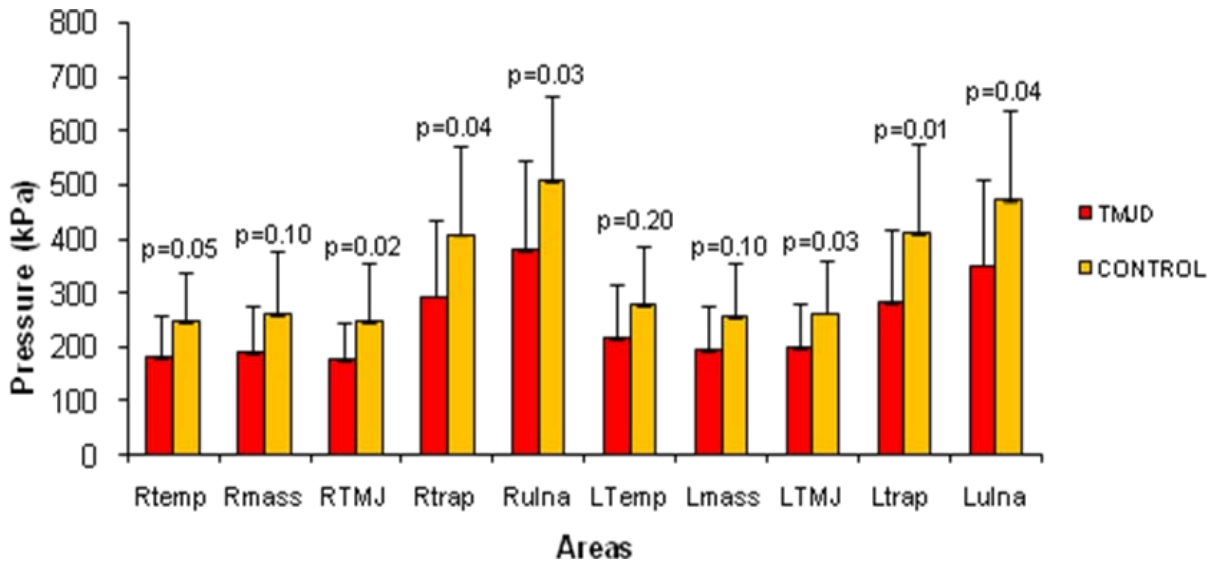


Figure 3-5. Pressure pain threshold procedure by group.

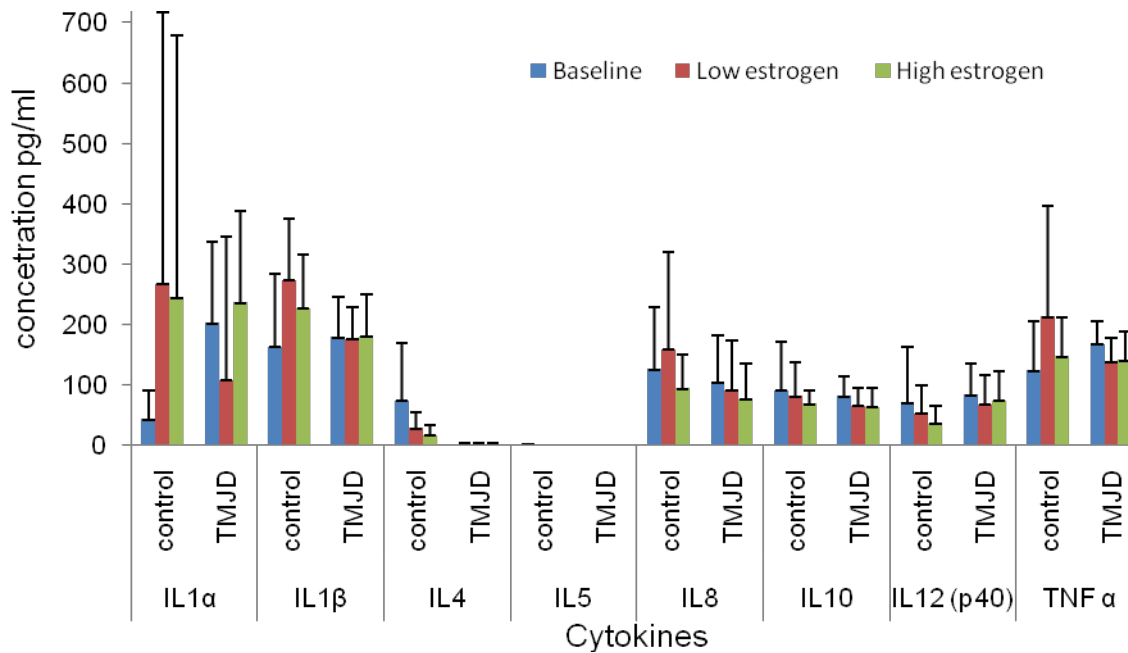


Figure 3-6. Cytokine responses at baseline and after stimulation with low and high estrogen across groups.

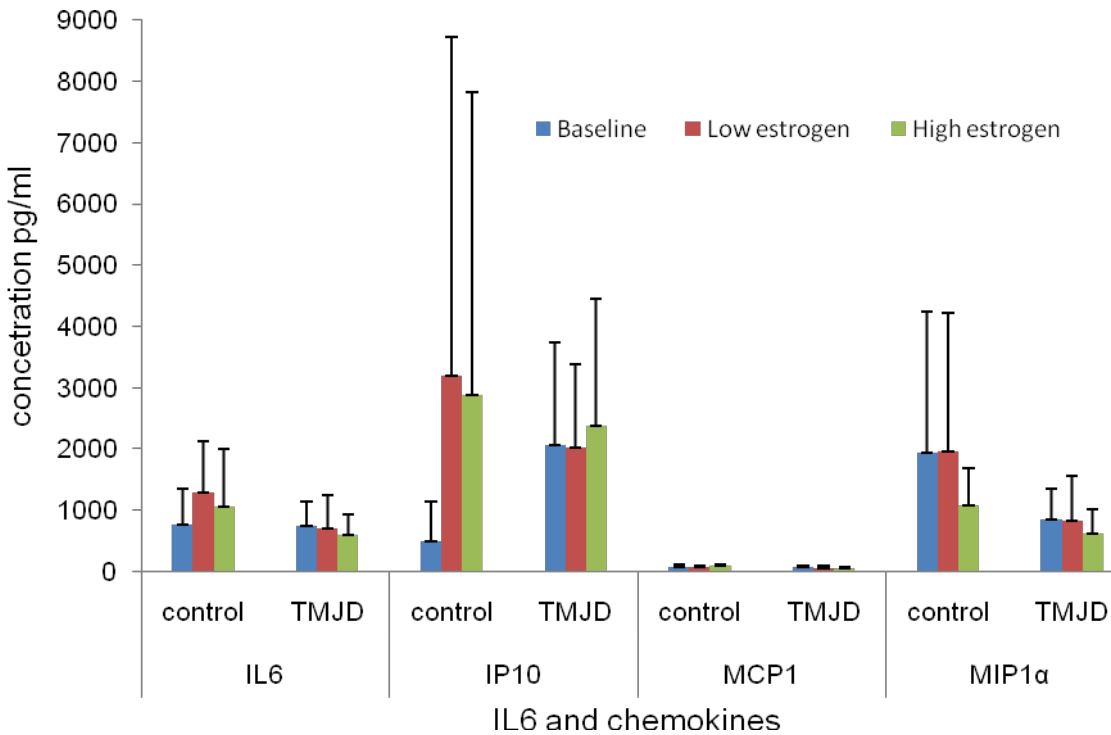


Figure 3-7. IL6 and chemokines responses at baseline and after stimulation with low and high estrogen across groups.

CHAPTER 4 DISCUSSION

This was a case control pilot study to examine sex-related influences on pain sensitivity and inflammation in participants with temporomandibular joint disorder. In general, the results confirm our hypotheses and are in accordance with previous findings that women are more sensitive than men to clinical TMJD pain and experimental pain. Also, these results provided us with novel preliminary data suggesting that estrogen may influence the inflammatory response in TMJD patients, which will serve to guide our future research.

Clinical Pain Response

As expected, there was a significant influence of gender in the report of clinical pain, such that TMJD women reported greater clinical pain than men. Also, women reported more interference of pain with their daily activities than men. This result is consistent with the literature that reports that women have worse pain intensity, and greater pain-related interference with function for chronic pain conditions.^{66, 67}

Clinical pain in TMJD participants was significantly correlated with palpation sensitivity, but interestingly, it was correlated only with palpation indices from the left side but not the right side. This may indicate that our population had more clinical pain sensitivity on the left side than the right side. This possibility is understandable from a clinical perspective because TMJD symptoms can be aggravated by parafunctional habits such as chewing on only one side of the mouth, sleeping on one side, or unbalanced occlusion that can lead to increased stress on one side of the joint and muscle, causing more symptomatology on that side. Indeed, some studies have shown using electromyography that an unbalanced occlusion in molar contact and unilateral

chewing pattern exhibit a greater asymmetry in activity of all the masticatory muscles.⁶⁸ However, we did not collect information regarding laterality of pain; therefore, we cannot confirm this explanation.

Experimental Pain Responses

Women showed greater sensitivity across all pain modalities than men independent of the group factor. This is highly consistent with an abundance of published literature on sex differences in experimental pain responses.^{11, 69,70,71,72} Also, TMJD participants showed greater sensitivity to pressure pain compared to controls. Previous findings have indicated more generalized hypersensitivity to experimental pain among TMJD patients.^{73, 74, 75} It may be that the relatively mild symptomatology of our TMJD group was related to lower experimental pain sensitivity than has been previously reported. Our findings also suggest that heat pain ratings are modestly associated with clinical pain severity among TMJD patients. Although of only borderline significance, this positive correlation between self-reported clinical pain and heat pain ratings is potentially interesting because it shows that experimental pain may be a useful predictor for clinical pain related to TMJD,⁷⁶ potentially implicating central pain amplification in TMJD pain.

Immunologic Assays

Although the results were not statistically significant due to the small sample size and the necessity of some adjustment in the protocol, we obtained potentially interesting results. Previous literature suggests that E2 exerts anti-inflammatory properties on LPS-activated monocyte/macrophage cell lines or microglial cells, macrophage cells of the CNS^{40, 41, 42} and estrogen supplementation also reduces TNF alpha levels in cells of monocytes/macrophage. The trend in our results indicated that estrogen may have an

anti-inflammatory action on the concentration of TNF α in the TMJD patients, independent of the dosage used for the in vitro stimulation (Table 3-2). It is interesting to note that TNF α is present in the synovial fluid of TMJD patients⁴⁸ and this cytokine induces the production of other cytokines such as IL1, IL6 and IL8.⁴⁷ Our results suggest an anti-inflammatory effect of estrogen on these 3 cytokines in TMJD participants, with the exception of IL1 alpha. However, for IL1 beta, estrogen was anti-inflammatory independent of the dosage. These data are very preliminary, but provide a foundation to continue the studies in this direction.

Study Limitations

Our study had several limitations related to the sample characteristics and immunological protocol. The sample size of male TMJD participants was much lower than the female TMJD, although we had predicted this due to the prevalence of this condition. In each sex the TMJD cases analyzed ranged from mild to moderate severity. This might have influenced the pattern of results, since experimental pain sensitivity may differentiate according to the level of clinical pain that these participants are feeling. Also, considering that TMJD may have an inflammatory component aggravating their condition, the lower presence of pain may be related to a lower inflammatory response.

Several problems emerged in developing the immunological protocol, due to the fact that it was a novel study and we could not rely on previously developed methods. Many samples were lost because of the time sensitive nature of this assay. Therefore, we ended up with a small sample size, which limited our ability to detect statistically significant results. However, the trends we observed are in agreement with the literature.

Future Directions

Although several lines of evidence suggest an influence of estrogen in inflammation, no investigator to date has determined whether presence or absence of a 'hyper-reactive' trait can influence pain sensitivity among TMJD patients or increase the risk of developing TMJD. Also, no study has been done in humans, to our knowledge, to determine if this trait is affected by estrogen levels, comparing cytokine expression between estrogen stimulated and unstimulated cultures of macrophages from patients with TMJD compared to a control group. Therefore in our next study, with a larger sample size, we intend to investigate with a more optimal immunological protocol how estrogen may influence the inflammatory markers not only in baseline but at different times during the experimental pain. We also intend to determine if the presence of increased levels of some cytokines are correlated with higher clinical and experimental pain. Moreover, we intend to analyze whether polymorphisms in the estrogen receptor and genes that regulate estrogen metabolism may be correlated with higher or lower inflammatory response.

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BIOGRAPHICAL SKETCH

Margarete Ribeiro-Dasilva was born in Sorocaba, Sao Paulo, Brazil in 1974. She attended dental school at the University of Campinas-Brazil (UNICAMP), where she received her Doctor of Dental Surgery as well as her Master of Science and PhD in the area of prosthodontics clinical research. She then relocated to the United States where she completed her post-doctoral program with the University of Florida College of Dentistry's Department of Community Dentistry and Behavioral Science under the mentorship of Dr. Roger Benton Fillingim. Margarete's current faculty position as Assistant Research Professor at the University of Florida College Of Dentistry came with her KL2 award from the Clinical and Translational Science Institute in 2009 and the completion of her MS in medical science with a concentration in clinical and translational science in May 2010. She currently resides in Ocala, FL with her husband Walter and son Joel Anthony.