Title
The role of female reproductive hormones in the etiopathogenesis of TMJ disorders

Author
Reed, Tina,

Publication Date
2000

Peer reviewed|Thesis/dissertation

THE ROLE OF FEMALE REPRODUCTIVE HORMONES IN THE ETIOPATHOGENESIS OF TMJ DISORDERS

by

TINA M. REED

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ORAL BIOLOGY

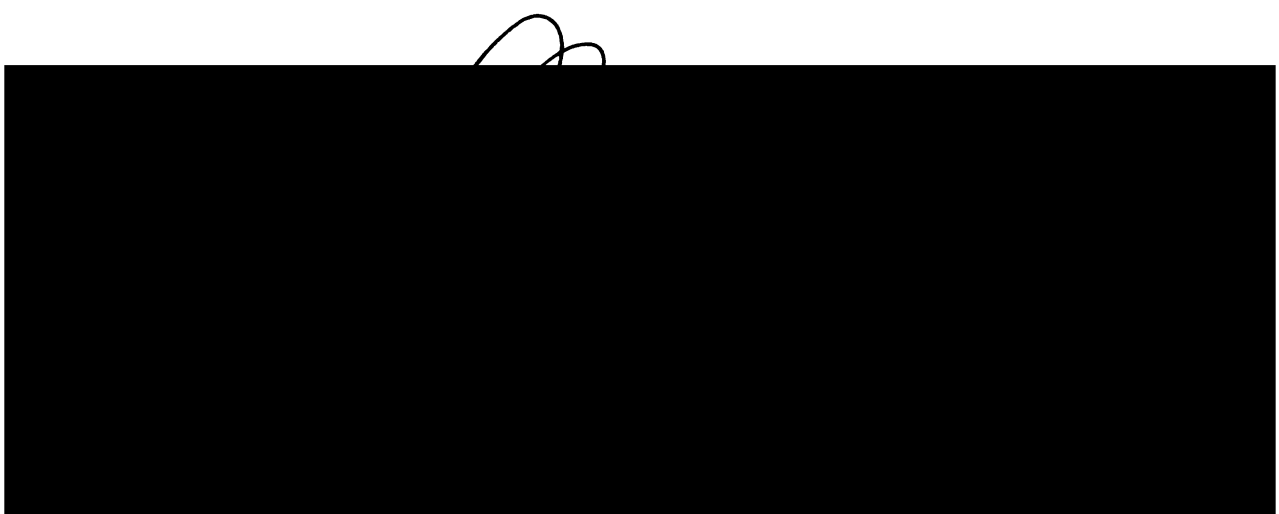
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



Date

University Librarian

ACKNOWLEDGEMENTS

I could not have completed this study without the support and dedication of many individuals. I am extremely grateful to every one of them.

I first want to thank my mentor, Sunil Kapila, who provided guidance and support throughout this project, as well as my entire orthodontic training. His dedication is always beyond the call of duty, and for that, many of us are thankful. Dr. Charles McNeill, Patricia Rudd and Dr. David Hatcher generously gave of their time to provide me with the training necessary to perform all of the clinical exams and radiographic evaluations. Dr. Stuart Gansky was invaluable in guiding our statistical investigations. I also thank the staff at the General Clinic Research Center for collecting the serum and saliva samples, and Madhavee Buddikot for performing the relaxin assays.

Finally, I'd like to thank my Mom and Dad, for twenty-eight years of unconditional love and support, and Joel, my best friend for bringing so much happiness to every day of my life.

THE ROLE OF FEMALE REPRODUCTIVE HORMONES IN THE ETIOPATHOGENESIS OF TMJ DISORDERS

Tina M. Reed, D.M.D.

ABSTRACT

Temporomandibular joint disorder (TMD) describes a spectrum of conditions that are associated with a wide range of clinical signs and symptoms involving the temporomandibular joint (TMJ) and muscles of mastication. TMDs have a very specific gender and age distribution. They are two to three times more likely to occur in women, usually between the ages of 18 and 45 years. Because of this gender and age predilection, some investigators have proposed that female reproductive hormones play a role in the etiology of these disorders. However current evidence to support this theory is of an epidemiologic nature. In addition, while it has been suggested that the predominately female hormone, relaxin, may be linked to increased systemic joint laxity (SJL), no study has examined the potential association between TMJ disease, hormone levels and SJL. This investigation is the initial component of a large case control study examining the relationship between TMDs, SJL and systemic levels of female reproductive hormones. The study also set out to examine whether levels of these hormones in serum correlate with levels in saliva.

Forty-seven subjects were initially recruited for the study and placed into a symptomatic or asymptomatic group on the basis of a questionnaire, clinical TMJ examination and radiographic evaluation. This resulted in twenty-six symptomatic and twenty-one asymptomatic subjects. All forty-seven subjects were also evaluated for systemic joint

hypermobility. In addition, sixteen of the symptomatic and eleven of the asymptomatic subjects provided serum and salivary samples at three time points in one menstrual cycle for the quantification of systemic levels of β-estradiol, progesterone and relaxin.

The quantitative findings on severity of TMJ disease, SJL and hormone levels were compared between the two groups using Student's two sample t-test, chi-square tests, and logistic regression with the significance level set at alpha = 0.05. The association between serum and salivary levels of the three hormones was assessed using linear regression (with 95% percent confidence intervals).

Due to the fact that this is the initial phase of a large study, the small sample size made it difficult to find statistically significant differences between the two groups. Moreover, this phase would have to formally use interim analysis adjustments before drawing any conclusions. The clinical exam showed that the symptomatic group had a smaller vertical range of motion, greater laterotrusive movement and a greater percentage of joint sounds than the asymptomatic group, however these differences were not significant. The symptomatic group demonstrated greater overall flexibility, with a nominally significant difference detected in the thumb (P = 0.016). The symptomatic group also demonstrated higher levels of β-estradiol, progesterone and relaxin, at specific time points in the menstrual cycle, although these differences were not statistically significant. The serum levels of progesterone and relaxin, but not β-estradiol showed a moderate (r = 0.66 to 0.80) correlation with levels in saliva. These findings show minor trends of a potential relationship between TMJ disease, SJL and female reproductive hormones. Further increases in sample size and

final analyses may help provide more conclusive evidence of any association between these variables.

Thesis Advisor

TABLE OF CONTENTS

TITLE PAGE i
ACKNOWLEDGEMENTS ii
ABSTRACT iii
TABLE OF CONTENTS vi
LIST OF FIGURES viii
LIST OF TABLES ix

I. BACKGROUND, SIGNIFICANCE AND SPECIFIC AIMS 1

A. Introduction 1
B. Temporomandibular Disorders 2
1. Disc Displacement 2
2. TMJ Dislocation 3
3. Inflammation and Arthralgia 3
4. Osteoarthrosis 4
5. Osteoarthritis 4
6. Ankylosis 5
C. Epidemiology 5
1. Prevalence 5
2. Gender Differences and TMDs 6
D. Proposed Etiologies 6
1. Occlusal Factors 6
2. History of Trauma 7
3. Psychological Factors 7
4. Systemic Joint Laxity 8
5. Endocrine Factors 8
E. Is Disease of the TMJ Associated With Systemic Levels of Relaxin? 9
F. Hypothesis, Specific Aims and Significance 11

II. MATERIALS AND METHODS 13

A. Recruitment and Criteria for Inclusion of Subjects 13
B. Procedures 14
1. Questionnaire 15
2. Clinical TMJ Evaluations 16
3. Systemic Joint Hypermobility 17

(a) Thumb 17
(b) Fifth Digit 18
(c) Elbow 18
(d) Knee 19
4. Radiographic Evaluation of the TMJ 19
5. Classification and Assessment of TMJ Status 21
6. Determining Time-Points for Collection of Blood and Saliva 21
7. Collection of Blood and Saliva 22
8. Processing of Blood and Saliva 23
C. Quantification Of Relaxin, β-Estradiol And Progesterone 24
1. Relaxin enzyme linked immonosorbent assay (ELISA) for serum and saliva 24
2. Progesterone and β-estradiol Assays 25
D. Statistical Methods And Analyses 25
1. Error of Method 26
2. Statistical Analyses 26

III. RESULTS 28

A. Profile of the Study Population 28
B. Clinical TMJ Findings 31
C. Radiographic TMJ Findings 35
D. Classification Of Tmj Disease 36
E. Systemic Hypermobility 37
F. Systemic Hormone Levels in Asymptomatic and Symptomatic Women 38
G. Correlations Between Serum and Salivary Hormone Levels 41

IV. DISCUSSION 43

V. CONCLUSION 49

VI. REFERENCES 50

VII. APPENDICES 55

LIST OF FIGURES

FIGURE **PAGE**

Figure 1 - Histogram of mean (± SD) age of individuals in the asymptomatic and symptomatic groups (N=47). 29
Figure 2 - Number of subjects in asymptomatic and symptomatic groups divided by ethnicities. 30
Figure 3 - Histogram of mean (± SD) unassisted opening in both groups (N=47)..... 32
Figure 4a-c - Histograms of mean (± SD), maximum (a), minimum (b) and mean (c) laterotrusion (mm) in asymptomatic and symptomatic subjects (N=47)... 34
Figure 5a-c - Graphs depicting mean levels of progesterone, relaxin and β- estradiol at the midfollicular (mf), ovulation -1 (ovul-1), and midluteal (ml) days of collection (N=27) 39
Figure 6a-f - Absolute differences between maximum and minimum levels of serum (a to c) and salivary (d to f) hormones within individuals in the symptomatic and asymptomatic groups (N=27). 40
Figure 7a-c - Scatterplot showing the relationship between serum and salivary relaxin at midfollicular, ovulation-1, and midluteal (N=27). 42

LIST OF TABLES

TABLE **PAGE**

Table I - Pearson's correlation coefficient for measured TMJ and Flexibility variables (N=15) 26
Table II - Number and mean age (± SD) of subjects in both groups of the study population. 29
Table III - Anticipated and current enrollment of subjects from various ethnicities (percentages) 30
Table IV - Percentage and number of subjects in each group reporting pregnancies and at least one successful childbirth. 31
Table V - Percentage and number of subjects in each group experiencing current facial pain. 31
Table VI - Age-adjusted odds ratio and 95% confidence intervals comparing symptomatic and asymptomatic subjects (N=47) 32
Table VII - Percentage and number of subjects in both groups demonstrating various TMJ sounds. 33
Table VIII - Percentage of subjects in both groups with positive finding for morphologic changes (N=40). 35
Table IX - Percentage and number of symptomatic subjects (N=26) in the TMJ disease categories 36
Table X - Percent of individuals demonstrating bilateral hypermobility in the fifth digit, thumb, elbow and knee and the mean and +SD of total flexibility score (N=47). 37
Table XI - Correlation coefficient (r) values depicting associations between serum and saliva hormone levels at the three days of sample collection and at all three days combined 41

I. BACKGROUND, SIGNIFICANCE AND SPECIFIC AIMS

A. INTRODUCTION

The term "temporomandibular disorder" (TMD) describes a wide array of diseases that involve the temporomandibular joint (TMJ) and muscles of mastication (reviewed in Bell, 1990; McNeill, 1993, Okeson, 1996). The triad of symptoms, joint sounds, locking and pain comprise the most common complaints associated with the temporomandibular joint. These clinical manifestations are often cyclical in nature, making it difficult for the clinician to properly diagnose and treat these disorders. In addition, radiographic evidence of these disorders is not present until advanced stages of the disease, which only adds to the frustration of both the clinician and patient when attempting to reach a correct, early diagnosis. Added to this already complex situation is the occasional occurrence of secondary psychological changes associated with TMJ problems. Thus, it would be extremely helpful to approach these disorders from a preventive standpoint by recognizing those individuals who are predisposed to TMDs. However this requires knowledge of the etiology and pathogenesis of TMDs, which have yet to be identified. Despite this limited information, there is currently some information on the particular sex and age distribution of patients seeking treatment for TMDs (LeResche et al., 1993) which may provide promising clues to the etiology and pathogenesis of TMDs. These disorders affect females more than males, usually between the ages of 18 to 45 years (Howard, 1991; McNeill, 1993). This may indicate that TMDs are linked to specific factors in women of childbearing age. This case control study examined the association between TMDs and systemic levels of female

reproductive hormones (β-estradiol, progesterone and relaxin). We then examined whether levels of these hormones in serum correlate with levels in saliva.

Secondly, we evaluated if an association exists between TMDs and joint hypermobility, due to the fact that, like TMDs, there is a higher predilection of systemic joint laxity (SJL) in women than in men, and that there is a potential association between SJL and the hormone relaxin.

B. TEMPOROMANDIBULAR DISORDERS

Temporomandibular disorders can be separated into masticatory muscle disorders and articular TM joint disorders of the TMJs. The common disorders of the joints include disc displacement, dislocation, inflammation, osteoarthritis, osteoarthrosis and ankylosis. All are described in further detail below.

1. Disc Displacement

The disc divides the joint capsule into upper and lower compartments. It is formed of dense, fibrous connective tissue, that provides stability between the condyle against the glenoid fossa of the temporal bone. The disc normally remains between these two bony structures during mandibular movement. However the disc can become displaced from this position. The usual direction for displacement is anterior or antero-medially (Isberg-Holm et al., 1982; Farrar et al., 1983). Displacement can occur either with or without reduction.

i) Disc displacement with reduction. The disc is temporarily misaligned during the translatory component of opening and closing but "reduces" or improves its relationship before the movement is complete. It usually presents with a clicking noise, as the disc re-establishes it's normal position. However, "reciprocal clicking" whereby a noise is heard during the opening and closing movement of the mandible, is also not uncommon.

ii) Disc displacement without reduction. The misalignment of the disc-condyle relation is maintained during translation.

2. TMJ Dislocation

Dislocation occurs when the condyle translates beyond the anterior band of the articular disc, getting stuck in this position. Sometimes this dislocation is momentary and is self-reducing in the patient. Other times it may require manual reduction by a dentist. The onset is often associated with yawning or when the muscles are fatigued from keeping the mouth open too long.

3. Inflammation and Arthralgia

Inflammatory conditions usually occur secondary to trauma, irritation, or infection (Clark and Solberg, 1987). Synovitis refers to inflammation of the synovial lining of the joint. It is characterized by local pain that is exacerbated by function and superior-posterior loading.

Swelling in the area can decrease the ability to occlude on the ipsilateral posterior teeth. Capsulitis is an inflammation of the capsule related to trauma of the capsule.

4. Osteoarthrosis

Osteoarthrosis is defined as a degenerative, yet non-inflammatory, condition of the joint characterized by structural changes of the bony surfaces (DeBont et al., 1985). Evidence of these changes is usually not detectable radiographically until later stages of the degenerative process. The condition is painless, but often is accompanied by crepitus and a limited range of mandibular movement. It is believed that osteoarthrosis is due to a physiologic imbalance between the forces applied to a joint and the ability of the soft tissue, cartilage and bone to withstand these forces (Bland et al., 1985).

5. Osteoarthritis

Osteoarthritis is a degenerative condition that is accompanied by secondary inflammation of the TMJ. It usually results from overloading of the joint structures. The identifying characteristics of TMJ arthritis are joint tenderness upon palpation, joint pain that increases with function, and crepitus. An acute malocclusion, such as a posterior open bite, may result from significant inflammation within the joint cavity.

6. Ankylosis

The primary characteristic of ankylosis is a complete or partial restricted joint movement. It can occur as a result of intracapsular fibrosis or bony fusion (Clark and Solberg, 1987). The adhesions usually occur when the posterior attachment and disc fibrose to the temporal bone. No pain is present unless the joint is forced beyond the end range of motion. Radiographs show osseous fusion in bony ankylosis or appear normal in fibrous ankylosis in the closed position of the jaw.

C. EPIDEMIOLOGY

1. Prevalence

Studies have shown that between 4 and 12 percent of various populations around the world suffer from TMJ problems. This translates to approximately 20 million people in the United States and 450 million worldwide (Drangsholt and LeResche, 1999). However, there is currently no universally accepted classification scheme and diagnostic criterion that exists for TMDs. This complicates the interpretation of results from various epidemiological and clinical studies on TMDs. Thus the true prevalence of TMDs is difficult to determine exactly.

2. Gender Differences and TMDs

In patient population studies, women seek treatment for TMDs in disproportionately far greater numbers than men, ranging anywhere from a ratio of 2:1 to 10:1 (Zarband Thompson, 1970; Cohen, 1978; Solberg, 1982; McNeill, 1993, Dragsholt and LeResche, 1999). Other studies examining clinical signs also showed significant gender differences, especially in regard to tender joints and TMJ clicking (Magnusson, 1978; Egermark et al., 1981; Helkimo, 1974; Rieder, 1983). The majority of these females are between the ages of 13 and 45 years (Carlsson et al., 1967; Isaacson et al., 1986). However, the reason for this disproportionate gender distribution is still unknown.

D. PROPOSED ETIOLOGIES

There are currently several theories on the etiology and pathogenesis of diseases of the TMJ. These range from anatomic factors, psychosocial factors, systemic joint laxity and endocrine factors. Several, but not all of the proposed etiologic factors are summarized below.

1. Occlusal Factors

Several studies have examined a link between TMDs and occlusal interferences (Roth, 1973; Magnussen and Carlsson, 1983; Forssell and Kangasniemi, 1986;). All found that the introduction of occlusal interferences to a stable occlusion of an asymptomatic person caused signs and symptoms of TMD to develop. Other studies suggest that discrepancies between

centric occlusion and centric relation could be a predisposing factor (Dubral, 1981; Roth, 1981). However other literature does not support this view (Carlsson and Droukas, 1984; Seligman and Pullinger, 1989; Rinchuse, 1996).

In recent years, orthodontic treatment has been postulated as a cause of TM disorders. However, two long-term studies indicated that comprehensive fixed appliance orthodontic therapy in adolescents did not alter the risk of TM disorders developing later in life (Sadowsky and Polson, 1984). Other studies that compared orthodontically treated patients versus controls found no significant differences in incidence of TM disorders (Gianelly et al., 1988, Dahl et al., 1988).

2. History of Trauma

The application of traumatic force can injure any or all components of the joint. One study reported that of 89 patients with arthrographically positive internal derangements, 25% had a history of jaw trauma immediately prior to the onset of the TMD (Katzberg et al., 1980). Another reported that a prior history of major trauma occurred in 30% of patients who reported to a temporomandibular and facial pain clinic (Pullinger et al., 1985). Fifty percent of patients who underwent TMJ surgery reported a prior history of facial trauma.

3. Psychological Factors

Persons with TMDs are often characterized in terms of their clinical symptoms, but less is documented about factors of social and psychological importance. A large case control study

found that the frequency of TMD was 1.4 times higher in groups with psycho-emotional tension (Wigdorowicz et al., 1979). In a study of 350 TMJ patients, higher levels of emotional stress were reported in those with the most severe local and general symptoms (Carlsson, Kopp and Wedel, 1982). More recent studies have shown that many TMJ patients report at least one previous episode of mental depression (Drangsholt and LeResche, 1999).

4. Systemic Joint Laxity

The prevalence of systemic hypermobility is higher in females than in males (Westling, 1992; Larsson et al., 1993; Pountain, 1992). Extreme laxity was found only in females, and was associated with more severe joint symptoms than seen in males.

Calguneri suggested that increased levels of certain hormones, particularly relaxin, could be the cause of such extreme laxity (Calguneri et al., 1982). As in any joint, increased laxity within the temporomandibular joint may make it less capable of tolerating normal mechanical loading.

5. Endocrine Factors

Several investigators have examined the potential role of female reproductive hormones in the etiology of TMDs. Recent studies have focused on the presence of estrogen receptors within cultured TMJ tissues (Aufdermorte et al., 1986; Milam et al., 1987; Campbell et al., 1993). Abubaker was one of the first investigators to find receptors in human TMJ specimens (Abubaker, 1991). Another investigation examined the role of exogenous

hormones from estrogen replacement therapy (ERT) and oral contraceptives (OC) in increasing the risk of developing TMJ pain (Le Resche, Sanders and Dworkin, 1993). This epidemiologic study found that ERT and OC users were 33% and 20%, respectively, more likely to seek care for facial pain than non-users.

E. IS DISEASE OF THE TMJ ASSOCIATED WITH SYSTEMIC LEVELS OF RELAXIN?

Although the above evidence points to a potential link between estrogen and TMDs, other hormones, such as relaxin, have also been implicated. Relaxin is a 6 kiloDalton (kDa) polypeptide hormone that is found systemically in ovulating and pregnant women. It exists as two gene products, H1 and H2. The function of H1 is to relax smooth muscle. H2 affects collagenous tissue by altering collagen turnover through an increase in degradation and decrease in synthesis of collagen in connective tissues (Unemori and Amento, 1990; Unemori et al., 1992; Unemori et al., 1993). Because of the known effects of relaxin H2 on connective tissue metabolism, the focus of this study is on this gene product.

Relaxin's highest serum concentrations occur during the first trimester of pregnancy, after which there is a steady decline until term (Bani, 1996). The primary sources are the corpus luteum, decidua and placenta. In cycling women, relaxin is produced mainly by the corpus luteum, which accounts for the small rise in serum relaxin that occurs during the midluteal phase of the menstrual cycle.

Traditionally it was believed that relaxin's main biologic function was to lengthen the interpubic ligament and soften the tissues of the cervix during the birthing process through the induction of collagen remodeling (reviewed in Sherwood, 1994). Relaxin's ability to induce metabolic changes in connective tissue has lead some investigators to search for other effects outside of the birthing process. In non-pregnant women, relaxin is regarded as one of several hormones that regulates the production of collagenolytic enzymes that weaken the ovarian follicle prior to its rupture at ovulation (Bryant-Greenwood, 1982). Relaxin can also mediate changes in molecules of the extracellular matrix of connective tissues. Several studies have found that relaxin and estrogen work synergistically to increase the content of glycosaminoglycans in the rat uterus and cervix (Bryant-Greenwood, 1982).

It was recently demonstrated in our lab that relaxin induces a matirx-degradative phenotype in cultured TMJ disc cells by increasing the expression of the matrix metalloproteinases (MMPs) collagenase and stromelysin (Kapila, 1993). However, it does not affect the tissue inhibitors of metalloproteinases, TIMP-1 and TIMP-2. Priming of these cells with β-estradiol potentiated their MMP-inductive response to relaxin. These findings are significant to the present study because they suggest that cells within the TMJ disc may be target sites for the matrix-degradative affects of relaxin, which is accentuated by β-estradiol. Both hormones are found in varying amounts in normally cycling women. Therefore, a potential link could exist between these hormones and TMJ diseases in women.

Because of the matrix catabolic effects of relaxin, an association between systemic joint hypermobility and relaxin has been suggested (Calguneri et al., 1985; MacLennan, 1991).

Studies have shown that a strong relationship exists between systemic levels of relaxin during pregnancy and postpartum pelvic joint hypermobility and pain (MacLennan et al., 1986; Saugstad, 1991). The highest levels of relaxin were observed in women who had experienced the most pain.

F. HYPOTHESIS, SPECIFIC AIMS AND SIGNIFICANCE

Due to the particular gender and age distribution of TMJ diseases, and the association between SJH and relaxin, we hypothesized that women of childbearing age with TMJ disease will demonstrate increased SJH and altered systemic levels of relaxin and other reproductive hormones than asymptomatic controls. We also hypothesized that serum levels of relaxin, β-estradiol and progesterone correlate positively with those in saliva. The specific aims of the study were to:

1. Determine and compare by objective and quantitative measures the presence and severity of clinical and radiographic signs of TMJ disease in female subjects with symptomatic and asymptomatic TMJs.
2. Evaluate and compare the magnitude of systemic joint laxity determined by an objective scoring criteria in female subjects with symptomatic and asymptomatic TMJs.
3. Quantify and compare the absolute and relative serum levels of relaxin, β-estradiol and progesterone in subjects with and without TMJ disease.

4. Determine if serum levels of relaxin, β-estradiol and progesterone correlate with salivary levels of these hormones.

The findings from this study may provide insight into the hormonal etiology of TMJ diseases of women. If an association exists between specific hormone levels, systemic joint hypermobility and disease severity, the findings of this study could lead to the development of accurate diagnoses and specific treatments that would focus on the underlying cause of TMDs. Secondly, if salivary levels of specific hormones correlate with serum levels, less invasive means other than venipuncture could be used pathogenesis of TMDs, coupled with simple screening methods, could lead to the implementation of screening individuals for TMDs. The identification of the etiology and implementation of preventive measures for those individuals deemed predisposed to TMJ diseases. Lastly, no similar studies have been performed on joints other than the temporomandibular joint. Thus the knowledge gained from this study could provide important information about the pathologies of other diarthrodial joints.

II. MATERIALS AND METHODS

A. RECRUITMENT AND CRITERIA FOR INCLUSION OF SUBJECTS

Methods for recruitment and investigation of subjects were carried out according to protocols for human research at the University of California - San Francisco. The Committee on Human Subjects at the University gave approval for all procedures rendered.

Normal cycling and ovulatory asymptomatic control and symptomatic women were recruited via flyers posted throughout the University of California - San Francisco campus (Appendix 1). Women interested in participating were to contact the examiner by telephone to receive further information about the study and to be screened for exclusion criteria prior to their enrollment as a participant.

Exclusion Criteria

The initial telephone screening incorporated a series of questions for the following exclusion criteria: current use of oral contraceptives or other hormonal therapies; systemic diseases that effect the joints and connective tissue metabolism (e.g., rheumatoid arthritis, diabetes); immuno-suppressive disorders; irregular menstrual cycles; history of trauma to the head and/or neck and pregnancy. A total of 56 women volunteered to participate in the study. Of these individuals, 8 were excluded because they had one or more of the exclusion criteria. Once a subject's eligibility for participation was established by telephone screening, an appointment was made for the TMJ and hypermobility examinations.

Anovulation was also an exclusion criterion. This was determined later via ovulation kits provided at the time of the initial examination. Only one woman who originally started the study had to be excluded later due to negative findings from the ovulation kit.

Inclusion Criteria

Women in both asymptomatic and symptomatic groups had to be between the ages of 18 to 40 years and have normal monthly menstrual cycles. Subjects were initially classified as symptomatic if they had present or a previous history of TMJ disease, which included one or more of the following symptoms: significant joint sounds, localized joint pain, or limited opening. Women with no previous history or present symptoms of TMJ diseases were initially classified as asymptomatic. This initial division was done in order to recruit approximately equal numbers of asymptomatic and symptomatic women. The subjects were later redistributed into the final asymptomatic and symptomatic groups on the basis of objectively defined clinical and radiographic criteria.

B. PROCEDURES

Information required to address the hypothesis was derived from a patient questionnaire, a systemic joint hypermobility test, clinical and radiographic TMJ examination, and quantitative assessment of serum levels of β-estradiol, progesterone and relaxin. In order to

determine if there is an association between serum and salivary levels of these hormones, we assayed levels from both serum and salivary samples.

Each subject was given a consent form that was signed by both the participant and examiner (Appendix 2) at the time of the examination appointment.

1. Questionnaire

Each subject completed a questionnaire (Appendix 3) which provided answers about the nature, frequency and severity of TMJ symptoms, and date when these were first noted by the subject or by a doctor. Information on history of facial trauma that could potentially impact joint function was sought, as well as that pertaining to systemic illnesses and problems with joints other than the temporomandibular joint.

Demographic information such as age, ethnicity, socio-economic status, marital status and level of education was also obtained. This information will help the examiners adjust for possible confounding variables that could be implicated in TMJ disease.

The questionnaire also included information about limitations in daily activities related to symptoms and questions pertaining to the subject's present psychological status. Such questions help to establish whether non-physiologic variables are associated with TMJ disease.

2. Clinical TMJ Evaluations

The clinical assessment of the TMJ was performed by a single examiner and followed the format provided by the Research Diagnostic Criteria (RDC) for TMDs (Dworkin, 1992-Appendix 4). The clinical assessment included determination of joint pain, range of motion, joint sounds and muscle examination.

The participant graded location of pain while being subjected to preauricular palpation, intraauricular palpation, jaw opening and jaw excursive movements. Location of pain (with the exception of muscle palpation) was scored in two ways: by side (left of right) and by specific location (whether or not the pain was in the joint). Pain in any particular area was recorded as none, left, right, or both. Secondly, pain was recorded as being present or absent from the joint. If the subject had no pain, "NA" was recorded.

Severity of pain was graded during preauricular palpation, intrauricular palpation, opening/closing, lateral excursive movements, intraoral muscle palpation and extraoral muscle palpation. Pain was recorded as absent (0), mild (1), moderate (2) or severe (3).

Range of motion was measured in millimeters with a disposable Therabite Gauge (MDC Inc.). Measurements included maximum unassisted and assisted opening, right and left lateral excursions, mandibular midline deviation, vertical incisor overlap and mandibular protrusion.

The presence of joint sounds was evaluated with a two-way stethoscope. The rubber tubing on the stethoscope from the contralateral joint was obstructed in order to discern sounds from a single joint. Sounds were recorded during opening/closing, lateral excursive movements and protrusion. All sounds were recorded as none, click, course crepitus or fine crepitus, and either reproducible or nonreproducible. In addition, opening/closing clicks were recorded as reciprocal or nonreciprocal.

Lastly, the subject's opening pattern was recorded as straight, lateral deviation (right or left), corrected deviation (right or left) or other, if more than one opening pattern existed.

3. Systemic Joint Hypermobility

Systemic joint hypermobility was measured using the methods developed by Beighton and Horan (1969). The exam bilaterally measures the flexibility of the thumb, fifth digit, elbow and knee. The range of motion for the elbow and knee was measured with a goniometer. The measurements were obtained by placing the two arms of the device along the proximal and distal bones adjacent to the joint under consideration. All measurements were made bilaterally. Further details of flexibility testing are described below and pictured in Appendix 5.

(a) Thumb

Flexibility was measured by evaluating passive opposition of the thumb to the flexor aspect of the forearm. The subject was instructed to extend the arm out ninety degrees from the

body. The palm of the hand faces the floor. The subject then uses the opposite hand to flex the thumb toward the forearm as far as possible. If the thumb can passively touch the flexor aspect of the forearm the wrist is considered hypermobile. When the thumb cannot make contact with the flexor surface of the forearm, wrist flexibility is not considered hypermobile.

(b) Fifth Digit

This measure is defined as the passive dorsiflexion of the fifth metacarpophalangeal joint. The subject is instructed to again extend the arm out ninety degrees from the body, with the palm facing and parallel to the ceiling. The opposite hand is then used to push the fifth digit dorsally to its end-feel position. The angle between the finger and the dorsum of the hand is measured with a finger goniometer. The fifth digit is considered to be hypermobile when it can be passively dorsiflexed to an angle of ninety degrees or less. An angle greater than ninety degrees is considered to have normal mobility.

(c) Elbow

Elbow flexibility is determined by the ability of the elbows to hyperextend. The subject is asked to extend the arm out ninety degrees from the body. The opposite hand is then used to "push the crease of the elbow up toward the ceiling." The angle from the styloid of the radius to the lateral epicondyle of the humerous to the acromion was measured with a goniometer. The angle beyond 0 degrees is recorded. An angle greater than or equal to 10 degrees is considered hypermobile. An angle of less than 10 degrees demonstrates normal flexibility.

(d) Knee

Flexibility is determined by measuring the magnitude of hyperextension of the knee. The subject is instructed to stand straight and "push the knee back" to a firm end-feel. The angle is measured by placing the joint of the goniometer on the lateral epicondyle of the femur, with the legs of the instrument extending toward the lateral malleollus distally and the greater trocanter proximally. An angle greater than or equal to 10 degrees is hypermobile. An angle of less than 10 degrees demonstrates normal flexibility.

Thus a total flexibility score was derived by giving a joint with normal mobility a score of 0 and a hypermobile joint a score of 1, providing a possible total flexibility score between 0 and 8 for each subject.

4. Radiographic Evaluation of the TMJ

The TMJ tomograms were used for radiographic quantification of TMJ disease. The set of radiographs taken included: a) submental vertex radiograph used for making corrected tomograms of the joint; b) center cuts of right and left A-P corrected tomograms in a closed position of the mandible; and c) center cuts of right and left lateral corrected tomograms in a closed position of the mandible.

Tomograms were taken with the head of the subject parallel to Frankfort-Horizontal Plane. Interpretation of the images was done by one examiner who was blinded to TMD symptoms.

The examiner was trained by a clinician, who is considered to be an expert in radiographic interpretation.

Intraexaminer reliability was established by the examiner evaluating a random sample of fifteen x-rays for a second time. The examiner was blinded for this test.

The tomograms were scored for condylar sclerosis, flattening, erosion and osteophyte formation on a scale of 0 to 3 (0 = normal, 1 = mild, 2 = moderate and 3 = severe) for a total score of 0 to 12 for each joint. Descriptions for the four above categories were as follows:

i. Condylar Sclerosis: A normal condyle has a thin "candy shell" cortical outline. Sclerosis is the first stage of abnormality. An adaptation to overbearing forces on the condyle cause trabeculae from the center of the bone to move toward the cortex, making it thicker.

ii Condylar Flattening: The normal anatomy of a condyle has a rounded outline in radiographic form. Flattening is evident when an articulating surface of a condyle is not rounded, but congruent with its opposing surface on the fossa or eminence.

iii Erosion: unlike the smooth cortical surface of an intact condyle, a condyle with erosion has concave interruptions in the cortical surfaces.

iv. Osteophyte formation: An osteophyte is an osseous projection of dense cortical bone, most commonly projecting from the anterior border of the articulating surface of the condyle. An osteophyte must, by definition, increase the articulating surface area of the condyle.

5. Classification and Assessment of TMJ Status

Subjects were classified into subsets of TMJ disease based on specific radiographic and clinical findings (as per Dworkin, 1992). These diagnoses were made on the basis of the following criteria:

i. No TMJ disease: No reported history and no radiographic or clinical signs or symptoms of TMJ disease.

ii. Previous history of TMJ disease: The subject is presently free of radiographic changes or pain in the TMJ but had a previous history of pain in the joints.

iii. Arthralgia: The subject presents with pain in the right and/or left joints.

iv. Osteoarthrosis: The individual presents with the absence of pain in the TMJ but demonstrates any on of the following scores radiographically: flattening $\geq$ 2; erosions $\geq$ 2; or osteophytes $\geq$ 2.

v. Osteoarthrosis: The subject experiences pain within the joint, in addition to the radiographic changes consistent with osteoarthrosis.

6. Determining Time-Points for Collection of Blood and Saliva

Blood and saliva samples were collected between 7:00 and 11:30 am during the midluteal, ovulation minus one day and midfollicular days of the menstrual cycle. These 3 time-points represent the days in a menstrual cycle when the levels of progesterone, β-estradiol or relaxin theoretically peak (Appendix 6). These days were determined through the use of ovulation predictor tests that were provided by the study. The kits detect the lutenizing hormone surge

from a urine sample, which occurs 24 hours prior to ovulation. This information, in addition to the length of each woman's menstrual cycle, was used to estimate the three time points for blood and saliva collection during the next month's menstrual cycle. Visit one was estimated as the halfway point between the first day of the cycle (marked as the onset of menstrual bleeding) and the predicted day of ovulation. The second visit was one day prior to the predicted day of ovulation (as ascertained by the ovulation kit). The third and final visit was the halfway point between the predicted day of ovulation and the end of the menstrual cycle (the last day before the onset of menstrual bleeding).

7. Collection of Blood and Saliva

The nursing staff at the General Clinic Research Center (GCRC) at the University of California-San Francisco performed all retrievals of blood and saliva. These collections were done during the morning hours in order to minimize diurnal variations in hormone levels. The protocol for saliva collection was as follows:

1. The subject was asked to fast for 90 minutes prior to the visit, which was verified by the nursing staff upon the subject's arrival.
2. A plastic funnel was placed within a 10ml plastic test tube. The tube was then immersed in a "Dixie" cup that was filled half way with ice.
3. The patient was given one pellet of unsweetened gum and asked to start chewing. The subject was asked to expectorate saliva into the funnel for ten continuous minutes. The staff person started a stopwatch as soon as the patient began chewing the gum.

4. At the end of ten minutes, the patient was asked to expectorate any remaining saliva, as well as the gum, into the funnel.
5. The plastic test tube was then capped and the funnel (with the gum) was thrown away.
6. The tube was then kept in the ice pack and immediately delivered to the General Clinical Research Center (GCRC) core laboratory for processing.

This was followed by the collection of blood. Ten milliliters of blood were drawn into a 15ml serum separating tube and immediately delivered to the GCRC core laboratory for processing.

8. Processing of Blood and Saliva

The protocol for the processing of saliva and serum began with the addition of three protease inhibitors to the sample in order to prevent the enzymatic degradation of relaxin. These inhibitors included pepsistatin A (final concentration 1mg/ml), phenylmethyl sulfonyl fluoride (PMSF, 100mg/ml) and ethylene ditrichloro acetic acid (EDTA, 1mM).

The saliva sample was then centrifuged at 12,000g for 20 minutes and the serous portion of the saliva removed, aliquoted into 1.5ml vials (1000µl per vial) and stored at –70 degrees Celsius. The serum sample was centrifuged at 1000g for 20 minutes. The supernatant was separated from the cells, aliquoted into 1.5µl vials and stored at –70 degrees Celsius for later analysis of β-estradiol, progesterone and relaxin.

C. QUANTIFICATION OF RELAXIN, β-ESTRADIOL AND PROGESTERONE

1. Relaxin enzyme linked immonosorbent assay (ELISA) for serum and saliva

Relaxin was assayed by an ELISA with reagents provided by Drs. Elaine Unemori and Mark Erikson of Connetics Corporations (Palo Alto, CA). The coating antibody, affinity purified anti-recombinant human relaxin polyclonal (0.7 mg/ml), was diluted 1:750 in coating buffer (0.05 M sodium bicarbonate buffer, pH 9.6). For ELISA on saliva samples, 300µl of the diluted antibody was coated on 96-well microtiter plates and incubated at 4^0C for 12-24 hours for saliva ELISA. For serum assays, 200µl of the diluted coating antibody was used. The plate was washed three times with wash buffer (PBS with 0.05 % Polysorbate) and incubated with 300µl per well of buffer diluent (PBS with 0.5% BSA and 0.05 % Polysorbate 20, 0.01% thiomersal) for 1 hour at room temperature and washed once with wash buffer.

Relaxin standards (1500,750, 375, 187.5,93.75,46.87, 23.43, 11.7 and 5.85 pg/ml) were prepared in buffer diluent for saliva samples. For serum samples, the standards were prepared in human male serum (Type AB, Atlanta Biologicals, Norcross, GA). A volume of 200µl of each standard was added per well. Three hundred µl of the saliva samples and 200 µl of the serum samples were added to the wells. Non-immune goat IgG, was added to all samples and standards to a final concentration of 0.02 mg/ml. The plates were incubated for 24 h at 4^0Celcius. The plates were washed three times with wash buffer. The signaling antibody, anti-recombinant human relaxin antibody conjugated to horseradish peroxidase, was diluted

1:100 and added to the wells. The plates were incubated at room temperature for 4 hours with shaking.

The wells were washed thrice with wash buffer to remove unbound antibody. A volume of 200 µl of the peroxidase substrate TMB 3,3' ,5,5'-tetramethylbenzidine with 0.01% H_2O_2 (TMB Microwell Substrate, Kirkegaard and Perry Laboratories, Galthersburg, MD) was added and the plates were incubated at room temperature for 20 minutes or until a blue color developed. The plates were read at 650 nm for absorbance on a Spectramax microtiter plate reader (Molecular Devices, Sunnyvale, CA). The relaxin concentration was determined from a standard curve using the software Sofimax Pro 3.1 (Molecular Devices).

2. Progesterone and β-estradiol Assays

Progesterone and β-estradiol levels in serum and saliva were determined using commercially available radioimmunoassay kits (DSL – 3400, and DSL –4800, respectively from Diagnostic Systems Laboratories, Inc. Webster, Texas) as per manufacturer's instructions. These assays were performed by a commercial laboratory (Diagnos Tech International Inc., Orcalo WI) on a contractual arrangement with GCRC.

D. STATISTICAL METHODS AND ANALYSES

Twenty-six symptomatic and twenty-one asymptomatic subjects were included in the study. This represents approximately one-fifth of the 290 subjects needed for the entire, ongoing

study. This latter sample size was determined to provide at least 80% power based on the previous data analyses from preliminary studies.

1. Error of Method

The method of error of the examiner was tested to verify the reliability of the measurements made. The examiner performed two repeated examinations of the TMJ and hypermobility tests on fifteen subjects, each done at least two weeks apart. The correlation between the two sets of observations was measured using Pearson's correlation coefficient. The results presented in Table I show a high degree of correlation between the measurements.

Table I – Pearson's correlation coefficient for measured TMJ and Flexibility variables (N=15)

	Variable	Pearson's Correlation Coefficient (r-value)
TMJ EXAM	Passive TMJ opening	0.90
	Laterotrusion	0.88
JOINT LAXITY	Wrist	0.96
	Fifth Digit	0.94
	Elbow	0.90
	Knee	0.86

2. Statistical Analyses

Two-sided statistical tests at the alpha = 5% level were used. All P values reported are unadjusted for the fact that this is technically an interim analysis. For a formal interim analysis, the significance level would be set at alpha = .00005. Two sample (unpaired) Student's t tests compared continuous measures between asymptomatic and symptomatic participants. These comparisons were made from data obtained from the questionnaire, clinical and radiographic TMJ exam, joint laxity exam and hormonal levels. These data

included information on age, vertical and lateral range of motion and systemic joint hypermobility. Chi-square tests compared categorical measures between groups. Bonferroni adjustments for multiple comparisons were used where appropriate. Linear regression models were used to compare salivary and serum hormone levels. Tests were adjusted for age with logistic regression models.

III. RESULTS

In this study we compared serum and salivary hormone levels between subjects with TMJ disease and asymptomatic controls. We then examined whether levels of these hormones in serum correlated with levels in saliva. We also compared the differences in systemic joint flexibility between the symptomatic and asymptomatic groups. The following provides our findings on the study population, as well as information obtained from questionnaires, clinical and radiographic examinations and hormone assays from the subjects in this study. It should be noted that the findings presented here are for the current enrollment of 47 subjects out of a required sample size of 290 subjects. As such, most of the statistical analyses were not expected to reveal significant differences.

A. PROFILE OF THE STUDY POPULATION

A total of 52 subjects responded to the recruitment flyer. Of these, three were excluded because of oral contraceptive use, and two due to a previous history of facial trauma at the time of the phone interview. The study was comprised of 47 subjects, 26 with symptomatic (S) TMJs and 21 with asymptomatic (A) TMJs. The mean ages of the symptomatic and asymptomatic groups were 31 and 26 years, respectively. These mean ages were significantly different as revealed by an unpaired t-test (P=.005) (Table II, Figure 1). Thus, subsequent analyses should adjust for this age difference. The overall ages of the study population ranged from 20.2 years to 39.9 years. All participants were experiencing regular menstrual cycles, as an entry requirement for the study.

Table II - Number and mean age (± SD) of subjects in both groups of the study population.

	Asymptomatic	Symptomatic
Number	21	26
Mean Age	26	31
Std Dev	5	6

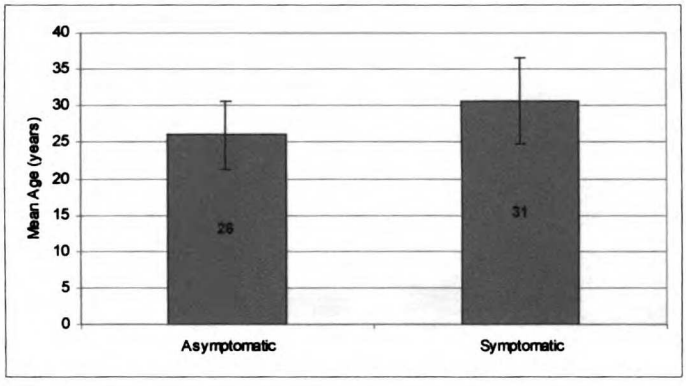


Figure 1 - Histogram of mean (± SD) age of individuals in the asymptomatic and symptomatic groups (N=47).

The patient questionnaire provided additional information regarding ethnicity and previous pregnancies. We used a one sample chi-square test to examine for differences between the numbers of subjects from the five ethnic categories in our study and the anticipated enrollment based on local figures from the 1990 census (χ^2 =1.41, df = 4, P=0.842). The number of subjects within each of the categories was similar to the anticipated enrollment (Table III). The current enrollment, based on ethnicity, between the two groups is presented in Figure 2 below.

Table III - Anticipated and current enrollment of subjects from various ethnicities (percentages).

Enrollment	WH	BL	HS	AP	OT
Anticipated *	58.6%	8.7%	8.1%	21.8%	2.7%
Current	55.3%	8.5%	12.8%	21.3%	2.1%

* Source: 1990 Census (http://venus.census.gov/cdrom/lookup/875128852)

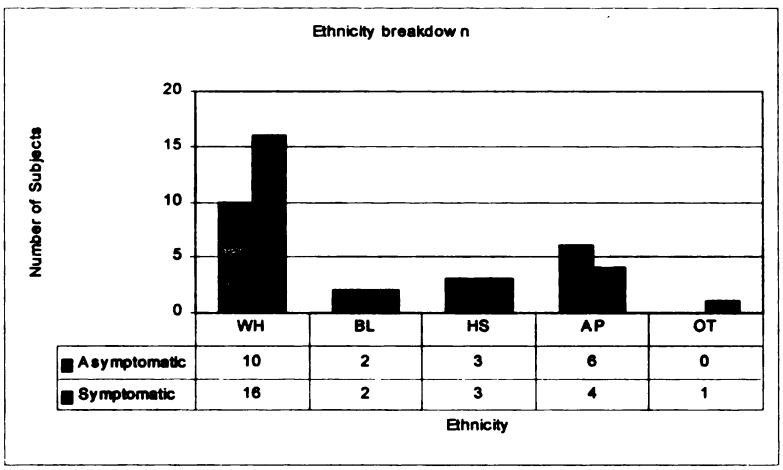


Figure 2 - Number of subjects in asymptomatic and symptomatic groups divided by ethnicities.

The percentage and number of subjects from both groups reporting previous pregnancies and the successful childbirths are presented in Table IV. Overall, four patients in the study reported at least one successful childbirth. Two were in the symptomatic group and two were in the asymptomatic group. An odds ratio of 1.19 revealed that women who reported a previous pregnancy were 19% more likely to be symptomatic than asymptomatic, but not to a statistically significant extent (P=0.770).

Table IV - Percentage and number of subjects in each group reporting pregnancies and at least one successful childbirth.

Group	Percentage of Women Reporting (N)	
	Previous History of Pregnancy	Successful Childbirths
Aysmptomatic (N=21)	38.1% (8)	9.5% (2)
Symptomatic (N=26)	42.3% (11)	7.7% (2)
Odds Ratio	1.19	1.26
P value	0.77	0.823

B. CLINICAL TMJ FINDINGS

The clinical TMJ examination involved the determination of current facial pain and its location in the TMJ and surrounding structures, vertical and lateral range of motion and joint sounds. The percentage of subjects reporting current facial pain, at the time of the examination, was 34.6% in the symptomatic group. Of these individuals, one was experiencing pain in the left joint only, five were experiencing pain in both joints and three were experiencing masticatory muscle pain (Table V). No subjects in the asymptomatic group reported current facial pain, as that was one of several criteria needed to be placed in that group.

Table V - Percentage and number of subjects in each group experiencing current facial pain.

	Percentage Reporting Pain (N)					
Group	No Pain	Current Facial Pain	Pain in One Joint Only	Pain in Both Joints	Pain in Muscle Only	Total
Asymptomatic	100.0% (21)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	100% (21)
Symptomatic	30.8% (8)	34.6% (9)	3.8% (1)	19.2% (5)	11.5% (3)	100% (26)

Assessment of the vertical range of motion, measured as the maximum unassisted opening, revealed that the asymptomatic group exhibited slightly greater opening than the

asymptomatic group (Figure 3). However, this difference was not statistically significant (P=0.132, adjusting for age, P=0.381). Due to the significant age difference between the symptomatic and asymptomatic groups, we used age-adjusted odds ratios and 95% confidence intervals from logistic regression models when comparing variables between these two groups. This did not reveal significant differences between the two groups for any of the variables measured. This information is presented in Table VI.

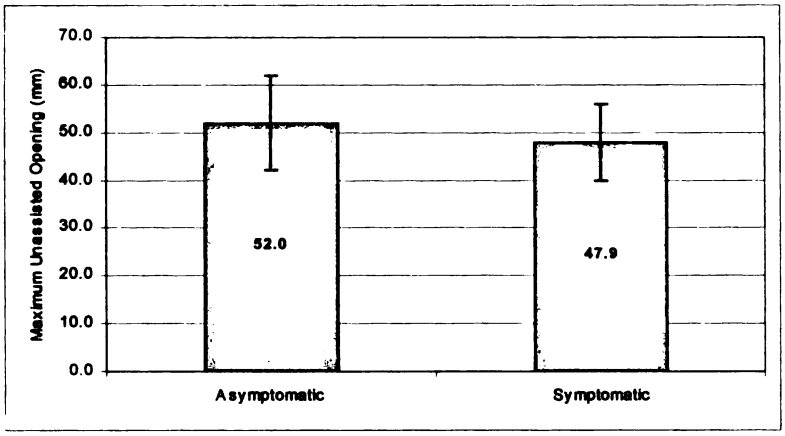


Figure 3 - Histogram of mean (+ SD) unassisted opening in both groups (N=47).

Table VI - Age-adjusted odds ratio and 95% confidence intervals comparing symptomatic and asymptomatic subjects (N=47)

Measured Variables	Odds Ratio	95% Confidence Interval	P-value
Maximum Opening	0.97	0.89 to 1.04	0.382
Laterotrusion			
Maximum	0.99	0.74 to 1.34	0.961
Minimum	0.99	0.70 to 1.40	0.937
Average	0.99	.070 to 1.40	0.947
Flexibility			
5th Joint	1.12	0.31 to 4.09	0.861
Thumb	2.56	0.52 to 12.50	0.247
Elbow	1.02	0.28 to 3.69	0.980
Knee	1.65	0.45 to 6.02	0.449
Total Flexibility	0.90	0.45 to 1.80	0.767
Absolute Hormone Levels			
Progesterone	1.14	0.97 to 1.34	0.121
Relaxin	1.00	0.99 to 1.01	0.661
Beta Estradiol	1.00	0.99 to 1.02	0.489

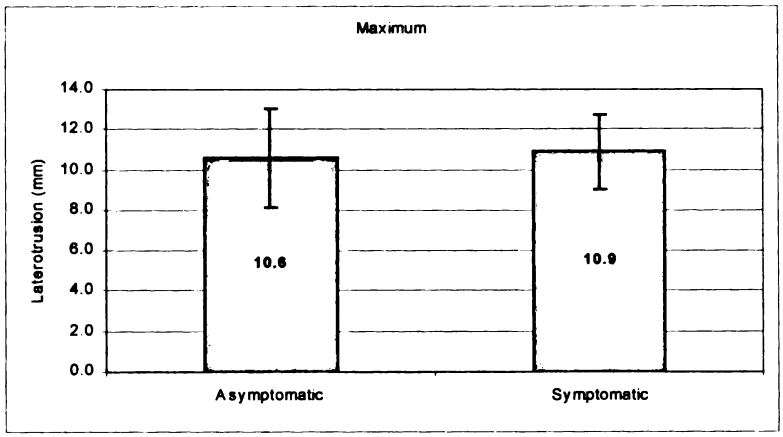
Assessment of laterotrusive movement involved examination of the mean, maximum and minimum movements between right and left joints for each subject. The symptomatic group had slightly greater mean, maximum and minimum laterotrusive movements (Figure 4a,b,c - shown on page 34). However, these differences were not statistically significant (P=0.132, 0.685 and 0.510, respectively).

Table VII lists the findings of joint noises for the two groups. Over half (52.4%) of the asymptomatic subjects displayed no joint noises, while 47.6% displayed some form of a click. Of the symptomatic subjects, only 23.1% exhibited no joint noise, 19.2% displayed a click, and 57.7% displayed crepitus. A chi-square test revealed that these differences between the two groups were significant (P<0.001), as would be expected since joint sounds were part of the criteria used to classify subjects as symptomatic or asymptomatic.

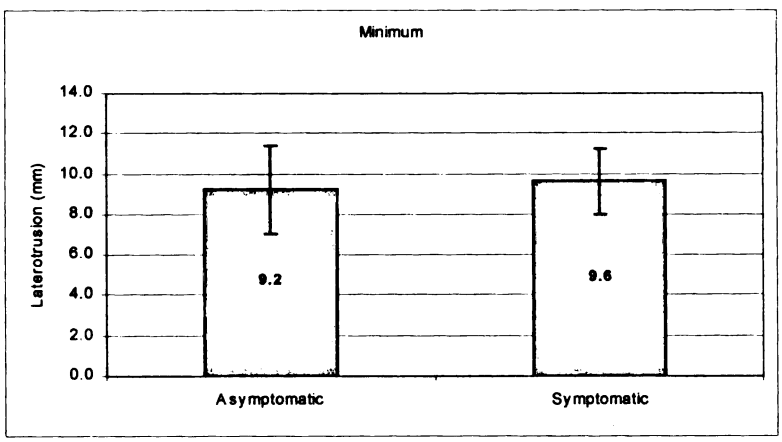
Table VII - Percentage and number of subjects in both groups demonstrating various TMJ sounds.

TMJ Sounds	Asymptomatic	Symptomatic	Total
No Click	52.4% (11)	23.1% (6)	**36.2% (17)**
Opening or Closing Click	28.6% (6)	7.7% (2)	**17.0% (8)**
Reciprocal Click	19.0% (4)	11.5% (3)	**14.9% (7)**
Crepitus	0.0% 0	57.7% (15)	**31.9% (15)**
Total	**100.0% (21)**	**100.0% (26)**	**100.0% (47)**

a.



b.



c.

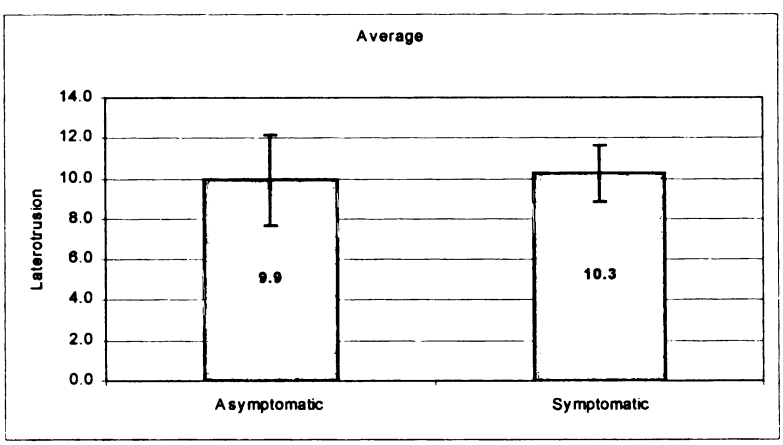


Figure 4a-c - Histograms of mean (± SD), maximum (a), minimum (b) and mean (c) laterotrusion (mm) in asymptomatic and symptomatic subjects (N=47).

C. RADIOGRAPHIC TMJ FINDINGS

Morphological changes in the TMJ (Table VIII), as ascertained by tomograms, revealed that the majority of the asymptomatic women (77.8%) demonstrated a mild form of sclerosis (grades 1 and 2), with the remainder of these subjects (22.2%) displaying completely normal joint morphology. The majority of symptomatic women (45.5%) demonstrated condylar flattening. Grade 3 sclerosis was observed in 18.2% of the symptomatic women. Condylar erosion was present in 13.6% of individuals in this group and osteophyte formation was detected in 22.7% of these women, as that was one of several criteria needed to be placed in that group.

Table VIII - Percentage of subjects in both groups with positive finding for morphologic changes (N=40).

Radiographic Findings	Percent Affected		Mean Score (± S.D.) from 1 to 3	
	Asymptomatic	Symptomatic	Asymptomatic (N=18)	Symptomatic (N=22)
Normal	22.2% (4)	0.0% (0)	N/A	N/A
Sclerosis	77.8% (14)	18.2% (4)	1.50 (.97)	2.00 (.71)
Flattening	0.0% (0)	45.5% (10)	N/A	1.60 (1.48)
Erosion	0.0% (0)	13.6% (3)	N/A	1.66 (.54)
Osteophyte	0.0% (0)	22.7% (5)	N/A	1.40 (.85)
Total	**100% (18)**	**100% (22)**		

We also examined for relationships between joint sounds (specifically crepitus) and radiographic findings. We found that out of the fifteen symptomatic subjects with crepitus, one displayed sclerosis, nine displayed flattening, three demonstrated erosions and two showed osteophyte formation.

Seven subjects dropped out of the study prior to radiographs being taken due to the commencement of oral contraceptive use.

D. CLASSIFICATION OF TMJ DISEASE

We further divided the symptomatic women into subsets of TMJ disease (previous history of pain, arthralgia, osteoarthritis and osteoarthrosis) based on clinical and radiographic findings. Table IX shows the percentages of the symptomatic women who fell into these four categories:

Table IX - Percentage and number of symptomatic subjects (N=26) in the TMJ disease categories

	Percentage	Number
Previous History of Pain	15.4%	4
Internal Derangements	7.7%	2
Arthralgia	7.7%	2
Osteoarthritis	50.0%	13
Osteoarthrosis	19.2%	5

E. SYSTEMIC HYPERMOBILITY

Generalized joint flexibility was assessed by examining the fifth digit, thumb, elbow and knee joints. Data for each joint was based on the absence (0) or presence (1) of hypermobility in either the right or left joint. A composite score was then given based upon the number of joints (out of 8) that exhibited positive findings of hypermobility (Table X).

Findings from all four joints show that a higher percentage of symptomatic women displayed hypermobility than the asymptomatic controls. Chi-square tests showed that these differences were statistically significant only for the thumb (P = .004); Bonferroni adjusted P = .016. The asymptomatic group also had a higher mean composite score. However, this difference was not statistically significant (P =0.637).

Table X - Percent of individuals demonstrating bilateral hypermobility in the fifth digit, thumb, elbow and knee and the mean and +SD of total flexibility score (N=47).

Joint	% of subjects with Hypermobility	
	Aysmptomatic (N=21)	Symptomatic (N=26)
5th Digit	57.1% (12)	61.5% (16)
Thumb	14.3% (3)	26.9% (7)
Elbow	38.1% (8)	38.5% (10)
Knee	33.3% (7)	46.2% (12)

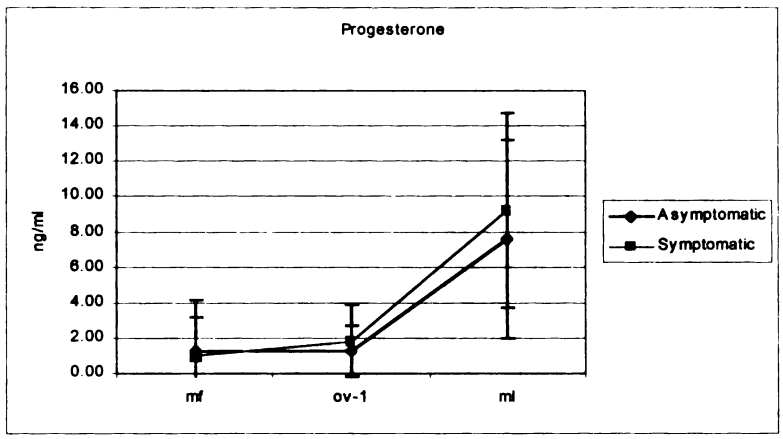
Total Hypermobility Score (out of 8)	Aysmptomatic (N=21)	Symptomatic (N=26)
Mean	2.38	2.65
Std Dev	1.91	2.02

F. SYSTEMIC HORMONE LEVELS IN ASYMPTOMATIC AND SYMPTOMATIC WOMEN

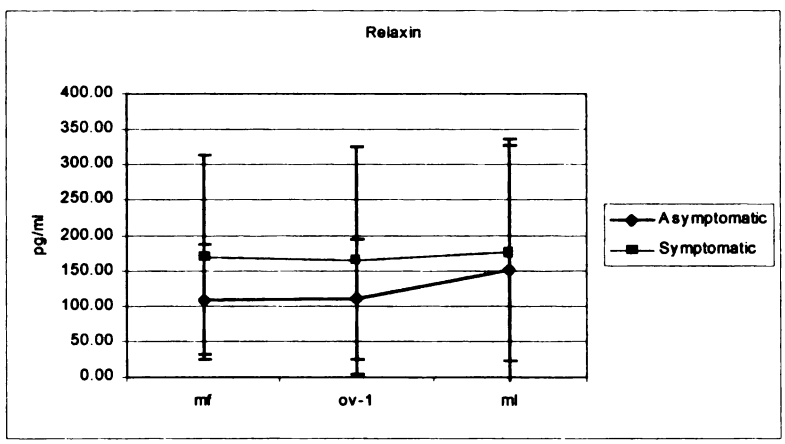
Figure 5a-c depicts mean levels of progesterone, relaxin and β-estradiol at the midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of collection for asymptomatic and symptomatic women. The sample size is smaller than the original 47 subjects, as some women had blood and saliva collected later than others. The hormonal findings from these women will be included in the continuation of this study. Baseline and peak levels of progesterone were fairly similar between the asymptomatic and symptomatic groups (Figure 5a). Baseline levels of β-estradiol were also very similar between the two groups, however peak levels at ovulation-1 day were higher, although not significantly, in the symptomatic group (Figure 5c). In contrast, while the peak levels of relaxin were similar in symptomatic and asymptomatic subjects, its baseline levels were slightly higher, but not significantly, in symptomatic women (Figure 5b).

We examined absolute differences between the maximum and minimum levels of all three hormones in serum and saliva due to the fact that there are substantial differences in hormone levels between individuals. These absolute differences in baseline and peak levels of hormones give insights the into variations of hormone levels within individuals. We then determined if there were any differences in these variables between symptomatic and asymptomatic women. Figure 6 shows that for both serum and saliva symptomatic women had higher levels of all three hormones. However, this difference was significant only for salivary progesterone (P=0.044).

a.



b.



c.

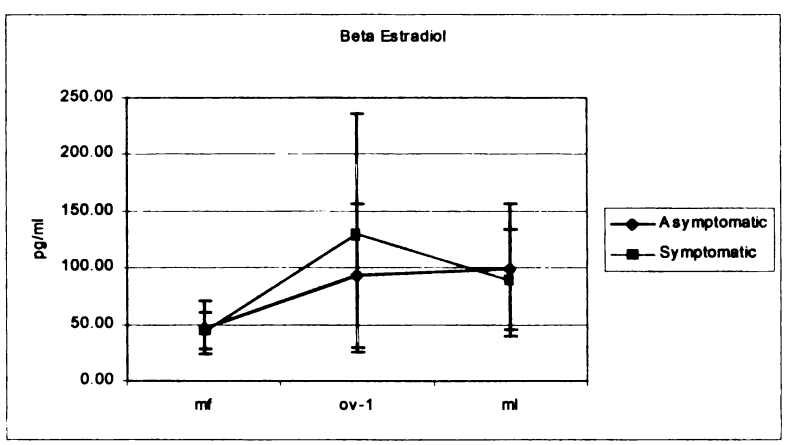


Figure 5a-c - Graphs depicting mean levels of progesterone, relaxin and β- estradiol at the midfollicular (mf), ovulation –1 (ovul-1), and midluteal (ml) days of collection (N=27).

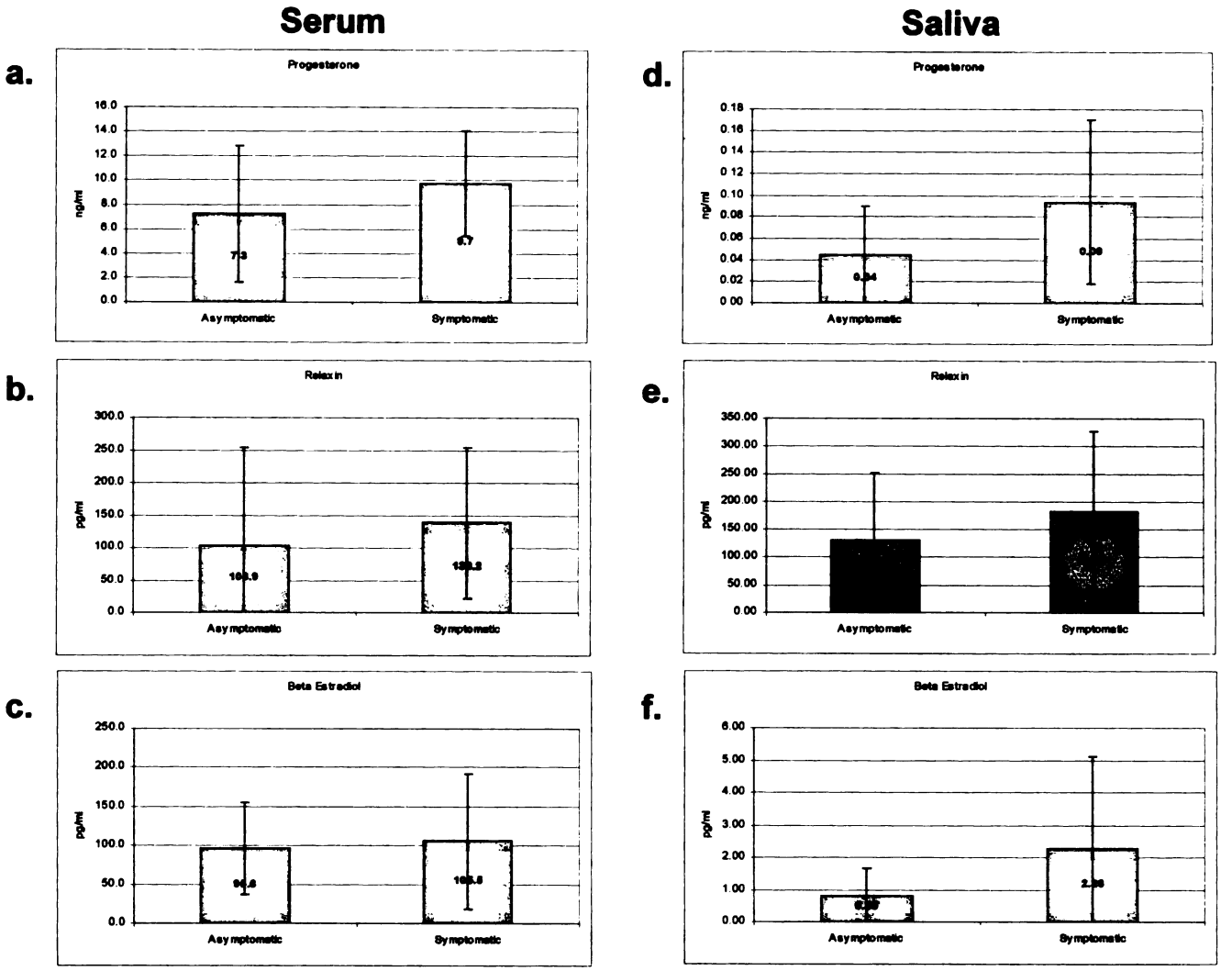


Figure 6a-f - Absolute differences between maximum and minimum levels of serum (a to c) and salivary (d to f) hormones within individuals in the symptomatic and asymptomatic groups (N=27).

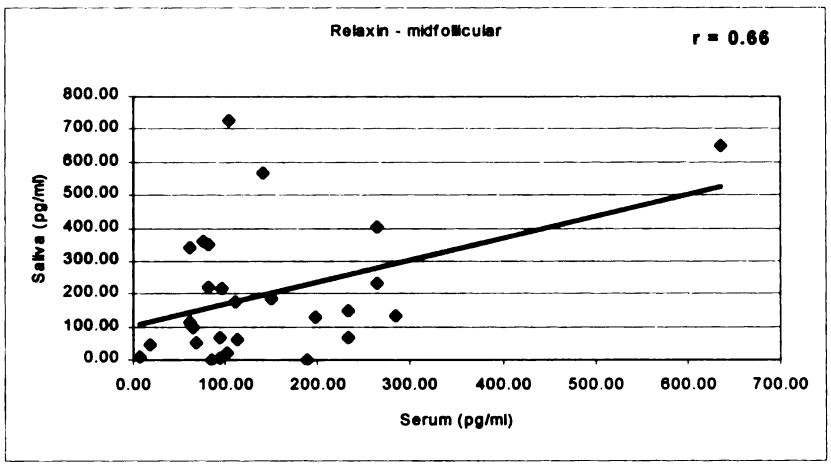
G. CORRELATIONS BETWEEN SERUM AND SALIVARY HORMONE LEVELS

In Table XI the correlation coefficient (r) values show the relationship between serum and salivary hormone levels (also see Figure 7). Correlations were moderately strong (r = 0.55 to 0.80) for progesterone at all three days of collection, and for relaxin at the midfollicular (r = 0.66) and midluteal (r = 0.60) days of collection. No correlation existed between serum and salivary levels of β-estradiol at all three time points.

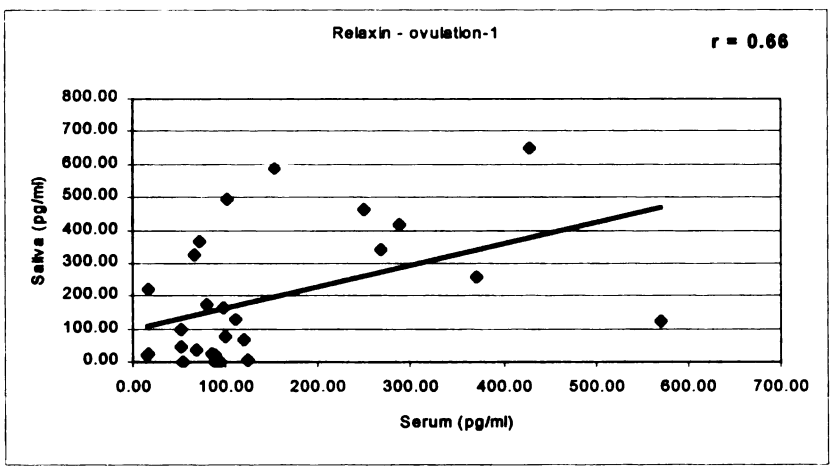
Table XI – Correlation coefficient (r) values depicting associations between serum and saliva hormone levels at the three days of sample collection and at all three days combined

	Progesterone	Beta Estradiol	Relaxin
Midfollicular	0.55	0.19	0.66
Ovulation-1	0.80	-0.20	0.34
Midluteal	0.64	0.20	0.60

a.



b.



c.

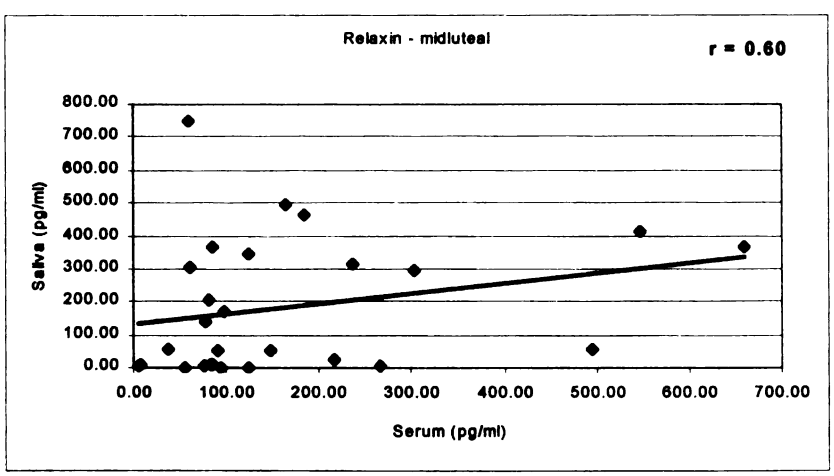


Figure 7a-c - Scatterplot showing the relationship between serum and salivary relaxin at midfollicular, ovulation-1, and midluteal (N=27).

IV. DISCUSSION

This study is one of the first to investigate the potential relationship between female reproductive hormones, TMJ status and systemic joint hypermobility. It was the first study to examine the association between levels of these hormones in serum and saliva. This investigation is considered to be a small component of a larger, ongoing study that plans to recruit 290 subjects over a 24-month period. The work presented here represents about one-fifth of the overall study, therefore the small initial sample reduced power to draw conclusions for many of the variables. However, the knowledge gained from this investigation will be a useful foundation for the overall study.

We compared the presence and severity of clinical and radiographic signs of TMJ disease in subjects with and without symptoms. We found no significant differences between the two groups for linear jaw opening and laterotrusive movements. This concurs with the findings of our original study (Peikoff, 1996). We originally expected an increased range of motion in subjects with greater joint laxity. However, the lack of a significant difference between the two groups may be explained by the fact that laxity of the joint ligaments may predispose an individual to dislocation, resulting in inflammation and physical limitation of jaw opening. In addition, alterations in hard and soft tissue morphology, or the presence of pain in a diseased joint may limit movement. Furthermore, although any symptomatic subject whose TMD was primarily muscular was excluded from our study, several of the women in this group demonstrated muscle soreness secondarily to positive joint findings. Such muscle pain may result in a lack of adequate muscle function, which would also reduce jaw movement.

When examining joint noises, we found that the majority (57.7%) of subjects in the symptomatic group displayed crepitus in one or both joints. This is similar to our original study, from 1996, which noted crepitus in 55% of the symptomatic subjects. Crepitus is demonstrative of structural changes in the bony surfaces of the joint (DeBont et al., 1985, McNeill, 1993). This coincides with our radiographic findings, as the majority of symptomatic women in the osteoarthritis and osteoarthrosis exhibited either flattening, erosions or osteophyte formation of the condyle. These three radiographic findings are considered to be abnormal adaptations to excessive mechanical loading in the joint. These adaptations can occur sequentially until the surface area of the bones within the joint increases enough to dissipate the higher forces (Ogus and Toller, 1986).

While a small majority of the asymptomatic women displayed no joint noises, however a substantial percentage (47.6%) of them had some form of a click. This finding is also similar to that found in our original study, where 42.9% of asymptomatic controls demonstrated an opening/closing click. This is lower than other studies that report close to 60% of the population having some form of click in one or both joints without the presence of any other signs or symptoms (Rugh, 1985, Bell, 1992, Tallents, 1993). The majority (77.8%) of the women in this group also demonstrated sclerosis of one or both condyles. This is considered to be a normal adaptation to increased strain placed on the condylar system (Faulkner, Hatcher and McEvoy, 1997). As mechanical loading increases, bony trabeculae within the center of the condyle begin to migrate toward the cortex, making it both thicker and stiffer. If this adaptation adequately dissipates the strain, the bony changes terminate at this level, and these findings of up to grade 2 sclerosis can then be considered a deviation from normal.

However, if the strain continues, the bony changes may continue and the condyle may eventually begin to flatten. The question arises as to whether the symptomatic group had more time for these clinical and radiographic signs to develop, since they were, on the average, older than the asymptomatic group. Long-term follow up could shed light on this issue, as could future longitudinal studies.

Systemic joint flexibility may vary within an individual for many reasons. One reason is the fact that most individuals have a dominant side, which equates to more muscle tone and therefore less joint laxity (Westling 1993). We attempted to circumvent this by examining all four joints bilaterally. Secondly, the magnitude of joint flexibility may vary depending upon the time of day. Unfortunately controlling the time of day that examinations were performed was logistically impossible. In this study we found that the symptomatic women demonstrated greater flexibility in all four joints than the asymptomatic controls. However, this difference was statistically significant only for the thumb. A previous study (Peikoff, 1996) also found significant differences in laxity of the thumb between symptomatic and asymptomatic women. Ericson and Lundberg (1968) found significant correlations between radiographic changes in the TMJ and finger joint flexibility. Beighton et al. (1973) found a correlation between laxity of the thumb and generalized joint laxity. Our symptomatic group also had a higher total composite score for hypermobility, however this was not significantly significant. One plausible explanation for the inability to derive statistical significance is the small sample size, thus resulting in low power. An interesting finding was that two of the subjects from the asymptomatic group, who were both from the same ethnicity, displayed total flexibility scores of 7 (out of a total of 8), which were the two highest scores out of all

47 subjects. Beighton found ethnic differences when examining joint flexibility (1973). Although no conclusions can be drawn from the findings of these two women, it indicates a need for further evaluation of the association between ethnic background, joint hypermobility and TMJ status in this ongoing study.

The results from hormone data were obtained by having each subject use an ovulation kit in order to determine ovulation day minus 24 hours. The three visits for serum and saliva collection were then calculated from this information and the length of the subject's cycle. Poor compliance with the ovulation kit or inaccurate recall of the length of one's menstrual cycle could easily result in the improper calculation of days for serum and saliva collection, which leads to variations in hormone levels. The results show that the mean peak in progesterone and β-estradiol for both symptomatic and asymptomatic women was similar to what would be anticipated, based on previous studies that have tracked the levels of these hormones over the course of the average woman's menstrual cycle (Schauf and Moffett, 1990). However, the peak for relaxin was not consistently observed as expected at the calculated midluteal time point. This could be due to poor compliance, as well as the fact that, unlike progesterone and β-estradiol, the time during which relaxin peaks during the average menstrual cycle is very short.

Thus a slight error in timing of the cycle could easily result to the appropriate time for sample collection being missed. This issue will be addressed in the continuation of this study by having each subject use the ovulation kit for two consecutive menstrual cycles, one before and one during the month of sample collection.

We also examined if the serum and salivary levels of these hormones are correlated. Strong correlations between serum and salivary levels would suggest that saliva may serve as a suitable alternative for determining systemic levels of these hormones. The correlations for both progesterone and relaxin were moderate to strong ($r = 0.60$ to .800). However, no correlation existed between levels of β-estradiol in serum and saliva since this could be due in part to a loss this hormone during processing. We may need to revise our protocol for handling β-estradiol salivary samples in the next phase of this study, as other studies have proven that salivary and serum estrogen and progesterone correlate significantly with each other (Hardiman, Thomas, Osgood and Vlassopoulou, 1990, Ellison, 1991). The fact that correlations were moderately strong for progesterone and relaxin suggests that our laboratory protocol for handling salivary samples is reasonable for these two hormones.

Although there was a significant mean age difference between the asymptomatic and symptomatic groups, (26 and 31 years, respectively) several studies (Schwartz 1982, Ellison 1990, Lipson 1991) have found that levels of progesterone and β-estradiol peak between the ages of 25 and 35 years. The two groups from our study have mean ages that fall into this age range. Thus the differences in hormone levels are not likely to be attributed to differences in the ages of the two groups. Further analyses with a bigger sample size will enable us to determine if the levels of these hormones are truly and significantly different between the asymptomatic and symptomatic women.

We recognize that several interesting trends can be investigated with the continuation of this study. In addition to expanding upon the findings presented here, we will look to identify associations among systemic joint hypermobility, range of motion, joint sounds, radiographic findings and hormone levels.

V. CONCLUSION

The work from this investigation is considered to be the initial component of an ongoing study that will eventually recruit approximately 290 subjects over a 24-month period. Despite some of the limitations that have been discussed, several conclusions were drawn from our initial investigation:

1. The symptomatic TMJ group defined by this study demonstrated slightly greater systemic joint laxity than the asymptomatic group in all four joints tested. However these differences were nominally significant in the thumb only.
2. Although not statistically significant, the symptomatic group demonstrated higher levels of β-estradiol at ovulation–1 day, and relaxin at the midfollicular and ovulation –1 day, than the asymptomatic group.
3. Correlations between serum and salivary hormone levels were moderately strong for both progesterone and relaxin, but were not for β-estradiol.

The findings gathered from this study will hopefully be expanded upon as the investigation continues. We anticipate that one day this information can shed more definitive light on the etiology and pathogenesis of TMJ diseases, thus enabling clinicians to provide more accurate diagnoses, treatments and even preventive measures for people suffering from or predisposed to these TMJ disorders.

VI. REFERENCES

Abubaker, A.O.: Estrogen and progesterone receptors in the temporomandibular disc of symptomatic and asymptomatic patients. AAOMS Oral Abstract Session VII: 111-2, 1991.

Aufdemorte, T.B., Van Sickels, J.E., Dolwick, M.F.: Estrogen receptors in the temporomandibular joint and systemic joint laxity. J Am Dent Assoc 109: 446-7, 1984.

Bani, D.: Relaxin, A pleiotropic hormone. Gen Pharmac 28:13-22, 1997.

Beighton, P., Horan, F.: Orthopaedic aspects of the Ehlers-Danlos syndrome. J Bone Joint Surg, 51:444-53, 1969.

Beighton, P., Solomon, L., Soskolne, C.L.: Articular mobility in an African population. Ann Rheum Dis 32:413-8,1973.

Beighton, P., Grahame, R., Bird, h.: Hypermobility of joints. New York: Springer Verlag, 1983.

Bell, W.E.: Management of Temporomandibular Disorders. Chicago: Year Book Medical Publ., 1982.

Bland, J.D., Stulberg, S.D.: Osteoarthritis: Pathology and Clinical Patterns. In Keely W.M. et al (eds.) Textbook of Rheumatology. 2nd ed. Philadelphia, W.B. Saunders Co, 1985.

Bryant-Greenwood, G.D.: Relaxin as a new hormone. Endocrine Rev, 3, 62-90, 1982.

Bryant-Greenwood, G.D., Schwabe, C.: Human relaxins: chemistry and biology. Endocrine Reviews 15: 5-26, 1994.

Calguneri, M.: Changes in joint laxity occurring during pregnancy. Ann Rheum Dis 41: 126-8, 1986.

Campbell, J.H., Courey, M.S., Browne, P.: Estrogen receptor analysis of human temporomandibular disc. J Oral Maxillofac Surg 51 (10): 1101-05, 1993.

Carlsson, G.E., Droukas, B.C.: Dental occlusion and the health of the masticatory system. J Craniomand Pract 2: 141, 1984.

Carlsson, G.E., Kopp, S., Wedeal, A.: Analysis of background variables in 350 patients with TMJ disorders as reported in self-administered questionnaire. Community Dent Oral Epidemiol 10: 47-51, 1982.

Clark, G.T., Solberg, W.T.; eds. Perspectives in Temporomandibular Disorders. Chicago: Quintessence Publishing Co., Inc. 1987.

Cohen, S.: Follow-up evaluation of 105 patients with myofascial pain dysfunction syndrome. J Am Dent Assoc 107: 420-4, 1978.

Dahl, B.L., Krogstad, B.S., Ogaard, B., et al.: Signs and symptoms of craniomandibular disorders in two groups of 19 year old individuals, on treated orthodontically and the other not. Acta Odontol Scand 46: 89, 1988.

DeKanter, et al.: TMJ guidelines, J Dent Res; 71:1607-12, 1996.

Debont, L.G.M., Boering, G., Liem R.S.B.: Osteoarthritis of the temporomandibular joint: a light microscopic and scanning electron microscopic study of the articular cartilage of the mandibular condyle. J Oral Maxillofac Surg 43: 481-488, 1985.

Dragsholt, M., LeResche, L.: Temporomandibular Disorder Pain, In: Epidemiology of Pain, edited by I.K. Crombie, IASP Press, Seattle, 203-233, 1999.

Dworkin, S.F., LeResche, L.: Research Diagnostic Criteria for Temporomandibular Disorders: review, criteria, examinations and specifications, critique. J Craniomand Disord Facial Oral Pain 6:301-355, 1992.

Egermark-Eriksson, I., Carlsson, Ingervall, B.: Prevalence of malocclusion dysfunction and orofacial parafunction in 7- 11- and 15- year old Swedish children. Eur J Orthod 3: 163-172, 1981.

Ellison, P., and Lager, C.: Reproductive ecology and human fertility. In Applications of Biological Anthropology to Human Affairs. G.W. Lasker and C.G.N. Mascie-Taylor, Eds.: 14-45. Cambridge University Press. London/New York, 1991.

Farrar, W.B., McCarty Jr., W.L.: A Clinical Outline of Temporomandibular Joint Diagnosis and Treatment. Montgomery, Ala.: Normandie Publ. 7th ed., 1983.

Faulkner, G., Hatcher, D., McEvoy, S., et al.: Craniofacial morphology and mechanical function of the TMJ. Tissue Response, 1998).

Forssell, H., Kangasniemi, L.: Mandibular dysfunction in patients with muscle contraction headache. Proc Finn Dent 50: 81-88, 1983.

Gianelly, A.A., Hughes, H.M., Wohlegmuth, P., et al.: Condylar position and extraction treatment. Am J Orthod Dentfac Orthop 93:205-210, 1988.

Goodard, G.: ed. TMJ - The Jaw Connection. Santa Fe: Aurora Press. 1991.

Hardiman, P., Thomas, M., Osgood, V., Vlassopoulou, V. and Ginsburg, J.: Are estrogen assays essential for monitoring stimulant therapy? Gynecol Endocrinol 4 261-9, 1990).

Helkimo, M.: Studies on function and dysfunction of the masticatory system. II. Index for anamnestic and clinical dysfunction and occlusal state. Swed Dent J 67: 101-121, 1974.

Howard, J.A.: Temporomandibular joint disorders, facial pain and dental problems of performing artists. In Sataloff R., Brandfonbrener A., Lederman R. (eds.). Textbook of Performing Arts Medicine. New York, Raven Press, 1991.

Isaacson, G., Isberg, A., Johansson, A.S.: Internal derangement of the temporomandibular joint: Radiographic and changes associated with severe pain. J Oral Maxillofac Surg 44: 771-8, 1986.

Isberg-Holm, A.M., Westesson, P.: Movement of the disk and condyle in temporomandibular joints with clicking: An arthrographic and cineradiographic study on autopsy specimens. Acta Odontolog Scand 40: 151-164, 1982.

Katzberg, R.W., Dolwick, M.F., Helms, C.A., et al.: Arthrotomography of the temporomandibular joint. Am J Roentgenol 134: 944, 1980.

Kapila, S., Lee, C., Wong, S.K., Richards, D.W.: Mechanisms of matrix degradation in the temporomandibular joint disc. J Dent Res 72(SI):301 (abstract), 1993.

Larsson, L.G., Baum, J., Mudholkar, G.S.: Hypermobility: prevalence and features in a Swedish population. Br J Rheumatol 32: 116-9, 1993.

LeResche, L., Saunders,K., Barlow, W., von Korff, M., Dworkin, S.F.: Does oral contraceptive use increase the risk of temporomandibular disorder (TMD) pain? Abstract 786, 7th World Congress on Pain. Seattle: IASP Press, 1993.

Lipson, S.F., O'Rourke, M.T., and Ellison, P.T.: Salivary progesterone profiles: reference data for anthropological studies of reproductive function. Am J Phys Anthropol, 12:115-116, 1991).

MacLennan, A.H.: The role of the hormone relaxin in human reproduction and pelvic girdle relaxation. Scand J Rheumatol 88:7-15, 1991.

McNeill, C., ed. Craniomandibular Disorders: Guidelines for Evaluation, Diagnosis and Management. The American Academy of Craniomandibular Disorders. Chicago: Quintessence Publishing Inc. 1993.

Magnusson, T., Carlsson, G.E.: Comparison between two groups of patients in respect of headache and mandibular dysfunction. Swed Dent J 2: 85-92, 1978.

Milam, S.B., Aufdemorte, T.B., Sheridan, P.J.: Sexual dimorphism in the distribution of estrogen receptors in the temporomandibular joint complex of baboons. Oral Surg Oral Med Oral Pathol 64: 527-32, 1987.

Okeson, P.: Orofacial Pain: Guidelines for Assessment , Diagnosis and Management. The Amer Aca of Orofacial Pain. Chicago: Quintessance Publishing Inc. 1996.

Ogus, H. and Toller P.: Common disorders of the temporomandibular joint. Bristol: John Wright & Sons Ltd. 1986.

Peikoff, E.: Systemic factors associated with temporomandibular joint disease in women. Masters Thesis, 1996.

Pountain, G.: Musculoskeletal pain in Omanis, and the relationship to joint hypermobility and body mass index. Br J Rheumatol 31: 81-85, 1992.

Pullinger, A.G., Monteiro, A.A., Lin, S.: Etiological factors associated with temporomandibular disorders. J Dent Res 64: 269, 1985.

Reider, C.E.: The prevalence of mandibular dysfunction, Part I: Sex and age distribution of related signs and symptoms. J Prosthet Dent 50:81-88, 1983.

Roth, R.: Temporomandibular pain dysfunction and occlusal relationships. Angle Orth 43:136-153, 1973.

Rugh, J.D., Solberg, W.K.: Oral health status in the United States. Temporomandibular disorders. J Dent Educ 49: 398-404, 1985.

Sadowsky, C., Polson, A.M.: Temporomandibular disorders and functional occlusion after orthodontic treatment: Results of two long-term studies. Am J Orthod 86: 386-390, 1984.

Saugstad, L.F.: Persistent pelvic pain and joint instability. Eur J Obstet Gynecol Reprod Biol 41:197-201, 1991.

Schiffman, E., Fricton, J.R.: Epidemiology of TMJ and craniofacial pain. In Fricton, J.R.; Kroening, R.J.; Hathaway, K.M. (eds.) TMJ and Craniofacial Pain: Diagnosis and Management. St. Louis, IEA Publ, 1988.

Schauff, C.L., Moffett, D.F., Mofett, S.B.: Human Physiology; Foundations and Frontiers. Times Mirror/Mosby College Publ. 1990.

Schwartz, D. and Mayaux, M.J.: Female fecundity as a function of age. N Engl J Med 306:404-406, 1982.

Seligman, D.A., Pullinger, A.G.: The role of intercuspal relationships in temporomandibular disorders: A review. J Craniomand Disord Facial Oral Pain 3:227-236, 1989.

Solberg, W.K.: Epidemiology, incidence and prevalence of temporomandibular disorders: a review. The President's Conference on the Examination, Diagnosis and Management of Temporomandibular Disorders. Chicago, ADA, 1982.

Tallents, R.H., Hatala, M., Katzberg, R.W., Westesson, P.: Temporomandibular joint sounds in asymptomatic volunteers. J Prosth Dent 69:298-304, 1993.

Westling, L. Helkimo, E.: Maximum jaw opening capacity in adolescents in relation to general joint hypermobility. J Oral Rehab 19: 485-494, 1992.

Wigdorwicz-Makowerowa, N., et al.: Epidemiologic studies on prevalence and etiology of functional distributions of the masticatory system. J Prosthet Dent 41: 76-82, 1979.

Unemori, E.N., Amento, E.P.: Relaxin modulates synthesis and secretion of procollagenase and collagen by human dermal fibroblasts. J Biol Chem 265:10681-85, 1990.

Unemori, E.N., Bauer, E.A.: Relaxin alone and in conjunction with interferon-gamma decreases collagen synthesis by cultured human scleroderma fibroblasts. J Invest Derm 99:337-42, 1992.

Unemori, E.N., Beck, L.S., Lee, W.P.: Human relaxin decreases collagen accumulation in vivo in two rodent models of fibrosis. J Investigat Derm 101(3):280-5, 1993.

Zarb, G.A., Thompson, G.W.: Assessment of clinical treatment of patients with temporomandibular joint dysfunction. J Prosth Dent 24: 542-554, 1970.

APPENDIX 1 - FLYER FOR RECRUITMENT

HEALTHY FEMALES NEEDED FOR STUDY OF TMJ (JAW JOINT) DISEASE IN WOMEN

Volunteers are needed to explore the possible hormonal causes of TMJ problems in women of childbearing age.

CRITERIA

- females between the ages of 18 - 40 years
- healthy
- no history of trauma to the jaw
- not currently taking oral contraceptives or corticosteroids

PARTICIPATION

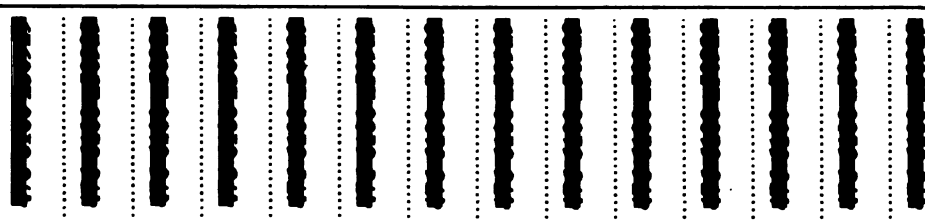
• completion of a health questionaire and TMJ exam	*45 min*
• X-rays	*30 min*
• collection of saliva and blood three times over the course of one menstrual cycle	*20 min ea*
• use of ovulation kit (provided by study) to obtain approximate day of ovulation	*at home*

SUBJECTS WILL RECEIVE

$100

FOR PARTICIPATION IN THE STUDY

If interested please contact Dr. Tina Reed at [redacted]



APPENDIX 2 - CONSENT FORM

UNIVERSITY OF CALIFORNIA SAN FRANCISCO
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

The Role of Reproductive Hormones in Temporomandibular Joint (TMJ) Disease in Women

A. PURPOSE AND BACKGROUND

Sunil Kapila, D.D.S, M.S., Ph.D., Tina Reed, D.M.D., and Dr. Kristen Miller, D.D.S, from the Department of Growth and Development, are conducting a study to help understand the causes of temporomandibular joint (jaw joint) problems in women. Because I am a healthy female between 18-40 years of age, I am being asked to participate in the study.

B. PROCEDURES

If I agree to be in this study, the following will happen:

1. Prior to inclusion in the study, I will be required to time my menstrual cycle and also determine my day of ovulation using an ovulation kit that will be provided. I will perform one test each day over 4 to 5 days around the middle of the menstrual cycle. Each test will take approximately 5 minutes of my time and will be performed at home.

2. I will complete a questionnaire regarding my age, number of pregnancies and history of pain in my joints. This should take about 30 minutes to complete.

3. I will be examined for flexibility in my jaw, wrist, elbow and knee joints. This will involve measuring the amount I can comfortably bend my little fingers backwards, bend my thumbs toward my forearm, extend my elbows, extend my knees, bend my upper body forward while my knees are straight and open my mouth. I will also receive a thorough examination of my jaw joints, the TMJ. These tests usually last 45 minutes.

4. I will have X-rays of my jaw joint (TMJ) taken. This will take approximately 30 minutes.

5. Three times in the month, at predetermined times in the morning (on about days 6, 13 and 21 of the menstrual cycle), approximately two teaspoonfuls (6 ml) of my saliva will be collected while chewing an unflavored, sugarless gum into a small cup. Also, approximately three teaspoonfuls (10 ml) of blood will be drawn from a vein in my arm. These procedures will take approximately 20 minutes at each visit. The blood and saliva samples will be tested for the amounts of certain hormones (called relaxin, progesterone,

E. COSTS

I will not be charged for any of the study treatments or procedures.

F. REIMBURSEMENT

I will be reimbursed for $100.00 for participating in this study. However, I will be reimbursed this amount only upon completion of my responsibilities for the study, as outlined above. Monetary reimbursement will be by check that will be issued within 60 days of completion of my participation in the study. If I fail to qualify for the study on the basis of findings of my ovulation test, I will be reimbursed $10.00. If I fail to complete the entire study, I will be reimbursed at the rate of $10.00 per visit. This payment will be made within 60 days after I inform you that I do not intend to complete the study.

G. QUESTIONS

This study has been explained to me by Dr. Reed or by Dr. Miller and my questions were answered. If I have any other questions about the study, I may call Dr. Reed at (415) 476-6100 (ext: 50929), Dr. Miller at (415) 476-6100 (ext: 53587) or Dr. Kapila at (415) 476-8401.

H. CONSENT

I have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care.

By signing below, I agree to participate in this study.

_____	_____
Date	Subject's Signature
_____	_____
Date	Person Obtaining Consent

APPENDIX 3 - QUESTIONNAIRE

ID= _____

Date: __ / __ / __

History Questionnaire

Please read each question and respond accordingly. For each of the questions below, circle only one response.

1. Would you say your health in general is excellent, very good, good, fair, or poor?
   Excellent 1
   Very good 2
   Good 3
   Fair 4
   Poor 5

2. Would you say your oral health in general is excellent, very good, good, fair, or poor?
   Excellent 1
   Very good 2
   Good 3
   Fair 4
   Poor 5

3. Have you had pain in the face, jaw, temple, in front of the ear, or in the ear in the past month?
   No 0
   Yes 1

   [If no pain in the past month SKIP to question 14]

   If Yes,

4.a. How many years ago did your facial pain begin for the first time?
   __ __ years

   [If one year ago or more SKIP to question 5]
   [If less than one year ago, code 00]

4.b. How many months ago did your facial pain begin for the first time?
   __ __ months

5. Is your facial pain persistent, recurrent, or was it only a one-time problem?
   Persistent 1
   Recurrent 2
   One-Time 3

6. Have you ever gone to a physician, dentist, chiropractor, or other health professional for facial ache or pain?
   No 1
   Yes, in the last 6 months 2
   Yes, more than 6 months ago 3

7. How would you rate your facial pain on a 0 to 10 scale at the present time, that is right now, where 0 is "no pain" and 10 is "pain as bad as could be"?

   No pain — Pain as bad as could be
   0 1 2 3 4 5 6 7 8 9 10

8. In the past six months, how intense was your worst pain, rated on a 0 to 10 scale where 0 is "no pain" and 10 is "pain as bad as could be"?

   No pain — Pain as bad as could be
   0 1 2 3 4 5 6 7 8 9 10

9. In the past six months, on the average, how intense was your pain rated on a 0 to 10 scale where 0 is "no pain" and 10 is "pain as bad as could be"? [That is, your usual pain at times you were experiencing pain].

   No pain — Pain as bad as could be
   0 1 2 3 4 5 6 7 8 9 10

10. About how many days in the last 6 months have you been kept from your usual activities (work, school, or housework) because of facial pain?
   _____ Days

11. In the past 6 months, how much has facial pain interfered with your daily activities rated on a 0 to 10 scale where 0 is "no interference" and 10 is "unable to carry on any activities"?

   No interference — Unable to carry on any activities
   0 1 2 3 4 5 6 7 8 9 10

12. In the past 6 months, how much has facial pain changed your ability to take part in recreational, social and family activities where 0 is "no change" and 10 is "extreme change"?

   No change — Extreme change
   0 1 2 3 4 5 6 7 8 9 10

13. In the past 6 months, how much has facial pain changed your ability to work (including housework) where 0 is "no change" and 10 is "extreme change"?

   No change — Extreme change
   0 1 2 3 4 5 6 7 8 9 10

14.a. Have you ever had your jaw lock or catch so that it won't open all the way?
   No 0
   Yes 1

   [If no problem opening all the way SKIP to question 15]

   If Yes,

14.b. Was this limitation in jaw opening severe enough to interfere with your ability to eat?
   No 0
   Yes 1

15.a. Does your jaw click or pop when you open or close your mouth or when chewing?
   No 0
   Yes 1

   b. Does your jaw make a grating or grinding noise when it opens and closes or when chewing?
   No 0
   Yes 1

   c. Have you been told, or do you notice, that you grind your teeth or clench your jaw while sleeping at night?
   No 0
   Yes 1

   d. During the day, do you grind your teeth or clench your jaw?
   No 0
   Yes 1

   e. Does your jaw ache or feel stiff when you wake up in the morning?
   No 0
   Yes 1

   f. Do you have noises or ringing in your ears?
   No 0
   Yes 1

   g. Does your bite feel uncomfortable or unusual?
   No 0
   Yes 1

16.a. Do you have rheumatoid arthritis, lupus, or any other systemic arthritic diseases?
   No 0
   Yes 1

16.b. Do you know of anyone in your family who has had any of these diseases?
   No 0
   Yes 1

Modified from: Research Diagnostic Criteria for Temporomandibular Disorders: review, criteria, examinations and specifications critique (Dworkin, S.F. and LeResche, L.; 1992; J Craniomandib Disord Facial Oral Pain)

16.c. Have you had or do you have any swollen or painful joint(s) other than the joints close to your ears (TMJ)? No ..... 0 Yes 1

[If no swollen or painful joints, SKIP to question 17.a.]

If Yes,

16.d. Is this a persistent pain that you have had for at least one year? No ..... 0 Yes 1

17.a. Have you had a recent injury to your face or jaw? No ..... 0 Yes 1

[If no recent injuries SKIP to question 18]

If Yes,

17.b. Did you have jaw pain before the injury? No ..... 0 Yes 1

18. During the last 6 months have you had a problem with headaches or migraines? No ..... 0 Yes 1

19. What activities does your present jaw problem prevent or limit you from doing?

a. Chewing	No 0	Yes 1
b. Drinking	No 0	Yes 1
c. Exercising	No 0	Yes 1
d. Eating hard foods	No 0	Yes 1
e. Eating soft foods	No 0	Yes 1
f. Smiling/laughing	No 0	Yes 1
g. Sexual activity	No 0	Yes 1
h. Cleaning teeth or face	No 0	Yes 1
i. Yawning	No 0	Yes 1
j. Swallowing	No 0	Yes 1
k. Talking	No 0	Yes 1
l. Having your usual facial appearance	No 0	Yes 1

20. In the last month, how much have you been distressed by

	Not at all	A little bit	Moderately	Quite a bit	Extremely
a. Headaches	0	1	2	3	4
b. Loss of sexual interest or pleasure	0	1	2	3	4
c. Faintness or dizziness	0	1	2	3	4
d. Pains in the heart or chest	0	1	2	3	4
e. Feeling low in energy or slowed down	0	1	2	3	4
f. Thoughts of death or dying	0	1	2	3	4
g. Poor appetite	0	1	2	3	4
h. Crying easily	0	1	2	3	4
i. Blaming yourself for things	0	1	2	3	4
j. Pains in the lower back	0	1	2	3	4
k. Feeling lonely	0	1	2	3	4
l. Feeling blue	0	1	2	3	4
m. Worrying too much about things	0	1	2	3	4
n. Feeling no interest in things	0	1	2	3	4
o. Nausea or upset stomach	0	1	2	3	4
p. Soreness of your muscles	0	1	2	3	4
q. Trouble falling asleep	0	1	2	3	4
r. Trouble getting your breath	0	1	2	3	4
s. Hot or cold spells	0	1	2	3	4
t. Numbness or tingling in parts of your body	0	1	2	3	4
u. A lump in your throat	0	1	2	3	4
v. Feeling hopeless about the future	0	1	2	3	4
w. Feeling weak in parts of your body	0	1	2	3	4
x. Heavy feelings in your arms or legs	0	1	2	3	4
y. Thoughts of ending your life	0	1	2	3	4
z. Overeating	0	1	2	3	4
aa. Awakening in the early morning	0	1	2	3	4
bb. Sleep that is restless or disturbed	0	1	2	3	4
cc. Feeling everything is an effort	0	1	2	3	4
dd. Feelings of worthlessness	0	1	2	3	4
ee. Feeling of being caught or trapped	0	1	2	3	4
ff. Feelings of guilt	0	1	2	3	4

21. How good a job do you feel you are doing in taking care of your health overall? Excellent ..... 1 Very good ..... 2 Good ..... 3 Fair ..... 4 Poor 5

22. How good a job do you feel you are doing in taking care of your oral health?
- Excellent ... 1
- Very good ... 2
- Good ... 3
- Fair ... 4
- Poor ... 5

23. When were you born? Month ___ Day ___ Year ___

24. Are you male or female?
- Male ... 1
- Female ... 2

25. Which of the following groups best represent your race?
- Aleut, Eskimo or American Indian ... 1
- Asian or Pacific Islander ... 2
- Black ... 3
- White ... 4
- Other ... 5

_____ (please specify)

26. Are any of these groups your national origin or ancestry?
- Puerto Rican ... 1
- Cuban ... 2
- Mexican/Mexicano ... 3
- Mexican American ... 4
- Chicano ... 5
- Other Latin American ... 6
- Other Spanish ... 7
- None of the above ... 8

27. What is the highest grade or year of regular school that you have completed?

Never attended or Kindergarten 00

Elementary School: 1 2 3 4 5 6 7 8

High School: 9 10 11 12

College: 13 14 15 16 17 18+

28a. During the past 2 weeks, did you work at a job or business not counting work around the house (include unpaid work in the family farm/business)?
- Yes ... 1
- No ... 2

[If Yes SKIP to question 29]

If No,

28b. Even though you did not work during the past 2 weeks, did you have a job or business?
- Yes ... 1
- No ... 2

[If Yes SKIP to question 29]

If No,

28c. Were you looking for work or on layoff from a job during those 2 weeks?
- Yes, looking for work ... 1
- Yes, layoff ... 2
- Yes, both on layoff and looking for work ... 3
- No ... 4

29. What is your marital status?
- Married—spouse in household ... 1
- Married—spouse not in household ... 2
- Widowed ... 3
- Divorced ... 4
- Separated ... 5
- Never Married ... 6

30. Which of the following best represents your total combined household income during the past 12 months?
- ___ $0–$14,999
- ___ $15,000–$24,999
- ___ $25,000–$34,999
- ___ $35,000–$49,999
- ___ $50,000 or more

31. What is your 5-digit zip code? ___ ___ ___ ___ ___

APPENDIX 4 - EXAMINATION FORM

Examination Form

1. Do you have pain on the right side of your face, the left side, or both sides? None ..... 0 Right ..... 1 Left ..... 2 Both 3

2. Could you point to the areas where you feel pain?

Right	*Left*
None 0	None 0
Jaw Joint 1	Jaw Joint 1
Muscles 2	Muscles 2
Both 3	Both 3

[Examiner feels areas subject points to if it is unclear whether it is joint or muscle pain]

3. Opening Pattern
Straight 0
Right Lateral Deviation (uncorrected) 1
Right Corrected ("S") Deviation ... 2
Left Lateral Deviation (uncorrected) 3
Left Corrected ("S") Deviation 4
Other 5
Type _____ (specify)

4. Vertical Range of Motion
Maxillary incisor used 8 9

a. Unassisted Opening Without Pain ___ ___ mm
b. Maximum Unassisted Opening ___ ___ mm
c. Maximum Assisted Opening ___ ___ mm
d. Vertical Incisal Overlap ___ ___ mm

Pain				Joint		
None	Right	Left	Both	Yes	No	NA
0	1	2	3	1	0	9
0	1	2	3	1	0	9

5. Joint Sounds (palpation)

		Right	Left
a. Opening	None	0	0
	Click	1	1
	Coarse Crepitus	2	2
	Fine Crepitus	3	3

Measurement of Opening Click ___ mm ___ mm

		Right	Left
b. Closing	None	0	0
	Click	1	1
	Coarse Crepitus	2	2
	Fine Crepitus	3	3

Measurement of Closing Click ___ mm ___ mm

		Right	Left
c. Reciprocal click eliminated on protrusive opening	No	0	0
	Yes	1	1
	NA	9	9

6. Excursions
a. Right Lateral Excursion ___ ___ mm
b. Left Lateral Excursion ___ ___ mm

Pain				Joint		
None	Right	Left	Both	Yes	No	NA
0	1	2	3	1	0	9
0	1	2	3	1	0	9

c. Protrusion ___ ___ mm

Right	Left
1	2

d. Midline Deviation ___ ___ mm

7. Joint Sounds on Excursions

Right Sounds:	None	Click	Coarse crepitus	Fine crepitus
Excursion Right	0	1	2	3
Excursion Left	0	1	2	3
Protrusion	0	1	2	3
Left Sounds:	**None**	**Click**	**Coarse crepitus**	**Fine crepitus**
Excursion Right	0	1	2	3
Excursion Left	0	1	2	3
Protrusion	0	1	2	3

Directions, Items 8–10:
The examiner will be palpating (touching) different areas of your face, head and neck. We would like you to indicate if you do not feel pain or just feel pressure (0), or pain (1–3). Please rate how much pain you feel for each of the palpations according to the scale below. Circle the number that corresponds to the amount of pain you feel. We would like you to make a separate rating for both the right and left palpations.
0 = No Pain/Pressure Only
1 = Mild Pain
2 = Moderate Pain
3 = Severe Pain

8. Extraoral Muscle Pain With Palpation:

	Right	Left
a. Temporalis (posterior) "Back of temple"	0 1 2 3	0 1 2 3
b. Temporalis (middle) "Middle of temple"	0 1 2 3	0 1 2 3
c. Temporalis (anterior) "Front of temple"	0 1 2 3	0 1 2 3
d. Masseter (origin) "Cheek/under cheekbone"	0 1 2 3	0 1 2 3
e. Masseter (body) "Cheek/side of face"	0 1 2 3	0 1 2 3
f. Masseter (insertion) "Cheek/jawline"	0 1 2 3	0 1 2 3

Modified from: Research Diagnostic Criteria for Temporomandibular Disorders: review, criteria, examinations and specifications critique (Dworkin, S.F. and LeResche, L.; 1992; J Craniomandib Disord Facial Oral Pain)

g. Posterior Mandibular Region (stylohyoid/posterior digastric region) "Jaw/throat region"	0	1	2	3		0	1	2	3			
h. Submandibular Region (medial pterygoid/suprahyoid/anterior digastric region) "Under chin"	0	1	2	3		0	1	2	3			

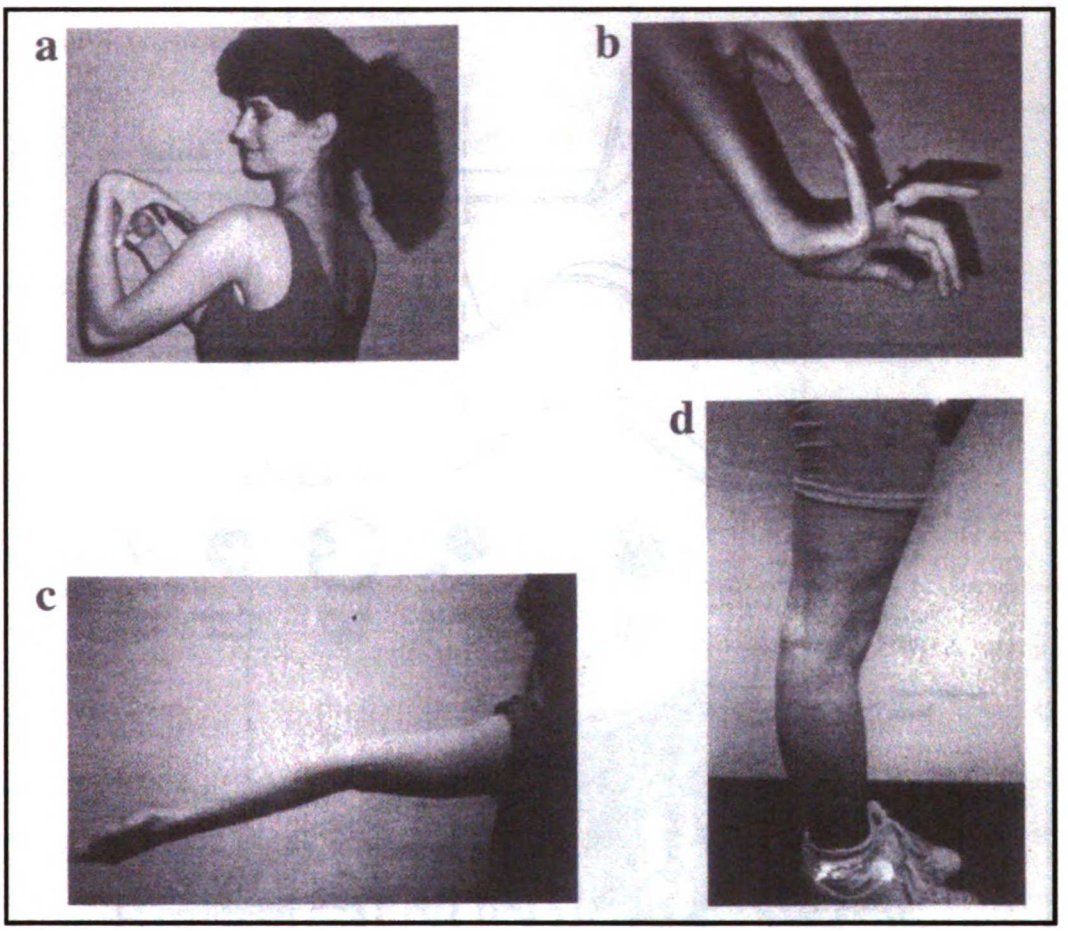
9. Joint Pain With Palpation:

	Right				Left			
a. Lateral Pole "Outside"	0	1	2	3	0	1	2	3
b. Posterior Attachment "Inside ear"	0	1	2	3	0	1	2	3

10. Intraoral Muscle Pain With Palpation:

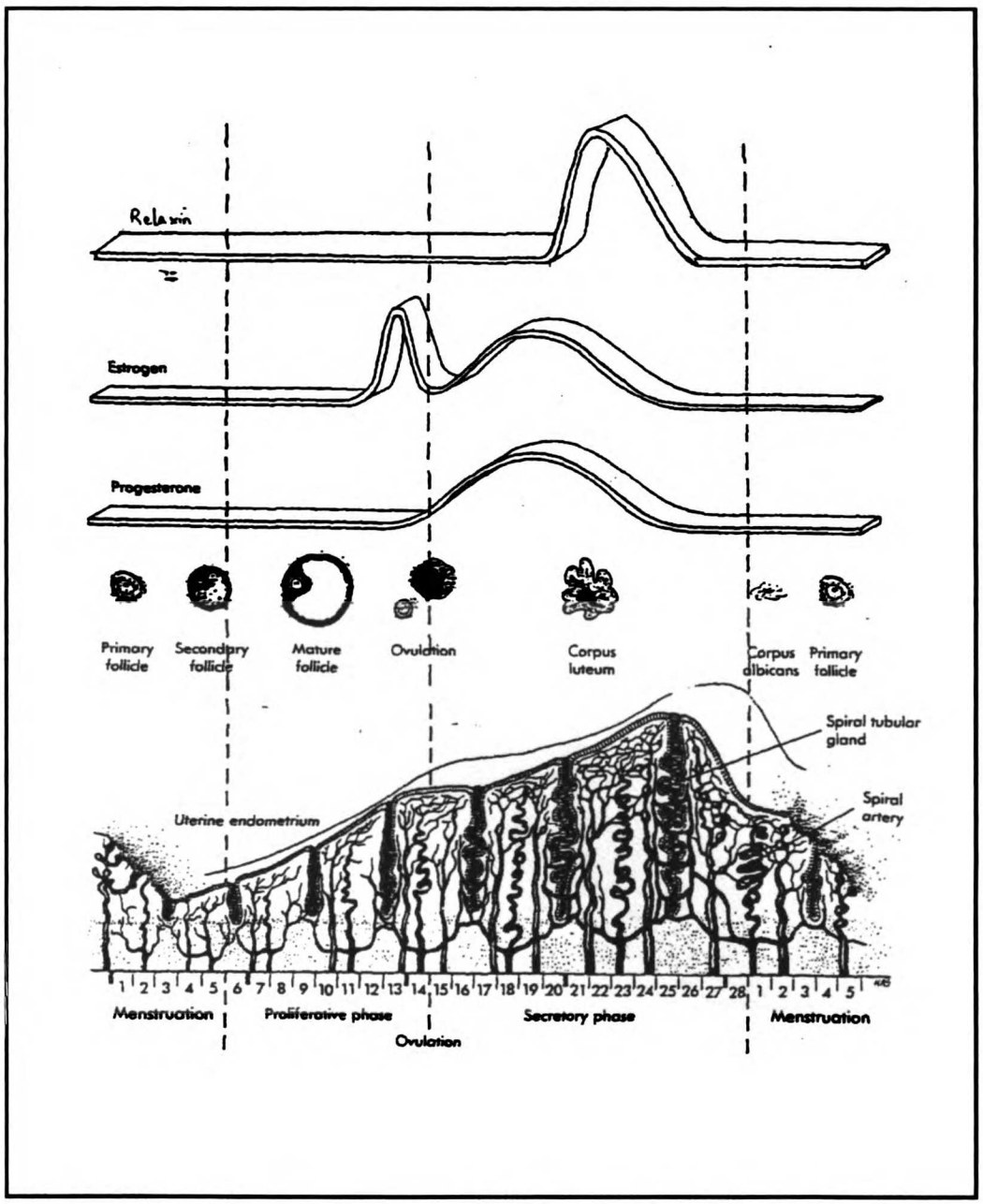
	Right				Left			
a. Lateral Pterygoid Area "Behind upper molars"	0	1	2	3	0	1	2	3
b. Tendon of Temporalis "Tendon"	0	1	2	3	0	1	2	3

APPENDIX 5 - DEMONSTRATIONS OF JOINT HYPERMOBILITY TESTS

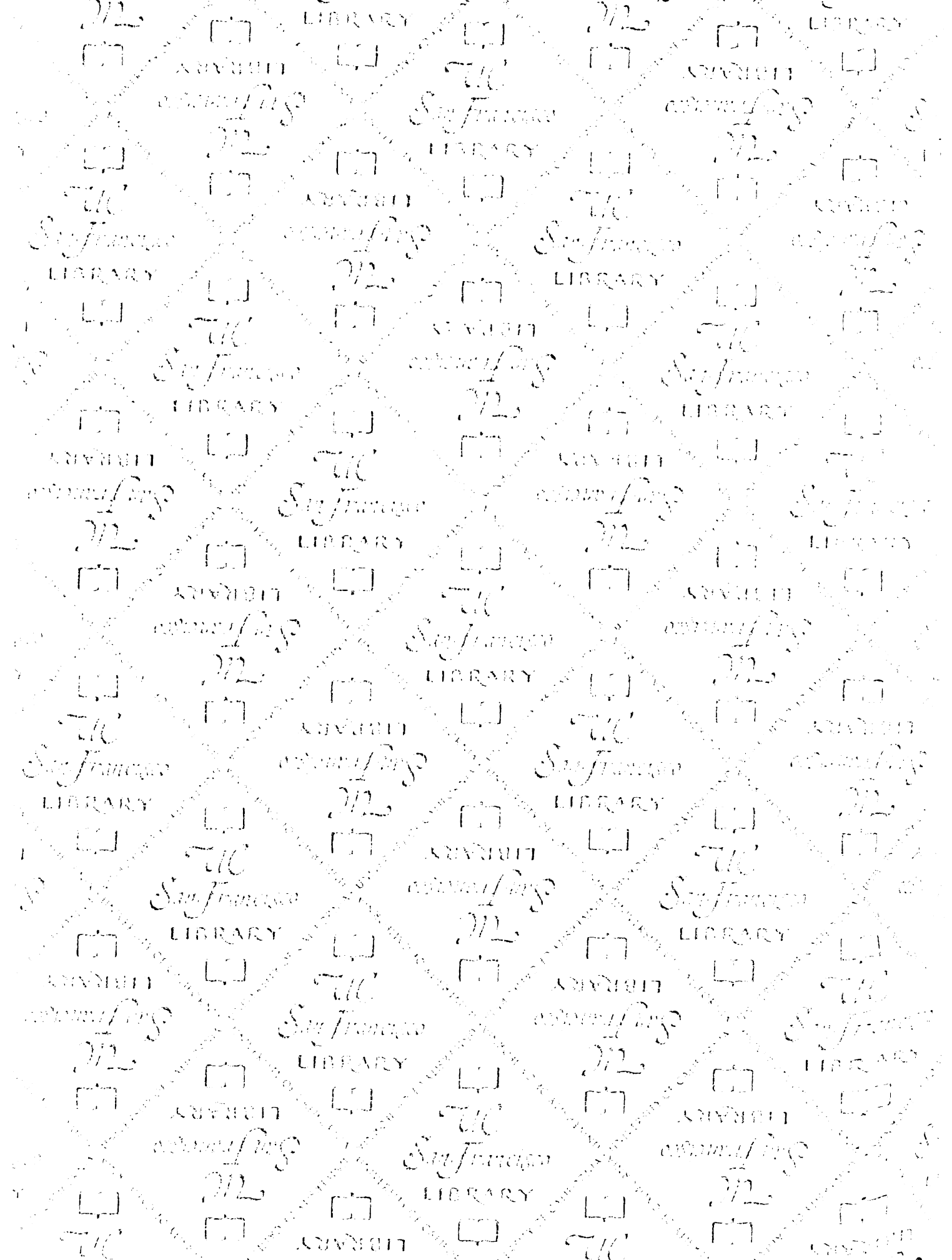


Modified from: Exercise and Total Well Being for Vertebral and Craniomandibular Disorders (Antonioth; T.; 1990; I for C Publications, pp. 16-17)

APPENDIX 6 – HORMONE PEAK LEVELS



Modified from: Human Physiology: Foundations and Frontiers (Schauf, C.L.; Moffett, D.F., Moffett S.B.; 1990 Times Mirror/Mosby College Publishing, pp. 666).


LIBRARY