Title
The role of female reproductive hormones in the etiopathogenesis of TMJ disease in women

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Publication Date
2002

Peer reviewed|Thesis/dissertation

THE ROLE OF FEMALE REPRODUCTIVE HORMONES IN THE ETIOPATHOGENESIS OF TMJ DISEASE IN WOMEN

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ORAL BIOLOGY

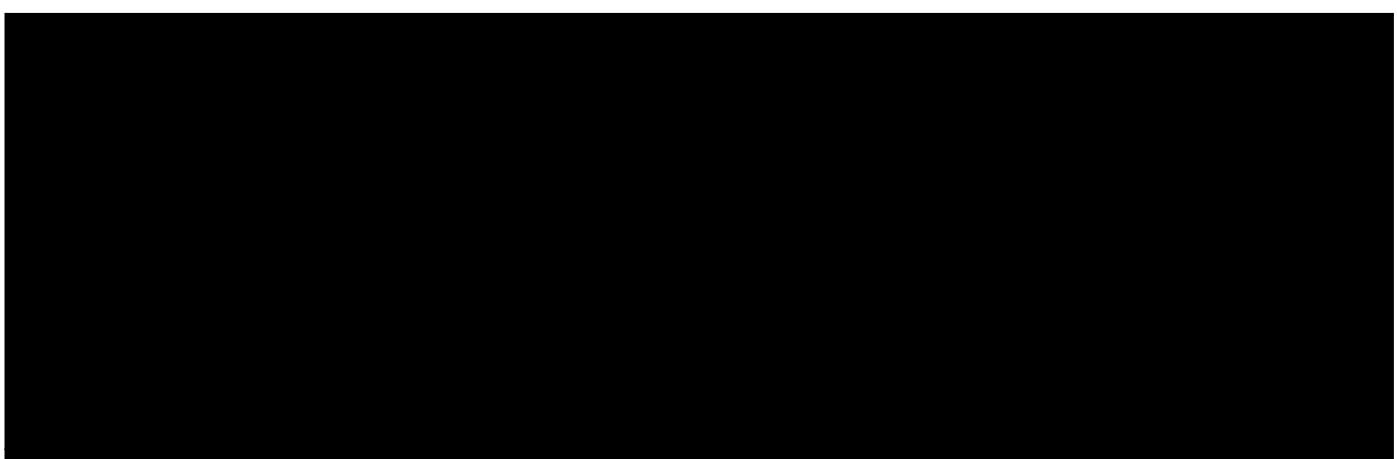
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



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ACKNOWLEDGEMENTS

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

My mentor, Dr. Sunil Kapila, dedicated his heart and soul to this project. His support and encouragement allowed me to successfully complete this study, as well as my orthodontic training. Dr. Charles McNeill, Dr. David Hatcher, and Patricia Rudd generously gave of their time to provide me with a foundation to perform the clinical exams and radiographic evaluations. Dr. Stuart Gansky and Sandra Sinisi were invaluable in guiding our statistical investigations. Dr. Gansky was very supportive and gave many valuable suggestions towards the written version of my work. I would also like to thank Dr. Art Miller who gave a generous amount of his time to help me get started with my writing. His encouraging words and kind nature are very much appreciated.

I would like to thank my Mom and Dad for their never-ending love and support. I could not have accomplished as much as I have without them. Finally, I would like to thank Bruce for his **patience**, mentorship, and encouragement through my entire dental/orthodontic education.

THE ROLE OF FEMALE REPRODUCTIVE HORMONES IN THE ETIOPATHOGENESIS OF TMJ DISEASE IN WOMEN

Kristen A. Miller, D.D.S.

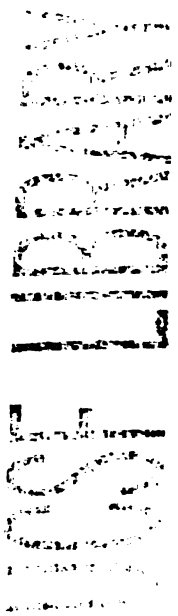
ABSTRACT

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. The etiology and pathogenesis of these diseases remains unknown, but they have a specific age and gender distribution. TMJ diseases are two to three times more likely to occur in women, usually between the ages of 18 and 45 years. This gender and age predilection has led some investigators to propose that female reproductive hormones play a role in the etiology of these disorders. To date there is little evidence to support this theory. Multiple studies have investigated the relationship of female hormones and systemic joint hypermobility (SJH), but no one has examined the potential association between joint disease, hormone levels, and SJH. The purpose of this study was to examine the relationship between TMDs, female reproductive hormones, and SJH. The study also examined whether levels of these hormones in serum correlate with levels in saliva.

116 subjects were initially recruited into the study. 95 of these subjects completed the TMJ/hypermobility exams, as well as tomograms of the TMJs, and were therefore able to be given a diagnosis of disease or non-disease. This division resulted in 47 disease and 48 non-disease participants. All of the subjects (116) were evaluated for systemic joint hypermobility. In addition, 40 disease and 37 non-diseased subjects provided serum and salivary samples at the predicted midfollicular, ovulation-1, and midluteal time

points in one ovulation cycle. These samples were used for quantification of systemic levels of β-estradiol and progesterone.

The quantitative findings on severity of TMJ disease, SJH, and hormone levels were compared between the two groups using Student's two sample t-tests, chi-square tests, and logistic regression with the significance set at alpha = 0.05. Bonferroni adjustments were used where necessary. Linear regression (95% confidence intervals) was used to analyze the association of serum and salivary levels of both hormones.

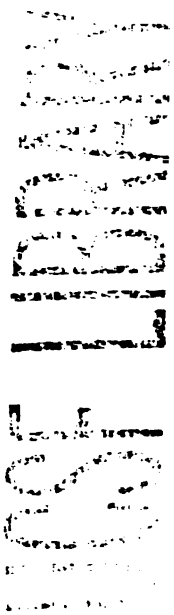


95 subjects with baseline questionnaires, clinical examinations, and radiographs were included in the study. This number represents approximately one-third of a total of 290 subjects that are meant to be included before completion of this continuing study. A statistically significant (P = .01) odds ratio of 3.97 revealed that women who reported a previous pregnancy were 4 times more likely to be diseased than non-diseased. The clinical exam showed that the diseased subjects had a smaller vertical range of motion, smaller laterotrusive movement, and a greater percentage of joint sounds compared to the non-disease group. The vertical range of motion differences were found to be statistically significant (P = .01). The diseased group demonstrated greater, but not statistically significant, overall systemic hypermobility. The diseased group demonstrated higher levels of β-estradiol at the ovulation-1 and midluteal time points and the non-diseased subjects showed higher levels of progesterone at all three time points. After Bonferroni adjustments, none of the differences in hormone levels were found to be statistically significant. The serum levels of progesterone, but not β-estradiol showed a moderate to strong (r = .69 to .85) correlation with levels in saliva at the ovulation-1 and midluteal time points. These findings show trends of a potential connection between SJH, TMJ disease, and female reproductive hormones. A

continuation of the study and subsequent increase in the sample size may provide conclusive evidence regarding the association between these variables.

Sunil Kapila

Thesis Advisor



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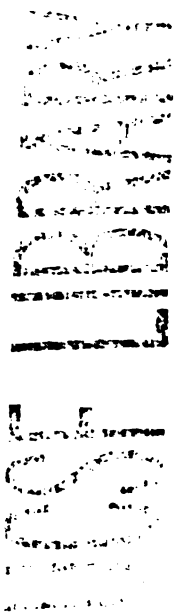
I. BACKGROUND, SIGNIFICANCE, AND SPECIFIC AIMS

A. INTRODUCTION

Temporomandibular disorders (TMDs) encompass a wide spectrum of diseases involving the temporomandibular joint (TMJ), muscles of mastication and associated structures. While the relative contribution of the muscles of mastication and the joint to TMDs remain unknown, in a substantial number of patients, TMDs result secondary to TMJ diseases. These include osteoarthritis, internal derangement, and joint hypermobility (Westling et al., 1992; McNeill, 1993). Additionally, women, between the ages 18 and 45 years of age, comprise a significantly greater proportion of patients seeking treatment for TMDs. This particular sex and age distribution may indicate that TMDs are linked to specific factors in women of child-bearing age. However, the etiology and pathogenesis of these diseases remain unknown.



From a clinician's perspective, patients experiencing ongoing TMJ disease present with variable clinical findings, a cyclical nature of symptoms, and inconsistent skeletal and dental relationships, making these patients some of the most difficult to diagnose and manage. This impacts on the ability of the clinician to provide optimal and timely treatment to patients with these arthropathies. Furthermore, current diagnostic procedures involving corrected tomograms or magnetic resonance imaging (MRI) (Helkimo, 1974; Raustia et al., 1994) provide important diagnostic information, but at best, detect the arthropathies at relatively advanced stages. From a preventive and early intervention standpoint it would be extremely valuable to have the ability to recognize those individuals who are predisposed to TMDs. This requires an

understanding of the etiology and pathogenesis of TMDs. However, at present, aside from trauma, no etiologies have been identified for non-rheumatoid diseases of the TMJ.

While the determination of causative factors remains elusive, the particular age and sex distribution of TMDs points towards some potential etiologic factors. In this study we examined the potential association between TMJ disease and systemic levels of the female hormones progesterone and β-estradiol. Furthermore, because of the higher prevalence of systemic joint hypermobility (SJH) in women than in men and the potential association between SJH and female hormone levels, the relationship of TMJ diseases and joint hypermobility was also assessed. Levels of the two hormones in serum were assessed for correlation with levels in saliva.

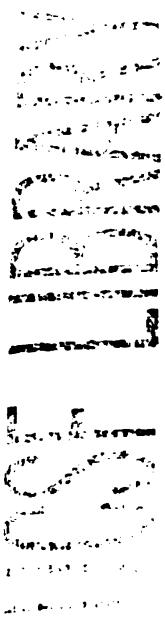
B. TEMPOROMANDIBULAR DISORDERS

Temporomandibular disorders (TMDs) have been described as a major source of non-dental pain occurring in the orofacial region. TMDs can be separated into masticatory muscle disorders and articular TM joint disorders. A substantial proportion of these disorders are related to diseases of the joint itself, which is the focus of this study. The common disorders of the joints include disc displacement, dislocation, inflammation, osteoarthritis, osteoarthrosis, and ankylosis. All of these disorders are described in further detail below.

1. Disc Displacement

The disc separates the joint capsule into lower and upper compartments. It is formed of dense, fibrous connective tissue that creates a cushion for the condyle against the

glenoid fossa of the temporal bone. The disc usually remains between the condyle and the glenoid fossa/articular eminence during normal mandibular movements. However, it is possible for the disc to be dislocated from this normal position. Displacement of the articular disc is the most common TMJ arthropathy and is characterized by several stages of clinical dysfunction involving the disc-condyle relationships. The disc is usually displaced in an anterior or antero-medial direction (Isberg-Holm et al., 1982; Farrar et al., 1983). Disc displacement is thought to result from the tearing or stretching of ligaments that attach the condyle to the disc (Stegenga, 1991). Depending on the clinical presentation of this condition, disc displacement may occur either with or without reduction.

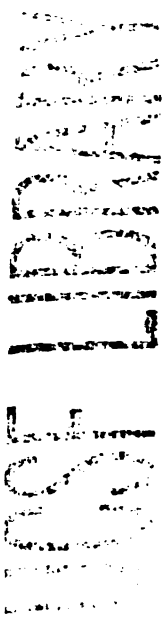


i) *Disc displacement with reduction.* This disorder involves an interference in the relationship between the disc and the condyle in its translation during opening and closing the mouth. A joint noise (click) is produced when this temporarily misaligned disc "reduces" or improves its structural relationship with the condyle. "Reciprocal clicking" occurs when a noise is produced during the opening movement and again in closing, just before the teeth occlude. If pain is present with this condition it is precipitated by the movement of the joint and usually occurs at the time of disc reduction. A painful disc displacement with reduction is thought to be due to gross injury that results in stretching or tearing of the disc ligaments and / or capsule of the joint.

ii) *Disc displacement without reduction.* In this disorder the disc is "non-reducing" or permanently displaced. Therefore, the disc-condyle relationship is maintained during mandibular translation. An acute stage of this disorder is characterized by pain with sudden limited jaw motion.

The disc becomes jammed as a result of disc adhesion, deformation and / or dystrophy (Stegenga, 1989). This situation manifests itself clinically as a straight-line deviation to the affected side on opening, a lack of joint noise, and a significantly limited laterotrusion to the contralateral side. In the chronic stage of this problem the jaw opening range will be close to normal and the pain is substantially reduced (McNeill et al., 1993).

2. TMJ Dislocation



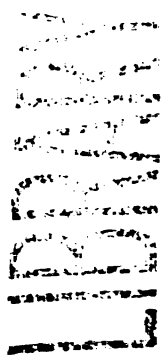
TMJ dislocation occurs when the condyle translates anterior to the articular eminence and is unable to return to its closed position. As a result the patient is not able to close his or her mouth. This dislocation may be momentary and self-reducing in the patient or it may require manual reduction by a clinician. People who experience this phenomena usually have a history of excessive range of motion without pain. Pain commonly results from a dislocation and may last for some time after it is reduced.

3. Inflammation and Arthralgia

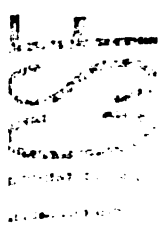
Inflammatory conditions usually occur as a result of trauma, irritation, or infection (Clark and Solberg, 1987). Synovitis and capsulitis are inflammatory conditions that may affect the TMJ. Synovitis is an inflammation of the synovial lining of the TMJ. It is characterized by localized pain that may be worsened by function and superior-posterior loading. This condition may be accompanied by swelling of the synovial lining and can decrease the ability to occlude on the ipsilateral posterior teeth. Capsulitis is an inflammation of the TMJ capsule which results from trauma. Clinically, it is difficult to differentiate between synovitis and capsulitis.

4. Osteoarthrosis

Osteoarthrosis is defined as a degenerative, non-inflammatory condition of the joint characterized by structural changes of the joint surfaces (DeBont et al., 1985). A radiograph may help detect these changes, but only at a later stage of the degenerative process. Clinically, it presents as a painless condition. It is often associated with crepitus, a deviation to the affected side during opening, and a limited range of motion (Rasmussen, 1981). It is believed that osteoarthrosis is due to a physiologic imbalance between the stress applied to a joint and the ability of soft tissue, cartilage, and bone to withstand the stresses of loading (Bland et al., 1985).



5. Osteoarthritis



Osteoarthritis is a degenerative condition which is accompanied by secondary inflammation of the TMJ. The disease is usually slow and progressive, but may show remissions and regeneration of cartilage which is characterized by deterioration of the articular cartilage and secondary bone formation. Osteoarthritis may result from the joint structures becoming overloaded during function. Clinical findings of this condition include crepitus, limited range of motion with deviation on opening to the affected side, joint tenderness on palpation, and radiographic evidence of structural bony changes.

6. Ankylosis

Complete or partial restricted joint movements are the primary characteristics of ankylosis. This condition may occur as a result of bony fusion or intracapsular fibrosis

(Clark and Solberg, 1987). Adhesions generally occur when the disc and posterior attachment fibrose to the temporal bone. Radiographs appear normal in fibrous ankylosis and will show osseous fusion in bony ankylosis in the closed position of the jaw. Pain may occur with this condition in patients who force the joint beyond its end range of motion.

C. EPIDEMIOLOGY

1. Prevalence

Cross-sectional studies of non-patient populations demonstrate that approximately 75% have at least one sign and 33% have at least one symptom of TMD (Rugh and Solberg, 1985; Schiffman and Fricton, 1988). More recent studies have demonstrated that between 4 and 12 percent of various populations suffer from some form of TMD around the world. In numbers this translates to approximately 450 million people around the world and 20 million people in the United States (Drangsholt and LeResche, 1999). Currently, a universally accepted diagnostic criterion and classification scheme does not exist for TMDs. This fact causes the interpretation of results from various clinical and epidemiological studies to be complicated. Therefore, it is difficult to determine the exact prevalence of TMDs in various populations.

2. Gender Differences and TMDs

In patient population studies, the percentage of women seeking treatment is disproportionately greater than men, ranging anywhere in a ratio from 2.5:1 to 10:1 (Zarb and Thompson, 1970; Cohen, 1978; Solberg, 1982; McNeill, 1993; Dragsholt and

LeResche, 1999). The preponderance of women in patient reports who suffer from TMDs has been striking (von Staplemohr, 1929; Schwartz and Cobin, 1957; Franks, 1964; Kruse 1965; Perry, 1968; Carraro et al., 1969; Carlsson et al., 1982). Studies observing non-patient populations found that women tend to have a greater prevalence of symptoms such as TMJ tenderness, TMJ clicking, facial muscle tenderness, and headaches (reviewed in Clark and Solberg, 1987; McNeill, 1993). Studies that focused on the specific diagnosis of internal derangement also found that women had this condition more often than men (Burman and Sinberg, 1946; Foged, 1949; Christie, 1953; Isaacson et al., 1989; Wilkes, 1989). The majority of these females who suffer from TMD are of child-bearing age, between 13 and 45 years (Carlsson et al., 1967; Isaacson et al., 1986). The reasons for these disparities in gender distribution have yet to be discovered.

D. PROPOSED ETIOLOGIES

Several theories currently exist on the etiology and pathogenesis of TMJ diseases. These range from history of trauma, anatomic factors, psychological factors, systemic joint laxity, and endocrine factors. Several of these proposed etiologic factors have been described below.

1. History of trauma

Traumatic injury may occur to individual, or all, components of the joint. The application of abnormally excessive forces may create these injuries. One study reported that 30% of patients who sought treatment at a temporomandibular and facial pain clinic had a prior history of major trauma (Pullinger et al., 1985). A history of facial trauma was

reported in 50% of patients who underwent TMJ surgery. Another study showed that 25% of 89 patients with arthrographically positive internal derangements had an incident of jaw trauma immediately prior to their onset of TMD (Katzberg et al., 1980).

2. Occlusal Factors

Since the 1930's some dental professionals have viewed occlusion as playing a primary role in the etiology of TMD. A link between occlusal interferences and TMDs has been examined in several studies (Roth, 1973; Magnussen and Carlsson, 1983; Forssell and Kangasniemi, 1986). All found that occlusal interferences introduced to a stable occlusion of an asymptomatic person caused TMD symptoms and signs to develop. Many studies have also suggested that a significant discrepancy in centric relation and centric occlusion may be a predisposing factor (Dubral, 1981; Roth 1981). Other literature does not support this view, and therefore the controversy of the discrepancy between centric occlusion and centric relation in causing TMD continues on (Carlsson and Droukas, 1984; Seligman and Pullinger, 1989; Rinchuse, 1996).

Orthodontic malocclusions were initially implicated in the etiology of TMDs. Extensive overbite was associated with joint sounds in one study (Runge et al., 1989), but these findings were not able to be reproduced by other investigators (Roberts et al., 1987; Cachiotti et al., 1991). Similarly the association between TMD and excessive overjet has not been substantiated (Lieberman et al., 1985; Castaneda et al, 1989). Unilateral maxillary posterior lingual crossbites have been found to be more common in TMD patients (Seligman, 1989). Overall, significant orthodontic malocclusions have not been found to be of primary importance in the etiology of TMD. It might be understood that a malocclusion may exacerbate symptoms when a patient has already developed a TMD.

In the recent years, orthodontic treatment has been postulated as a possible cause of TM disorders. One study compared control patients with those that had undergone orthodontic treatment and found no significant differences in the incidence of TM disorders (Gianelly et al., 1988; Dahl et al., 1988). Long-term studies have also shown that comprehensive orthodontic therapy with fixed appliances did not alter the risk of developing TM disorders later in life (Sadowsky and Polson, 1984).

3. Psychological Factors

People that experience TMDs are often described in terms of their clinical symptoms, but less is documented about factors of psychological and social importance. A large case control study found that groups of people who experienced psycho-emotional tension were more likely to experience TMD compared to controls (Wigdorowicz et al., 1979). Similarly it has been found that patients with TMD experience more anxiety than healthy control groups (McCreary et al., 1991). In a study of 350 TMJ patients, those who experienced the most severe general and local symptoms had the highest levels of emotional stress (Carlsson, Kopp and Wedel, 1982). Some of the most recent studies had demonstrated that TMJ patients are more likely to have experienced at least one previous episode of mental depression (Drangsholt and Le Resche, 1999).

4. Systemic Joint Hypermobility

Joint mobility is the maximum range of motion in all directions for a given joint. Joint laxity is characterized by increased distensibility of passive joint movements and by hypermobility in active movements. Unduly lax joints may become injured by normal forces placed on them which would be harmless to joints of normal stability. Hypermobile joints are therefore more liable to develop traumatic synovitis which may later progress to osteoarthritis (Kirk et al., 1967; Bird et al., 1978; Grahame, 1981; Scott et al., 1979; Bird and Wright, 1989; Finsterbush and Pogrund, 1982).

Females have a higher prevalence of systemic and joint hypermobility compared to males (Westling, 1992; Larsson et al., 1993; Pountain, 1992). Only females were found to have extreme laxity, which was associated with increased joint symptoms in those between the ages of 16 to 25 years (Pountain, 1992). These findings may help explain why women experience significantly higher clinical incidence of osteoarthritis and TMJ internal derangement (Bates et al., 1984; Westling, 1992). During pregnancy it has been shown that hypermobility develops in the peripheral joints of women. It has been suggested that such extreme laxity could result from increased levels of certain hormones during pregnancy, particularly relaxin (Calguneri et al., 1982). Increased laxity within the TM joint, as in any joint, may make it unable to withstand normal loading forces.

A recent systematic review (Dijkstra et al., 2002) sought to analyze the conflicting evidence in the literature regarding the association between TMJ disorders and generalized joint hypermobility. Fourteen papers were reviewed and 4 were included in the review (208 subjects). According to the pooled data (113 cases and 95 controls) it

was concluded that it was unclear whether generalized joint hypermobility is clinically associated with TMJ disorders. The inconclusiveness of this study was due to the poor methodological quality of the currently available studies.

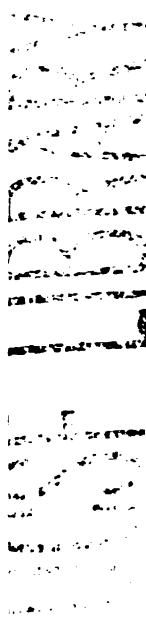
5. Endocrine Factors

The potential role of female reproductive hormones in the etiology of TMDs has been of interest due to the age and gender distribution of its signs and symptoms. The presence of estrogen receptors within cultured TMJ tissues has been the recent focus of many studies (Aufdemorte et al., 1986; Milam et al., 1987; Abubaker, 1991; Chander and Specter, 1991; Campbell et al., 1993). Abubaker was one of the first investigators to find receptors in human TMJ specimens (Abubaker, 1991). This study reported that symptomatic females contained higher numbers of estrogen receptors in the joint (Abubaker, 1991). The role of exogenous hormones from oral contraceptives (OC) and estrogen replacement therapy (ERT) have also been examined and found to increase the chances of developing TMJ pain (LeResche et al., 1993). OC and ERT users were 20% and 33% more likely to seek care for facial pain compared to non-users, respectively (LeResche et al., 1993). On the other hand, a recent study found no association between OC and TMD (Hatch et al., 2001). 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). Recent work (Kapila and Xie, 1998) suggests that cells within the TMJ disc may be target sites for the matrix-degradative affects of relaxin, which is accentuated by β-estradiol. Since these hormones are found in varying amounts in normally cycling women a potential link could exist between these hormones and TMJ diseases in women. Further studies (Kapila and Hashem, unpublished data) also suggest that progesterone decreases the induction

of tissue degrading enzymes by relaxin. Together these studies suggest that altered absolute or relative levels of one or more of these hormones may predispose to TMJ disease in women of reproductive age.

E. HYPOTHESIS, SPECIFIC AIMS, AND SIGNIFICANCE

Due to the association between SJH and β-estradiol and progesterone, and the particular age and gender distribution of TMJ diseases, women of childbearing age with TMJ disease were hypothesized to demonstrate increased SJH and altered systemic levels of reproductive hormones compared to non-diseased controls. Serum levels of β-estradiol and progesterone were also hypothesized to correlate positively with those in saliva. The specific aims of the study were to:



1. Determine by objective and quantitative measures the presence and severity of clinical and radiographic signs of TMJ disease in female subjects with and without TMJ disease.
2. Evaluate and compare the magnitude of systemic joint laxity determined by objective scoring criteria in female subjects with and without TMJ disease.
3. Quantify and compare the absolute and relative serum levels of β-estradiol and progesterone in subjects with and without TMJ disease.
4. Determine if serum levels of β-estradiol and progesterone correlate with salivary levels of these hormones.

The results from these studies may provide insight into the hormonal etiology of TMJ diseases in women. If an association exists for levels of specific hormones or systemic joint hypermobility with severity of disease, the findings of this study could lead to the

development of accurate (perhaps earlier) diagnoses and specific treatments that would address the underlying cause for the disorder. This would be a superior therapy compared to the current method that includes treating the signs and symptoms of the disorder and not the cause. Also, if salivary levels of these hormones correlate to those in serum then a less invasive means (saliva collection) of screening individuals for TMDs could be utilized. A dentist would potentially be able to use this as a screening tool to make an accurate assessment of the status of TMJ disease before, during, and after dental treatment. These studies will provide new information on the pathology of the TM joint, as well as provide insight to 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 Research at the University gave approval for all procedures rendered.

Normal cycling and ovulatory 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. During the telephone interview potential participants were screened for exclusion and inclusion criteria prior to their enrollment into the study.

Exclusion and Inclusion Criteria

A series of questions were incorporated in the initial telephone screening and included the following exclusion criteria: current/recent use of hormonal contraceptives or other hormonal therapies; history of trauma to the head and/or neck; systemic diseases that affect the joints and connective tissue metabolism (e.g., rheumatoid arthritis, diabetes); immuno-suppressive disorders; autoimmune disorders; irregular menstrual cycles and currently pregnant. All women included in the study had to be between the ages of 18 to 40 years and have normal (approximately equal in length) monthly menstrual cycles. A flow diagram demonstrates the recruitment of all subjects and their progress in the stages of the study (Figure 1). 215 women were interviewed to potentially take part in

the study. Fifty four of these 215 women chose not to participate in the study for various reasons (Table I). A total of 161 women volunteered to participate in the study. Of these 161 individuals, 45 were not recruited because they failed to meet exclusion criteria as listed in Table II. Once a subject's eligibility for participation was verified by telephone screening an appointment was made for the clinical TMJ and hypermobility examinations. One hundred and sixteen subjects started in the study and a total of 97 women completed the clinical exam (TMJ and hypermobility exams), as well as tomograms of the TMJ. Seventy nine of these 97 subjects completed all 5 visits of the study.

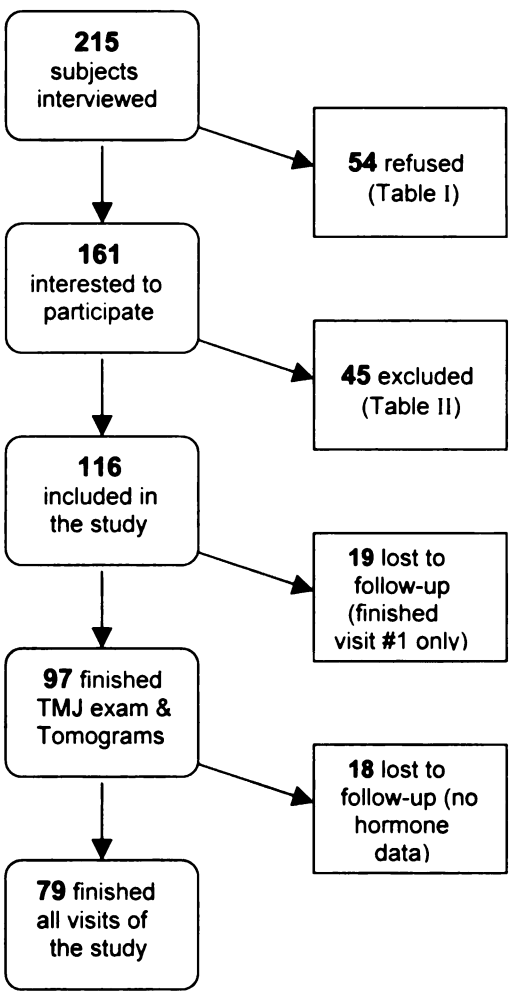


Figure 1. Flow diagram of subject recruitment/progress in the study.

Table I - Breakdown of reasons interviewees gave for not participating in the study.

Reasons for disinterest in study	Number (N=54)
Too busy for 5 visits	23
Too many visits/not interested	16
Live too far away	9
Radiation exposure concerns	3
Afraid of blood collection	3

Table II - Breakdown of subjects excluded from the study.

Reasons for Exclusion	Number Excluded (N=45)
Taking hormonal birth control	15
Irregular menstrual cycle	12
Not within 18-40 years of age	8
Contraindicated medication	6
Disqualifying medical condition	3
Pregnant	1

Women with no previous or current symptoms of TMJ disease, including significant clicks, pain, and impairment of TMJ function, were initially classified as asymptomatic. Subjects were assigned to the symptomatic group if they had present or previous history of TMJ disease, which included one or more of the following symptoms: localized joint pain, significant joint sounds, or limited opening. The final classification of TMJ disease or no TMJ disease was given to each subject on the basis of objectively defined clinical and radiographic criteria as described later.

B. PROCEDURES

Specific data required to address the hypothesis was derived from a patient questionnaire, a systemic joint hypermobility examination, radiographic and clinical TMJ examination, and quantitative assessment of serum levels of β-estradiol and progesterone. Both serum and saliva samples were assayed in order to determine if there is an association between serum and salivary levels of these hormones. The TMJ questionnaire and clinical assessment used was that of the Research Diagnostic Criteria (RDC) (Dworkin et al., 1992), with minor modifications as described later.

Each subject was given a consent form that was signed by the participant and examiner (Appendix 2) at the time of the examination appointment. A total of five appointments were required for each participant to complete the study. The breakdown of the five visits is outlined in Table III.

Table III - Summary of the 5 visits required for each subject to complete the study.

	Examination	Additional information
Visit #1	**--TMJ exam** **--Hypermobility exam**	**--Study further explained** **--Consent form signed** **--TMJ questionnaire completed**
Visit #2	**--TMJ Tomograms**	
Visit #3	**--1st blood/saliva collection**	**--Blood taken at predicted midfollicular time point**
Visit #4	**--2nd blood/saliva collection**	**--Blood taken at predicted day before ovulation time point**
Visit #5	**--3rd blood/saliva collection**	**--Blood taken at predicted midluteal time point**

1. Questionnaire

Each subject completed a questionnaire (Appendix 3) which provided information on the prevalence, signs and symptoms, severity, and frequency of TMJ symptoms. 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.

The questionnaire also required each subject to answer how her TMJ symptoms affect different aspects of daily life. This aspect of questioning was meant to allow the examiners some insight into the psychological status of each subject. Such information may help to establish whether TMJ disease may be associated with non-physiologic variables.

Demographic information such as ethnicity, age, marital status, socio-economic status, and level of education was also obtained. This information may allow the examiners, as well as future studies, to have further insight into the factors related to TMJ disease.

The final questions, which were not contained within the standard RDC questionnaire, requested information pertaining to pregnancy and childbirth. The questions focused on number of successful deliveries (parity) as well as whether TMJ pain was worse before, during, or after pregnancy. The information on childbirth and pregnancies could be utilized to evaluate if these events contribute to present levels of hormones and to any association with TMJ disease.

2. Clinical TMJ Evaluation

A single examiner performed the clinical assessment of the TMJ and followed a modified format of the Research Diagnostic Criteria (RDC) for TMDs (Dworkin, 1992-Appendix 4). The exam was modified to exclude the intraoral muscle palpations (lateral pterygoid and tendon of temporalis). The exam also did not include the millimeter measurement of the open or closing click for each subject. The clinical assessment did include an extraoral muscle examination, as well as an evaluation of joint pain, joint noises, and range of motion.

Location and severity of pain was graded by the participant while being subjected to the following procedures: jaw opening, jaw excursive movements, preauricular palpation, and intraauricular palpation. Location of pain was scored in two ways: by side (right or left) and by specific location (whether or not pain was present in the joint). Pain in any particular region was ranked by the subject as none, right, left, or both sides. Pain was also specified as either being present or absent from the joint. "NA" was recorded in subjects without pain.

Pain severity was quantified by the subject as absent (0), mild (1), moderate (2), or severe (3). The severity of pain was evaluated during extraoral muscle palpation, preauricular palpation, intraauricular palpation, opening/closing, and lateral excursive movements.

A disposable Therabite Gauge (MDC Inc.) measured the range of motion, in millimeters, for each subject. Measurements included passive jaw opening, maximum assisted and

unassisted opening, right and left maximum lateral excursions, vertical incisor overlap, mandibular protrusion, and deviation of the mandibular midline.

Each subject's pattern of opening was recorded as straight, right or left lateral deviation, and right or left corrected ("S") deviation. It was also evaluated if a subject had more than one opening pattern.

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

3. Systemic Joint Hypermobility

The methods used for measurement of joint laxity are based on those developed by Beighton and Horan (1969). The exam bilaterally measures the flexibility of the elbow, knee, fifth digit, and thumb. A goniometer was used to give a range of motion value (angle measurement in degrees) for the fifth digit, elbow, and knee. These measurements were obtained by placing the two arms of the goniometer along the proximal and distal bones adjacent to the joint under consideration. All measurement were made bilaterally. The details of flexibility testing for all joints as well as determination of total systemic flexibility are described below and pictured in Appendix 5.

(a) Thumb

Thumb flexibility was derived by evaluating passive opposition of the thumb to the flexor aspect of the forearm. This measurement was determined without the use of the goniometer. With the palm facing down, the subject was instructed to hold her arm out ninety degrees perpendicular to the body. The subject's opposite hand was then used to flex the thumb as far as she could towards the forearm. When the thumb did not contact the flexor surface of the forearm the flexibility of the wrist was considered normal and given a value of 0. If the thumb was passively able to touch the flexor aspect of the forearm then the thumb was considered to be hypermobile and given a score of 1 (Appendix 5, Fig A).

(b) Fifth Digit

Fifth digit flexibility is defined as the passive dorsiflexion of the fifth metacarpophalangeal (MCP) joint. The subject was instructed to hold her arm out ninety degrees perpendicular to the body, with the palm facing the ceiling. The opposite hand was then used to extend the fifth digit dorsally to its end-feel position. The angle between the dorsum of the hand and the finger was measured (degrees) and recorded using a finger goniometer. The fifth digit is considered to have normal mobility when it can be passively dorsiflexed to an angle of ninety degrees or less, and is given a score of 0. An angle greater than or equal to ninety degrees is considered hypermobile and given a score of 1 (Appendix 5, Fig B).

(c) Elbow

Elbow flexibility is determined by the ability of the elbows to hyperextend. Once again the subject was asked to extend her arm ninety degrees perpendicular to the body (Appendix 5, Fig C). Next, she was instructed to push the crease of the elbow towards the ceiling. The landmarks utilized to acquire this measurement were the lateral epicondyle of the humerus, the styloid of the radius, and the acromion. The joint of the large goniometer was held at the styloid of the radius. The two legs of the goniometer were extended toward the acromion proximally, and the lateral epicondyle of the humerus distally. The angle beyond 180 degrees was recorded. An angle of less than 190 degrees demonstrated normal flexibility (score=0). If the angle was greater than or equal to 190 degrees then the subject's elbow was considered hypermobile (score=1).

(d) Knee

Knee flexibility was measured by determining the magnitude of knee hyperextension. This measurement was made with the subject standing up. The subject was then instructed to "push the knee back" to a firm end-feel (Appendix 5, Fig D). The angle was measured by placing the joint of the goniometer on the lateral epicondyle of the femur, with the legs of the goniometer extending toward the greater trocanter proximally, and the lateral malleollus distally. The angle beyond or less than 180 degrees (straight line through the legs, above & below the knee) was measured. An angle measured at less than 190 degrees was considered to have normal flexibility and scored as a 0. An angle measurement of 190 degrees or greater was considered hypermobile and given a score of 1.

(e) Total Flexibility Score

A total flexibility score was derived by assigning each of the 4 joints bilaterally a score of 0 if it demonstrated normal mobility or a score of 1 if hypermobile. Therefore, a total flexibility score was achieved ranging from 0 to 8 for each individual.

4. Radiographic Evaluation of the TMJ

TMJ tomograms were utilized for the radiographic quantification of TMJ disease and to determine if pathologic changes were present in asymptomatic joints. The standard set of diagnostic radiographs were taken by the same technician and included: a) submentovertex radiograph used for making corrected tomograms of the TMJ; b) center cuts of right and left A-P corrected tomograms in a closed and protruded mandibular position; and c) center cuts of right and left lateral corrected tomograms in an open and closed position of the mandible.

Tomograms were taken with the subject's head parallel to the Frankfort-Horizontal plane. One examiner who was blinded to the subject's TMD symptoms interpreted the images. The examiner is considered to be an expert in radiographic interpretation.

The tomograms were scored for condylar sclerosis, flattening, erosion and osteophyte formation on a severity scale of 0 to 3 (0 = normal, 1 = mild, 2 = moderate, and 3 = severe). Each joint was given a score ranging from 0 to 12. Descriptions for the four above categories were as follows (shown in Figure 2):

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.

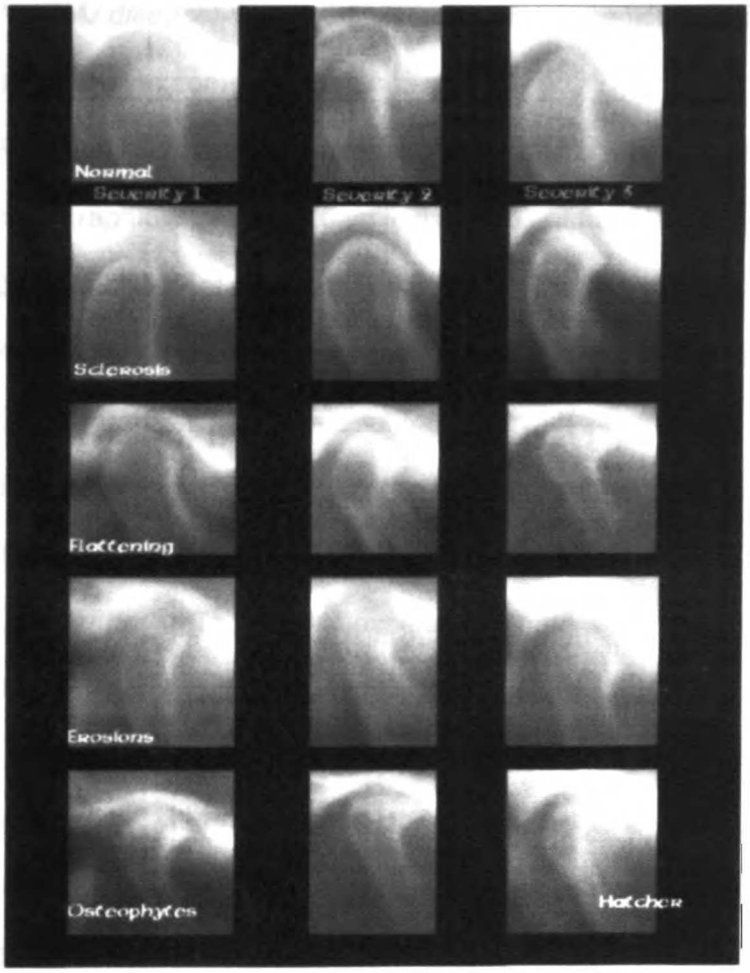


Figure 2. Gradations of condylar images

5. Classification and Assessment of TMJ Status

Subjects were classified into subsets of TMJ disease based on clinical findings as per the RDC index (Dworkin, 1992) that was modified by inclusion of radiographic findings. A composite and objective TMJ diagnosis was assigned to each subject based on findings from both the clinical examination and the radiographic evaluation. 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 individual is currently free of radiographic changes or pain in the TMJ but had a previous (lifetime) positive history of pain and / or locking of the TMJs.

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 of the following scores radiographically: flattening ≥ 2; erosion ≥ 1; or osteophyte ≥ 1.

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

A cut off of flattening of ≥ 2 was selected as indicative of a diseased joint since approximately 60% of totally asymptomatic subjects demonstrated radiographic changes of flattening ≤ 1.

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

Blood and saliva samples were collected between 7:00 am and 11:30 am during the midfollicular (mf), ovulation day minus one (ov-1), and the midluteal (ml) days of the menstrual cycle. These three time-points were selected since the mf samples would provide data on baseline levels of estrogen and progesterone, and the ov-1 and ml samples would provide data on the peak levels of estrogen and progesterone, respectively (see Appendix 6). For each subject, these 3 time-points were determined through the use of ovulation predictor test kits (Walgreens; Deerfield, IL) that were provided by the study. The kits work by detecting the lutenizing hormone surge from a

urine sample, which occurs 24 hours prior to ovulation. During an individual subject's first menstrual cycle she was to notify the examiner of the first day of her period and the day of the positive result obtained with the ovulation kit. The subject then notified the examiner of the first day of her second menstrual cycle on the same day as it occurred. At that point the length of the woman's menstrual cycle as well as its midpoint (ovulation day) was determined. This information was used to estimate the three time-points for blood and saliva collection during the second menstrual cycle. Visit one, the mf day, was estimated as the halfway point between the first day of the cycle (marked by the onset of menstrual bleeding) and the predicted day of ovulation. The second visit was one day prior to the predicted day of ovulation (ov-1) ascertained by the ovulation kit. The third and final visit was on the ml day which is halfway between the predicted day of ovulation and the end of the menstrual cycle (last day before the onset of menstrual bleeding). To verify that each subject's blood/saliva was actually collected on the correct day a second ovulation kit was used during the second ovulation cycle. The subject contacted the examiner on the second cycle ovulation day as well as on the first day of the subject's third menstrual cycle. Therefore, the subject's three blood/saliva collections during the midfollicular, ovulation day minus one, and the midluteal could be cross-checked to determine if they were taken on the correct days.

7. Collection of Blood and Saliva

All retrievals of blood and saliva were performed by the nursing staff at the General Clinic Research Center (GCRC) at the University of California San Francisco. 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 subject 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 subject began chewing the gum.
4. At the end of ten minutes, the subject 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 GCRC core laboratory for processing.

A collection of blood occurred after the collection of saliva. 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 (to be assayed in future studies). 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 and progesterone.

C. QUANTIFICATION OF β-ESTRADIOL AND PROGESTERONE

1. 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. GROUPING OF SUBJECTS

Due to the complex nature of temporomandibular disorders a large effort was made to divide the subjects into proper groups for comparison. Each subject was initially described by the signs and symptoms obtained from the TMJ exam. The subjects without any clinical signs or symptoms of TMJ disease were classified as non-diseased. Subjects with clicking (with reducing discs) were also placed in the non-diseased category. The subjects classified as diseased had one or more of the following signs/symptoms: 1) TMJ pain; 2) past (lifetime) history of TMJ pain or open/closed

locking; 3) coarse crepitus; 4) restricted jaw opening (due to a non-reducing disk); and 5) radiographic changes including erosion or osteophytes. There was a group of subjects who were found to have fine crepitus during the TMJ exam. It was determined that fine crepitus may have been overdiagnosed considering the large number of subjects who were included with this description. Radiographic images were utilized to further subdivide these subjects with fine crepitus. To confirm the diagnosis of crepitus, the subject's tomographic image had to display moderate to severe condylar changes such as flattening ≥ 2 (moderate/severe flattening), erosion ≥ 1 (erosions described as mild, moderate, or severe), and/or osteophyte formation ≥ 1 (mild, moderate, or severe osteophyte formation). A subject with fine crepitus and the previous description of moderate to severe condylar changes was described as "diseased." Of a total of 47 subjects with fine crepitus, 25 (53.2%) were classified into the category of disease. Subjects with fine crepitus and condylar changes including flattening ≤ 1, or sclerosis were to be included in the "non-diseased" group. There were 22 subjects (46.8%) with fine crepitus who were grouped into the non-disease category. The "fine crepitus" heard when examining these subjects was probably artifact from the two-way stethoscope. There were two subjects with myalgia only (no other signs/symptoms of TMJ disease) who were excluded from the subsequent analysis, due to the focus of this study on the TMJ. The description of the disease and non-disease subject divisions is included in Table IV.

Table IV - Number and description of subjects in the non-disease and disease groups.

Groups	Number of Subjects	Description of Subjects
Non-disease	**48**	**1) No clinical signs or symptoms of TMJ disease** **2) Clicking/Reciprocal clicking** **3) Fine crepitus (mild radiographic changes)**
Disease	**47**	**1) TMJ pain** **2) Past history of TMJ pain or open/closed locking** **3) Coarse crepitus** **4) Restricted jaw opening (due to non-reducing disk)** **5) Radiographic changes including erosion or osteophytes** **6) Fine crepitus (severe radiographic changes)**

E. STATISTICAL METHODS AND ANALYSES

97 subjects with baseline questionnaires, clinical examinations, and radiographs were included in the study. This number represents approximately one-third of a total of 290 subjects that are meant to be included before completion of the entire study. The goal to achieve 290 subjects was determined to provide at least 80% power based on previous data from preliminary studies.

1. Error of Method

The method of error was determined to evaluate the examiner's ability to achieve similar repeated measurements. The TMJ and hypermobility exams were performed twice on 11 subjects. The second examination was completed at least two weeks after the first. Lin's concordance coefficient was used to measure the agreement between measurements taken at each exam. The Bland-Altman method was used to demonstrate the strength of agreement for the measurements of maximum TMJ opening

(Appendix 7), laterotrusion (Appendix 8), fifth digit (Appendix 9), elbow (Appendix 10), and the knee (Appendix 11). The kappa statistic was used to determine the agreement of the measures for the thumb. Table V demonstrates that there was very good intra-examiner reliability.

Table V - Lin's concordance coefficient or Kappa Statistic for measured TMJ and flexibility variables (N=11).

	Variable	Lin's concordance coefficient	Kappa statistic
TMJ EXAM	Maximum TMJ opening	0.95	---
	Laterotrusion	0.79	---
JOINT LAXITY	Thumb	---	1.0
	Fifth Digit	0.92	---
	Elbow	0.88	---
	Knee	0.75	---

2. Statistical Analyses

Two-sided statistical tests were used at the alpha = 5% level. All P values reported are unadjusted for the fact that the results are those before completion of the planned sample size. This study comprises one-third of the total subjects required (290) to complete the study. The continuous measures between the control and TMD groups were evaluated with the two sample (unpaired) Student's t test. This test was used to compare data taken from the questionaire, clinical and radiographic TMJ exam, joint hypermobility exam, and hormone levels, including information on age, systemic joint hypermobility (for the fifth digit, elbow, and knee), and vertical/lateral range of motion of the jaw. Chi-square tests compared categorical measures between groups (systemic joint hypermobility of the thumb, fifth digit, elbow, and knee). Bonferroni adjustments for multiple comparisons were utilized when appropriate (for the four hypermobility sites and for the 3 time-points of the hormone levels). The correlation between serum and salivary

hormone levels were evaluated with linear regression models and the Pearson product-moment correlation coefficient.

III. RESULTS

The following section provides information on the study population that was acquired from questionnaires, clinical and radiographic examinations, and hormone assays from the subjects in this study. Differences in systemic joint hypermobility were compared between the non-disease and disease groups. Serum and salivary hormone levels were also compared between the non-disease and disease groups. Then, serum hormone levels were examined for correlation with the salivary hormone levels.

A. PROFILE OF THE STUDY POPULATION

A total of 215 subjects responded to the recruitment flyer (Appendix 1). Of the 215 women screened, 54 chose not to participate in the study for various reasons outlined in Table I. Another group of 45 women were not recruited because they failed to meet one of the following exclusion/inclusion criteria described in Table II. A total of 116 subjects completed the questionaire, as well as the TMJ and hypermobility exams. Nineteen of these 116 subjects did not continue past visit 1 (questionnaire, TMJ, and hypermobility exams).

1. Drop out Analysis

A drop out analysis was completed on the 19 subjects that did not continue past visit 1 of the study. These 19 women were compared with the remaining 97 subjects who completed visit 1 and 2 of the study (TMJ/hypermobility exam and tomograms). The 97 women who continued on with the subsequent analysis were able to be separated into the disease or non-disease groups of the study. The comparisons between the 19 and

97 subjects were made on ethnicity (Figure 3), average age, percent (#) reporting facial pain on exam, percent (#) reporting a previous TMJ history, percent (#) reporting ever being pregnant (gravidity), and percent (#) ever having a baby (parity) (Table VI). The 19 subjects who were lost to follow-up were slightly younger, less likely to report facial pain at the initial exam, and were less likely to ever have been pregnant or had a baby compared with the 97 women who continued on with the study.

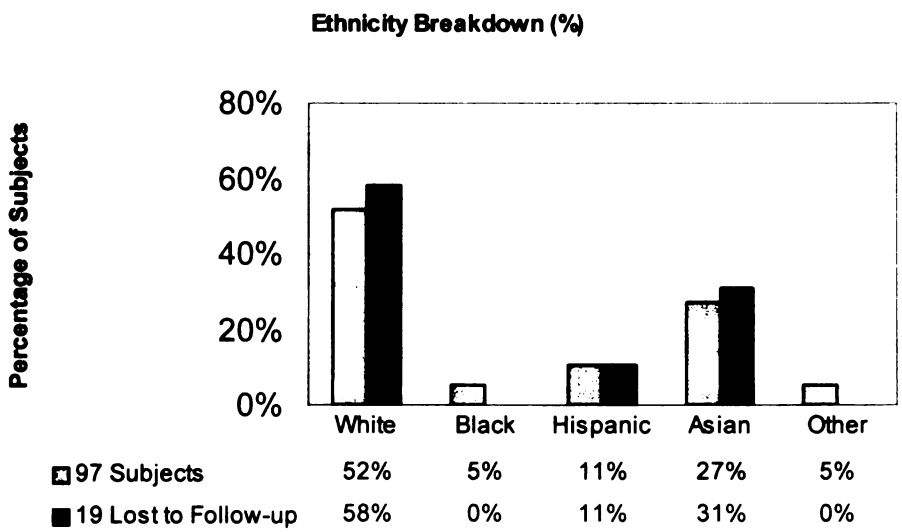


Figure 3 - Percent (%) comparison on race/ethnicity between the 19 lost to follow-up subjects and the 97 who finished the first two visits of the study (TMJ/hypermobility exam and tomograms).

Table VI - Comparison of 97 subjects with 19 lost to follow-up subjects on the basis of average age, percent (#) reporting facial pain at the exam, percent (#) reporting a previous TMJ history, gravidity, and parity.

	97 Subjects	19 Lost to Follow-up Subjects
Average Age (years)	28.8	27.5
Percent and number reporting facial pain on exam	40.2% (39)	26.3% (5)
Percent and number reporting previous TMJ history	42.3% (41)	42.1% (8)
Percent and number who have ever been pregnant (gravidity)	26.8% (26)	10.5% (2)
Percent and number who have ever had a successful childbirth (parity)	13.4% (13)	5.3% (1)

2. Continuation of the Analysis (97 subjects)

The remaining 97 subjects completed the questionaire, TMJ/hypermobility exams, and tomograms of the TMJ. Two of the 97 subjects were diagnosed with myalgia only, and due to the focus of this study on the jaw joint, were therefore excluded from the subsequent analysis. Analyses were carried out on the remaining 95 subjects. Of these 95 subjects, 47 were defined as diseased (TMJs) and 48 as non-diseased. The mean ages of the non-diseased and diseased groups were 28 and 29 years, respectively. These ages were not significantly different by an unpaired t-test (P=.289) (Table VII, Figure 4). The overall ages for all subjects in the study ranged from 18.1 years to 40.6 years. All participants were meant to have regular menstrual cycles, as this was an inclusion requirement to participate in the study.

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

	Non-disease	Disease
Number	48	47
Mean Age	28.3	29.5
Std Dev	5.1	5.3

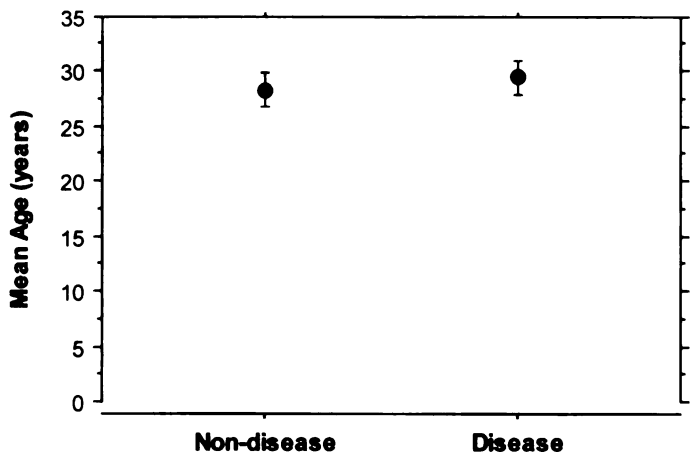


Figure 4 - Distribution of mean age by TMD status and 95% confidence intervals (N=95).

Additional information was also acquired from the patient questionaire in regards to ethnicity and previous pregnancies. A one sample chi-square was used to examine for the differences between the number of subjects in the five racial/ethnic categories in our study compared with the anticipated enrollment based on local figures from the 2000 U.S. census for San Francisco County (χ^2=3.44, df=4, P=0.487). The number of subjects within each racial/ethnic group was found to be similar to the anticipated enrollment (Table VIII). The current enrollment, comparing the two groups based on race/ethnicity is presented in Figure 5 below.

Table VIII - Anticipated and current enrollment of all subjects in the study from various race/ethnicities (number and percentages).

Enrollment	White	Black	Hispanic	Asian/Pacific	Other
Anticipated*	43.6%	7.5%	14.0%	30.7%	4.2%
Current (N=95)	49 (52.5%)	5 (5.2%)	10 (10.3%)	26 (26.8%)	5 (5.2%)

* Source: 2000 Census for San Francisco County (http://venus.census.gov/cdrom/lookup/875128852

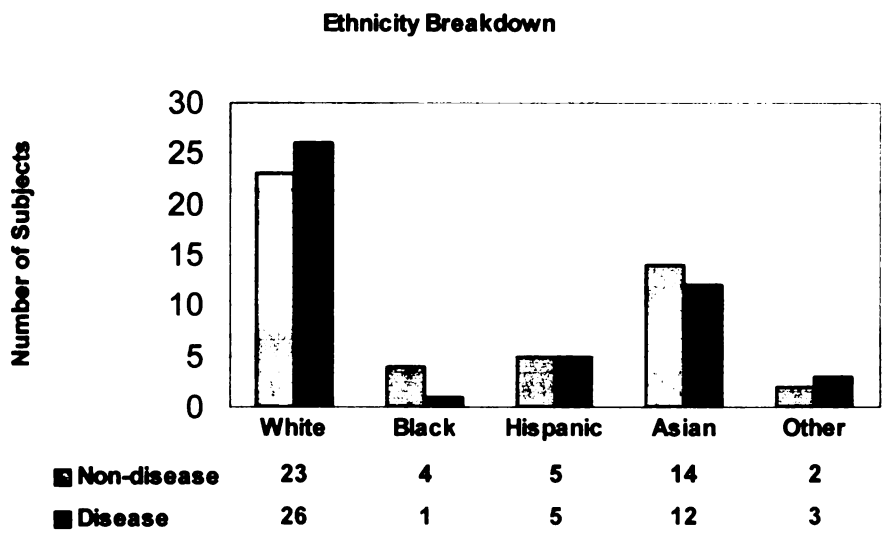


Figure 5 - Number of subjects in the non-disease and disease groups divided by race/ethnicity.

The number and percentage of subjects from both groups who reported previous pregnancies and successful childbirths are presented in Table IX. 26 subjects in the study reported having one or more pregnancies in their lifetime (gravidity). 19 subjects were in the disease group and 7 were in the non-disease group. A statistically significant (P=.01) odds ratio of 3.97 revealed that women who reported a previous pregnancy were about 4 times more likely to be diseased than non-diseased. Thirteen subjects reported having at least one successful childbirth (parity). Nine of these 13

subjects were diseased, compared to the 4 that were non-diseased. An odds ratio of 2.61 was not statistically significant (P=.13).

Table IX - Percentage and number of subjects in each group reporting pregnancies (gravidity) and at least one successful childbirth (parity).

Group	Percentage of Women Reporting (N)	
	Previous History of Pregnancy	Successful Childbirths
Non-disease (N=48)	14.6% (7)	8.3% (4)
Disease (N=47)	40.4% (19)	19.1% (9)
Odds Ratio	3.97	2.61
P value	0.01	0.13

B. CLINICAL TMJ FINDINGS

The clinical TMJ examination involved the determination of each subject's lateral and vertical range of motion, joint sounds, and the presence of current facial pain and its location in the TMJ and surrounding structures. Thirty one (66%) subjects in the diseased group reported current facial pain at the time of the examination. Of these individuals, sixteen were experiencing pain in the muscles and joints, six experienced pain in one joint only, three were experiencing pain in both joints, and six experienced pain in the masticatory muscles (Table IX). By definition, the subjects in the non-diseased group were not experiencing any current facial pain. Current facial pain involving one or both joints was one of several criteria for a subject to be placed in the diseased group. The 14 women who experienced facial muscle pain at the exam (6=disease and 8=non-disease) were not excluded from the study due to the fact that

they had one or more signs or symptoms (click, fine/coarse crepitus, etc...) of TMJ disease.

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

	Percentage Reporting Pain (N)					
Group	No Pain	Pain in Muscle and Joint	Pain in One Joint Only	Pain in Both Joints	Pain in Muscle Only	Total
Non-disease (N=48)	83.3% (40)	0.0% (0)	0.0% (0)	0.0% (0)	16.7% (8)	100% (48)
Disease (N=47)	34.0% (16)	36.2% (17)	12.8% (6)	4.2% (2)	12.8% (6)	100% (47)

The maximum unassisted opening was used to assess each subject's vertical range of motion. The mean and standard deviations for the maximum unassisted jaw opening for both groups are listed in Table XI. The non-diseased group had a statistically significant greater maximum jaw opening (P=.01) compared to the diseased group (Figure 6).

Table XI - Maximum jaw openings (mean ± SD) of non-disease and disease subjects.

Groups	Mean (mm)	Standard deviation (mm)
Non-disease (Maximum Opening)	51.7	6.0
Disease (Maximum Opening)	48.7	5.3

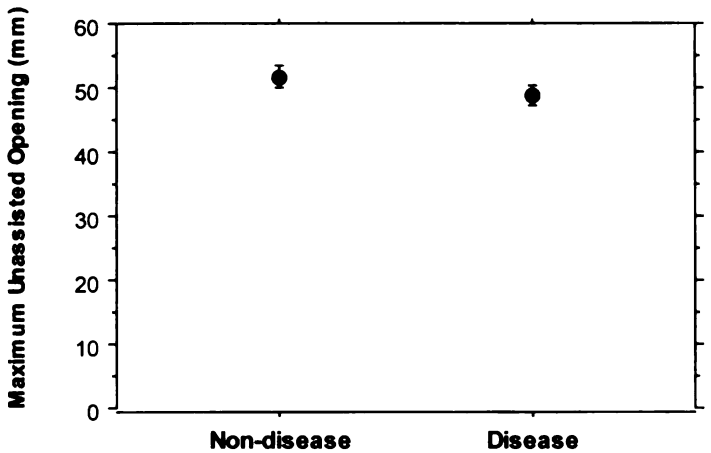
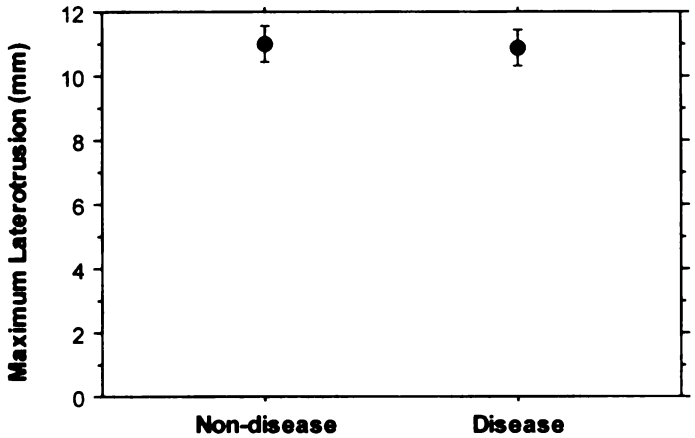


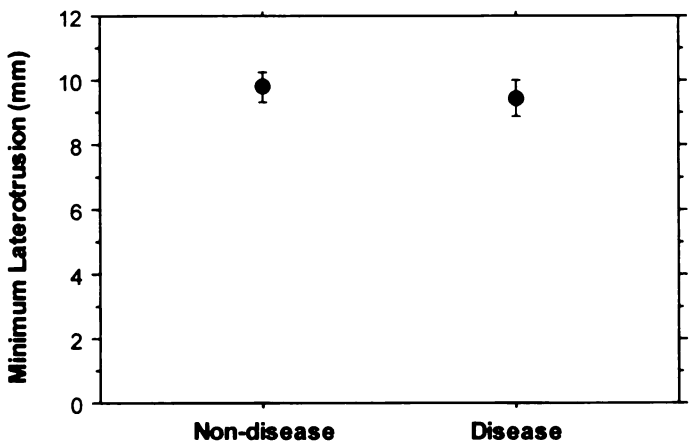
Figure 6 - Distribution of maximum openings by TMD status, including mean and 95% confidence intervals (N=95).

Right and left lateral jaw movements were also evaluated for all subjects. This assessment of laterotrusion involved examination of the maximum, minimum, and mean movements for each subject. The non-diseased group had a slightly greater, but not significant, maximum (P=.75), minimum (P=.34), and mean (P=.40) laterotrusive movements (Figure 7a, b, c - shown on page 41).

a.



b.



c.

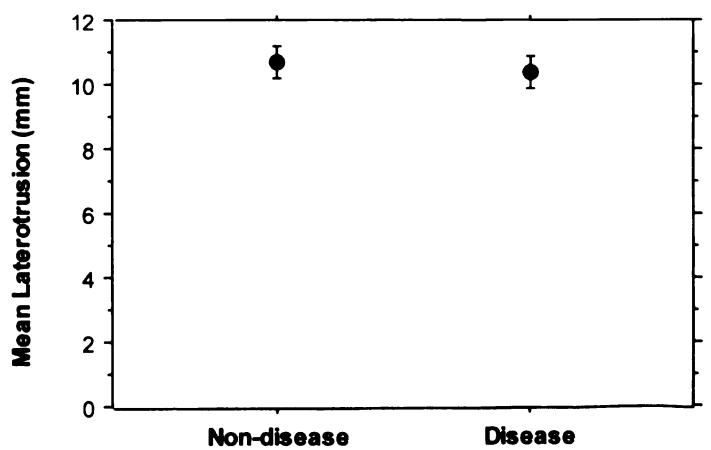


Figure 7a-c - Distribution of maximum (a), minimum (b), and mean (c) laterotrusion (mm) by disease status, including mean and 95% confidence intervals (N=95).

The breakdown of various joint noises for the subjects are listed in Table XII. Only 14.8% of diseased subjects exhibited no joint sounds, while 12.8% displayed an open or closing click, 2.1% had reciprocal clicking, 4.3% had crepitus, 12.8% had coarse crepitus and a click, 25.5% demonstrated fine crepitus (with severe boney changes confirmed by tomograms), and 27.7% demonstrated fine crepitus and clicking. In the non-diseased group 31.3% of the subjects displayed no joint sounds, 18.7% had an open or closing click, 4.2% had reciprocal clicking, 18.7% demonstrated fine crepitus (fine crepitus with mild boney changes determined from tomograms, by definition), and 27.1% displayed fine crepitus and clicking.

Table XII - Percentage and number of non-disease (N=48) and disease (N=47) subjects demonstrating various TMJ sounds.

TMJ Sounds	Non-disease	Disease	Total
No TMJ Sounds	31.3% (15)	14.8% (7)	23.2% (22)
Opening/Closing click	18.7% (9)	12.8% (6)	15.8% (15)
Reciprocal Click	4.2% (2)	2.1% (1)	3.2% (3)
Coarse crepitus	0.0% (0)	4.3% (2)	2.1% (2)
Coarse crepitus/click	0.0% (0)	12.8% (6)	6.2% (6)
Fine crepitus	18.7% (9)	25.5% (12)	22.1% (21)
Fine crepitus/click	27.1% (13)	27.7% (13)	27.4% (26)
Total	100.0% (48)	100.0% (47)	100.0% (95)

C. RADIOGRAPHIC TMJ FINDINGS

Tomograms were used to ascertain the morphological changes in the TMJ for each subject (Table XIII). These evaluations revealed that the majority of non-diseased subjects displayed mild to moderate flattening (60.4%) or sclerosis (33.3%). The

majority of diseased women displayed flattening (53.2%), erosion (25.6%), and osteophytes (10.6%). 10.6% of the diseased subjects displayed sclerosis and there were no women in the same group with normal joint morphology. Only 3 non-diseased subjects (6.3%) displayed normal joint morphology.

Table XIII - Percentage of subjects in both groups with positive findings for morphologic changes (N=95).

Radiographic Findings	Percent Affected		Median Score (1 to 3)	
	Non-disease	Disease	Non-disease (N=48)	Disease (N=47)
Normal	6.3% (3)	0.0% (0)	N/A	N/A
Sclerosis	33.3% (16)	10.6% (5)	1.0	1.0
Flattening	60.4% (29)	53.2% (25)	1.0	2.0
Erosion	0.0% (0)	25.6% (12)	N/A	3.0
Osteophyte	0.0% (0)	10.6% (5)	N/A	1.0
Total	100% (48)	100% (47)		

D. CLASSIFICATION OF TMJ DISEASE

The diseased group of subjects were further divided into subsets of TMJ disease (previous history of pain, arthralgia, osteoarthritis, and osteoarthrosis). This assessment was based on clinical and radiographic findings. The number and percentage of the diseased subjects that fall into each category are listed in Table XIV.

Table XIV - Percentage and number of diseased subjects (N=47) in the TMJ disease categories.

	Percentage	Number
Previous History of Pain	0.0%	0
Arthralgia	21.3%	10
Osteoarthritis	31.9%	15
Osteoarthrosis	46.8%	22

E. SYSTEMIC HYPERMOBILITY

The systemic joint hypermobility was evaluated by examining the fifth digit, thumb, elbow, and knee joints. Data for each joint was based on the presence (1) or absence (0) of hypermobility in either the right or left joint. The hypermobility of the most flexible (bilateral measurements) joint was used for the analysis (Table XV). There were no differences between diseased and non-diseased subjects in hypermobility for the fifth digit, thumb, elbow, and knee.

Table XV - Percent of individuals demonstrating hypermobility (right or left) in the fifth digit, thumb, elbow, and knee (N=95).

Joint	% of subjects with Hypermobility		
	Non-Disease (N=48)	Disease (N=47)	P-Value
5th Digit	14.6% (7)	17.0% (8)	.79
Thumb	25.0% (12)	19.1% (9)	.62
Elbow	52.1% (25)	44.7% (21)	.54
Knee	16.7% (8)	27.7% (13)	.22

The degrees of hypermobility were also measured for the fifth digit, elbow, and knee. For the analysis, a flexibility measurement of zero was considered to be hypermobile. A positive degree value represented a greater amount of hypermobility and an increasingly negative value represented less hypermobility. T-tests for the three joints showed that there were no differences between the diseased and non-diseased groups for flexibility of the 5th digit, elbow, and knee (Table XVI, Fig 8a-c). Additionally there was no difference between the diseased group and non-diseased group in mean composite score for total hypermobility (P=.77) (Table XVII).

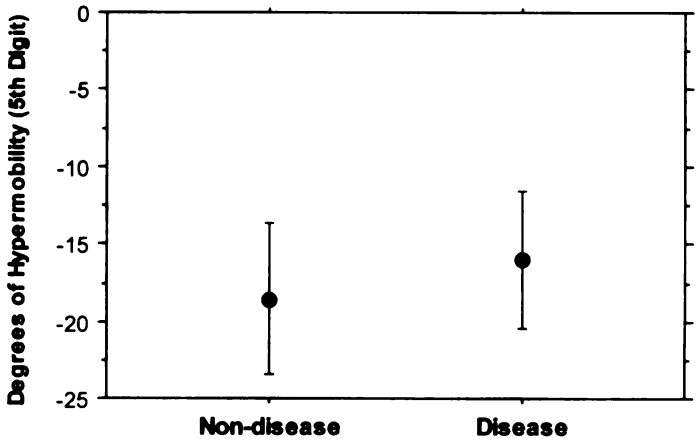
Table XVI - Mean, standard deviation, and P-value for the degrees of hypermobility in the fifth digit, elbow, and knee for all subjects (N=95).

Joint	Degrees of hypermobility (0 degrees=hypermobile joint)	
	Non-disease (N=48)	Disease (N=47)
Fifth Digit		
Mean	-18.6	-16.0
Std Dev	16.7	14.9
P-value	0.43	
Elbow		
Mean	-1.0	-1.6
Std Dev	5.2	5.7
P-value	0.61	
Knee		
Mean	-3.3	-2.7
Std Dev	3.5	3.0
P-value	0.45	

Table XVII- Mean and standard deviation of total flexibility score for both groups (N=95).

Total Hypermobility Score (0-8)	Non-diseased (N=48)	Disease (N=47)
Mean	1.8	2.0
Standard Deviation	1.7	2.0
Median	2	2

a.



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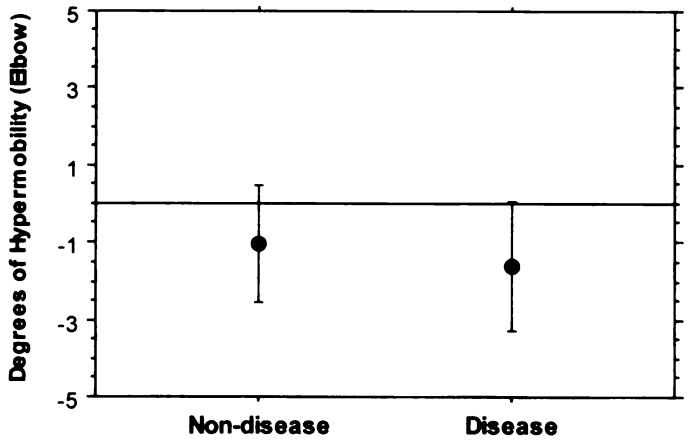

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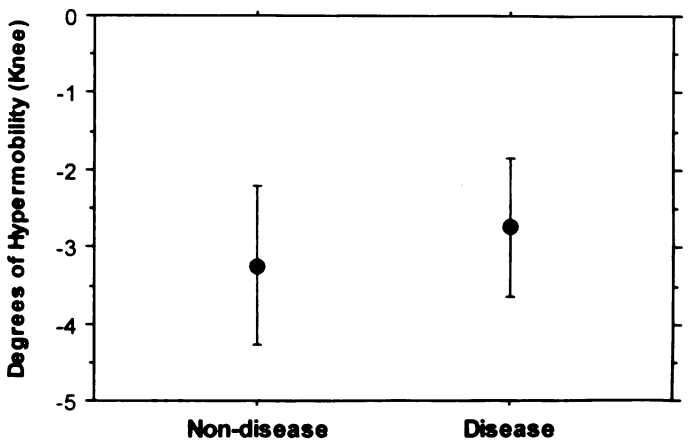


Figure 8a-c - Distribution showing the degrees of hypermobility (right or left) for the (a.) fifth digit, (b.) elbow, and (c.) knee including mean and 95% confidence intervals (hypermobile joint is ≥ 0).

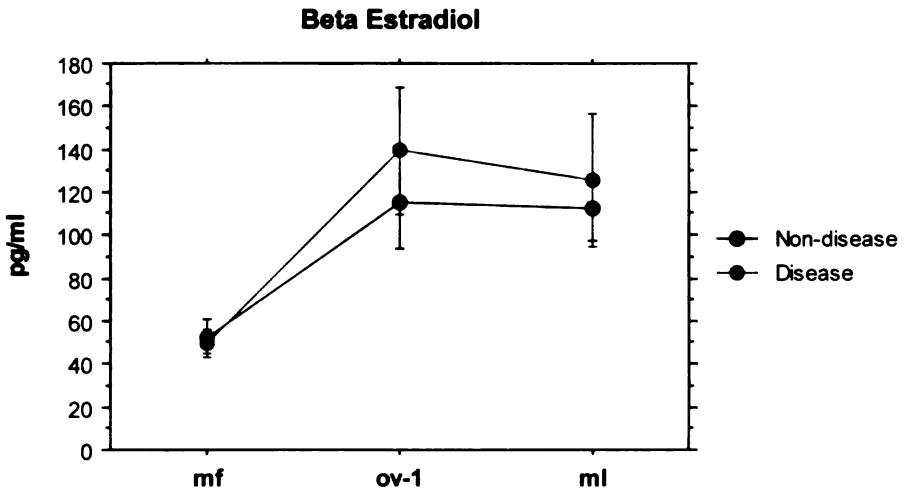
F. SYSTEMIC HORMONE LEVELS IN NON-DISEASE AND DISEASE WOMEN

The mean (± SD) levels of β-estradiol and progesterone were determined at the midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of one menstrual cycle for the non-diseased and diseased subjects (Table XVIII, Figure 9a-b). 79 subjects completed blood and saliva collections with hormone information being available for 77 women for the current analysis. Of these 40 women had no TMJ disease while 37 had TMJ disease. Figure 9a displays that peak (ov-1) and midluteal (ml) levels of β-estradiol were higher, although not statistically significant, in the diseased group (ov-1, P=.18; and ml, P=.41). Although non-diseased subjects had higher baseline estrogen (mf) levels these were not statistically significant (P=.54). The non-disease subjects also had higher progesterone levels at all time points (mf, ov-1, and ml) compared with the diseased group. This difference was statistically significant for baseline (mf) progesterone (P=.03), but after Bonferroni adjustment for 3 time points, this is not significant at the .05/3=.0167 level.

Table XVIII - Mean, standard deviation, and P-value for serum β-estradiol and progesterone at the midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of collection (N=77).

	Beta Estradiol (pg/ml)			Progesterone (ng/ml)		
	mf	ov-1	ml	mf	ov-1	ml
Non-diseased (N=40)						
Mean	53.0	115.1	112.1	.62	1.7	10.6
Std Dev	24.2	67.9	46.0	.28	1.9	6.0
Diseased (N=37)						
Mean	49.8	139.4	125.7	.50	1.3	10.1
Std Dev	20.6	88.2	92.0	.17	1.8	5.1
P-value	.54	.18	.41	.03	.32	.70

a.



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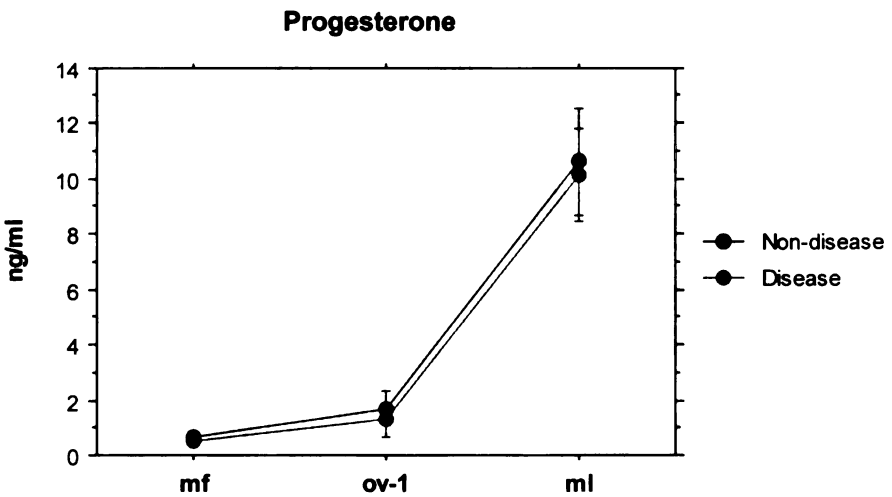
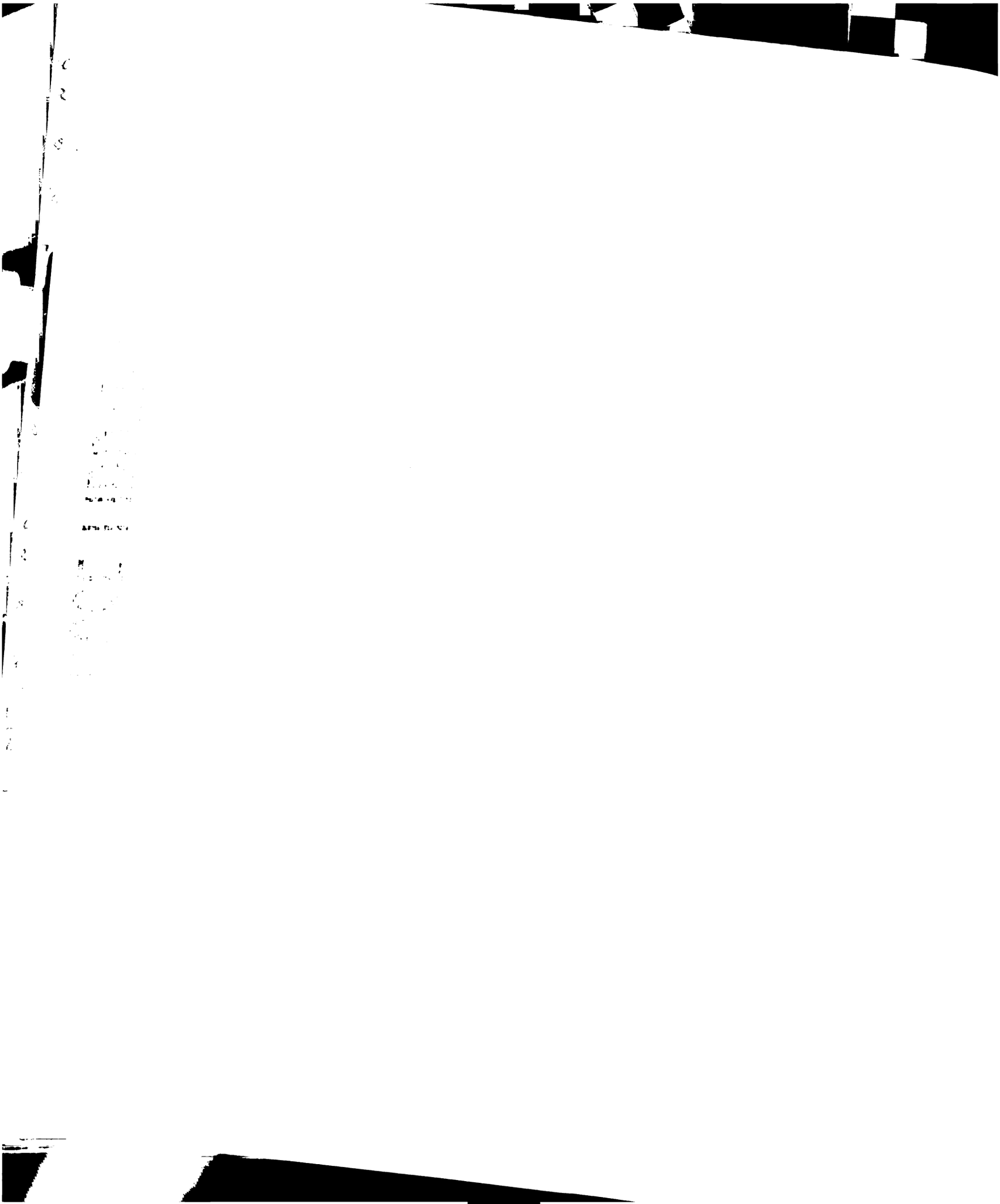


Figure 9a-b - Graphs depicting mean and 95% confidence intervals of serum β-estradiol (a.) and progesterone (b.) at the midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of collection (N=79).



To confirm that each subject had her blood/saliva taken at the correct time (mf, ov-1, ml) a second ovulation kit was used during the cycle of collection. The subject contacted the examiner when she obtained the positive ovulation test result (ov-1) during the cycle of collection. By knowing this information, along with the first and last day of each subject's collection month, the examiner was able to determine how accurately each subject (at the mf, ov-1, and ml timepoints) was at giving a sample on the target date (0). Table XIX summarizes how many samples (blood/saliva) were collected on the target date (0), within one day (± 1) of target, within two days (± 2) of target, and over 2 days (± 3 or more days) from the target collection date. On the basis of this assessment the mean (± SD) levels of β-estradiol and progesterone were determined at the midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of collection for all samples of blood and saliva that were collected within one day (± 1) of target collection (Table XX).

Table XIX - Percentage and number of subjects who had blood/saliva collected on target date (0), within 1 day of target (± 1), within 2 days of target (± 2), and over 2 days of target (± > 2).

	Time points of cycle (Percentage and number of Subjects)		
	(mf)	(ov-1)	(ml)
Blood/saliva collected on Target date (0)	**46.8% (36)**	**20.8% (16)**	**35.0% (27)**
Blood/saliva collected within 1 day of target (± 1)	**24.7% (19)**	**33.7% (26)**	**14.3% (11)**
Blood/saliva collected within 2 days of target (± 2)	**20.8% (16)**	**23.4% (18)**	**24.7% (19)**
Blood/saliva collected over 2 days of target (± > 3)	**7.7% (6)**	**22.1% (17)**	**26.0% (20)**

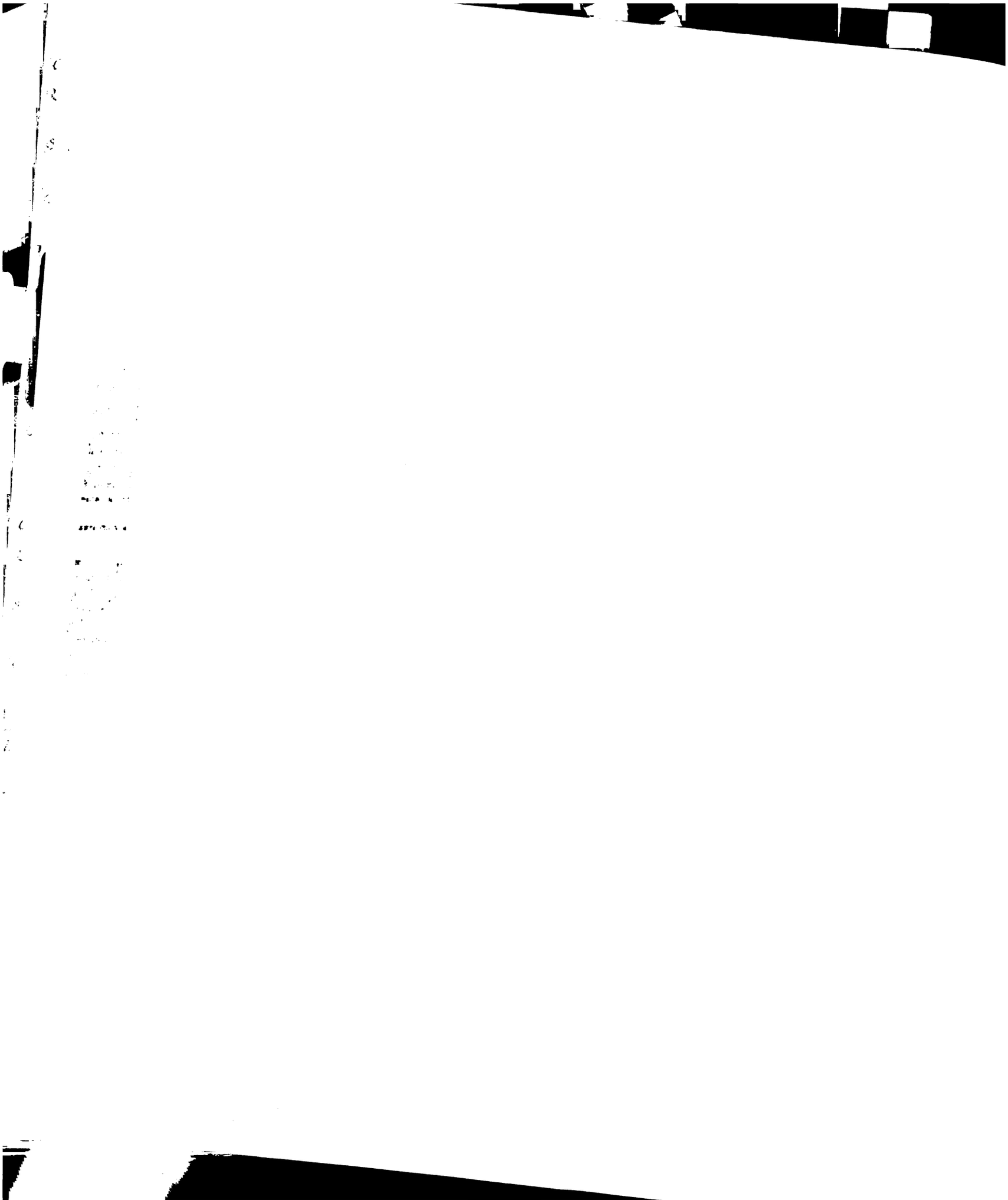


Table XX - Mean, standard deviation, and P-value for serum β-estradiol and progesterone of all samples collected within 1 day (± 1) of the target midfollicular (mf), ovulation-1 (ov-1), and midluteal (ml) days of collection.

	Beta Estradiol (pg/ml)			Progesterone (ng/ml)		
	mf	ov-1	ml	mf	ov-1	ml
Non-diseased						
Number of Subjects	27	19	18	27	19	18
Mean	50.7	131.4	111.0	.59	1.5	11.8
Std Dev	22.3	80.1	52.1	.20	2.1	6.6
Diseased						
Number of Subjects	28	23	20	28	23	20
Mean	49.5	164.7	141.5	.50	.96	10.5
Std Dev	21.6	98.2	118.7	.18	.83	4.5
P-value	.85	.24	.32	.08	.25	.50

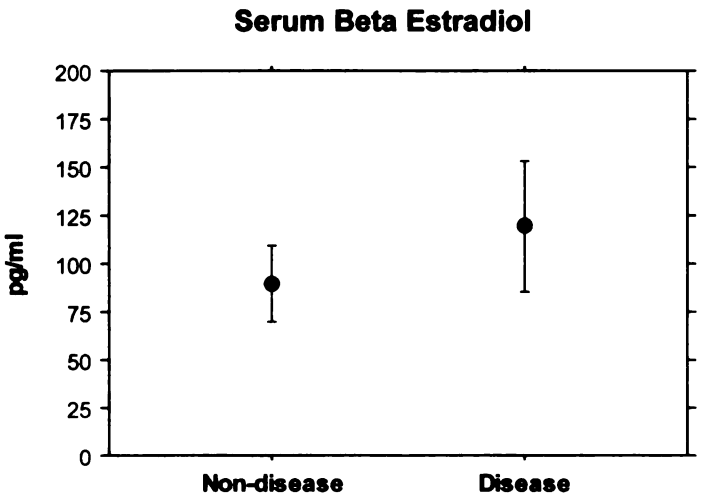
We also determined the mean (± SD) levels of β-estradiol and progesterone in the women who were within 2 days (± 2) of their target midfollicular (mf) collection, within 1 day (± 1) of their target ovulation-1 (ov-1) collection, and who were within 1 day (± 1) of their midluteal blood and saliva collection (Table XXI). We extended the time in the baseline (mf) collection to be within 2 days (± 2) of target due to non-fluctuating levels of hormones at this point in the cycle (Appendix 6). The peak levels of hormones at the ovulation-1 (ov-1) and midluteal (ml) time points have a shorter period of time at their maximum levels in the cycle (Appendix 6). The assumption was made that blood/saliva collections made within a day (± 1) of these target collections (ov-1 and ml) would provide accurate information on peak hormone levels. The hormonal trends are similar between table XX and XXI, as well as when analyzing all the blood/saliva samples, collected at the three time points, for every subject (Table XVIII). None of the findings were statistically significant, but the non-disease subjects had higher progesterone levels at all time points, and the diseased subjects had higher estrogen values at the ovulation-1 (ov-1) and midluteal (ml) time points.

Table XXI - Mean, standard deviation, and P-value for serum β-estradiol and progesterone of subjects who had their blood/saliva samples collected within 2 days (± 2) of the target midfollicular (mf), and within 1 day (± 1) of their target ovulation-1 (ov-1), and midluteal (ml) days of collection.

	Beta Estradiol (pg/ml)			Progesterone (ng/ml)		
	mf	ov-1	ml	mf	ov-1	ml
Non-diseased						
Number of Subjects	10	10	10	10	10	10
Mean	44.2	116.4	95.1	.57	.97	11.6
Std Dev	16.5	63.0	54.9	.23	.59	5.1
Diseased						
Number of Subjects	17	17	17	17	17	17
Mean	48.0	176.0	146.1	.46	.96	10.6
Std Dev	18.3	100.8	128.7	.13	.91	4.3
P-value	.59	.11	.25	.14	.97	.62

We examined absolute differences between the maximum and minimum levels of both hormones in serum and saliva due to the fact that there were substantial differences in hormone levels between individuals. These absolute differences in baseline and peak levels of hormones give insights into the individual variation of hormone levels within the subjects. Next, it was determined whether there were any differences in these hormone changes between the disease and non-disease women. Figure 10 and figure 11 show that for both serum and saliva no statistically significant differences in absolute differences between maximum and minimum levels of both hormones existed between diseased and non-diseased women (Figure 10, P=.12 for serum, and P=.64 for saliva) (Figure 11, P=.64 for serum, P=.79 for saliva).

a.



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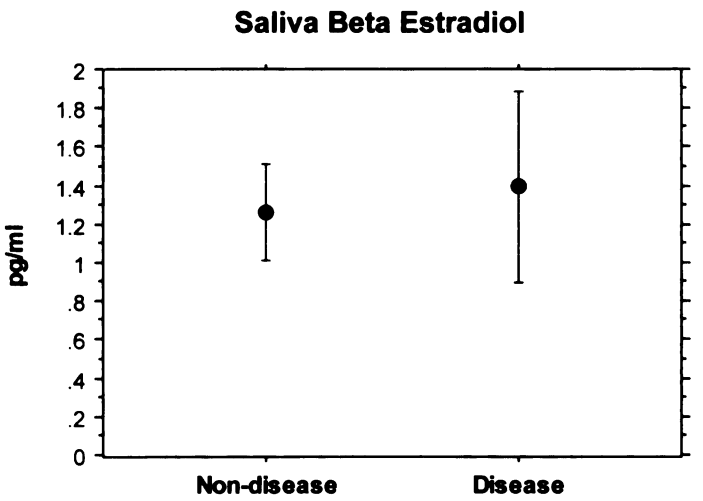
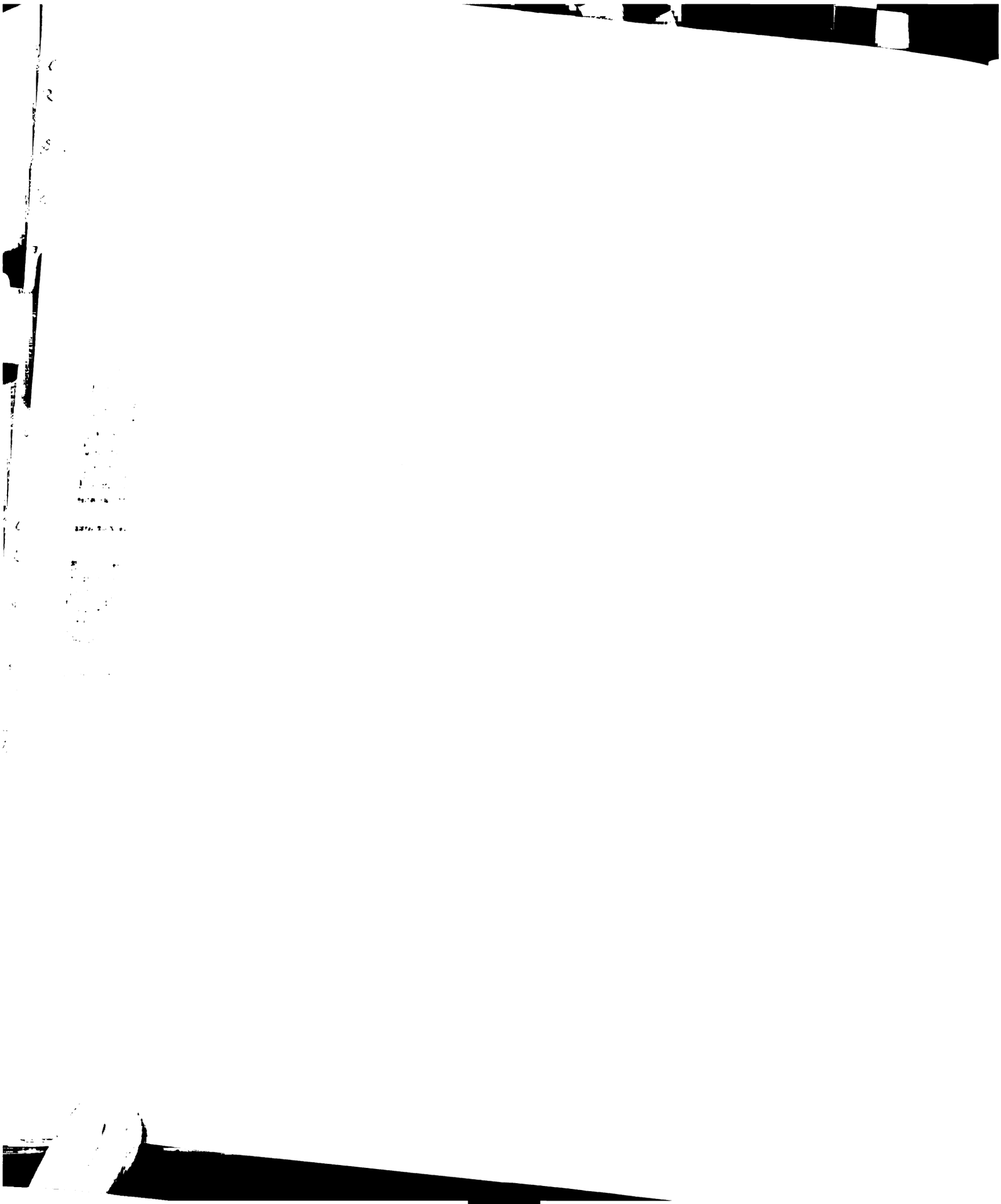


Figure 10a-b - Absolute differences (95% confidence intervals) between maximum and minimum levels of serum (a.) and salivary (b.) Beta estradiol within individuals in the disease (N=37) and non-disease (N=40) groups (serum P=.12 and saliva P=.64).



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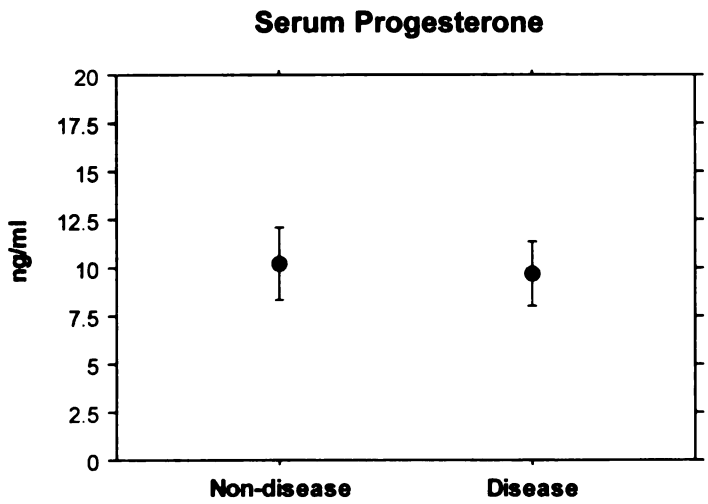

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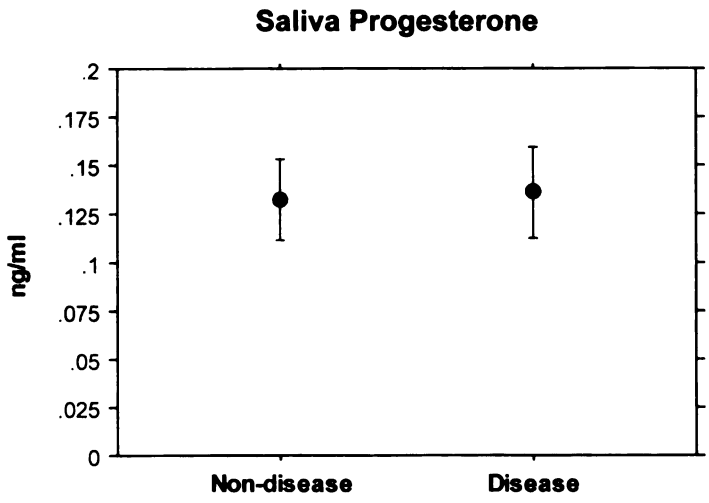
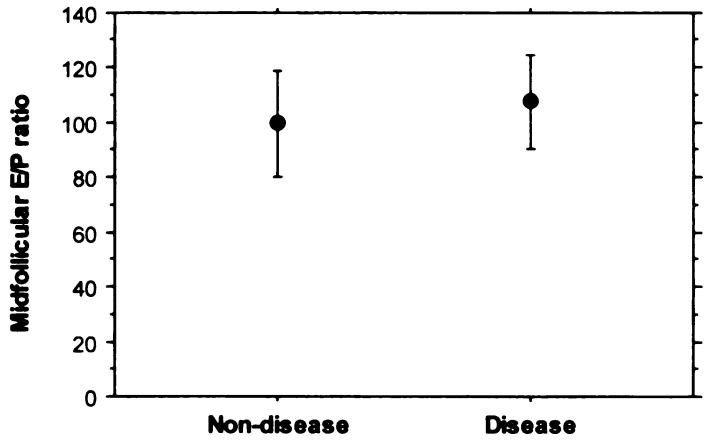


Figure 11a-b - Absolute differences (95% confidence intervals) between maximum and minimum levels of serum (a.) and salivary (b.) progesterone within individuals in the disease (N=37) and non-disease (N=40) groups (serum P=.64 and saliva P=.79).

Since the relative levels of hormones may impact TMJ disease by differentially modulating TMJ degradation we determined any differences in relative levels of estrogen and progesterone between the non-disease and disease groups. Figure 12 displays the estrogen to progesterone ratios (E/P) for all time points in the cycle (mf, ov-1, and ml). The diseased subjects (N=37) were found to have greater estrogen to progesterone (E/P) ratios, which were not statistically significant, at all time points (mf=.54; ov-1=.06, and ml=.14) in the ovulation cycle.

a.



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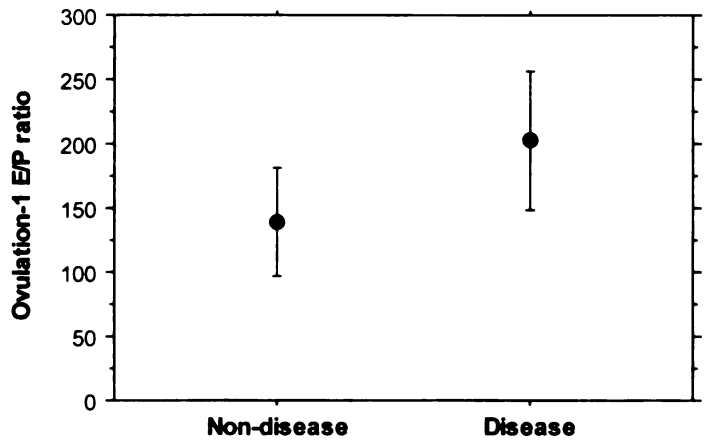

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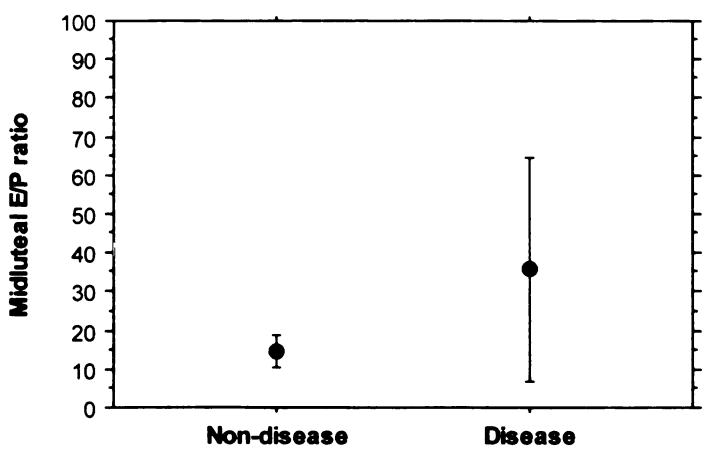


Figure 12a-c - Absolute differences (95% confidence intervals) of estrogen to progesterone ratios (E/P) between the non-disease and disease groups at the midfollicular (a.), ovulation-1 (b.), and midluteal (c.) time points

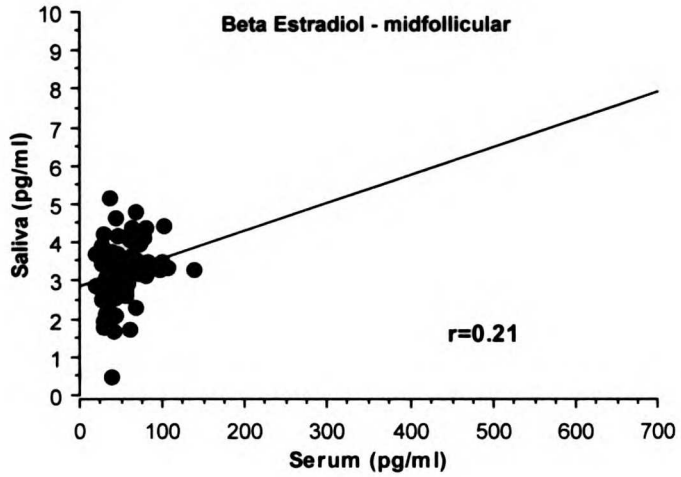
G. CORRELATIONS BETWEEN SERUM AND SALIVARY HORMONE LEVELS

The Pearson correlation coefficient (r) values in Table XXI demonstrate the relationship between serum and salivary hormone levels (Figure 13, Figure 14). Correlations were moderate to strong (r=0.69 to 0.85) for the ovulation-1 and midluteal days of collection for progesterone. There was no correlation between serum and salivary levels of β-estradiol at any of the three time-points.

Table XXII - Correlation coefficient (r) values depicting associations between serum and saliva hormone levels at the three days of sample collection.

	Beta Estradiol	Progesterone
Midfollicular	0.21	0.19
Ovulation-1	0.33	0.69
Midluteal	0.19	0.85

a.



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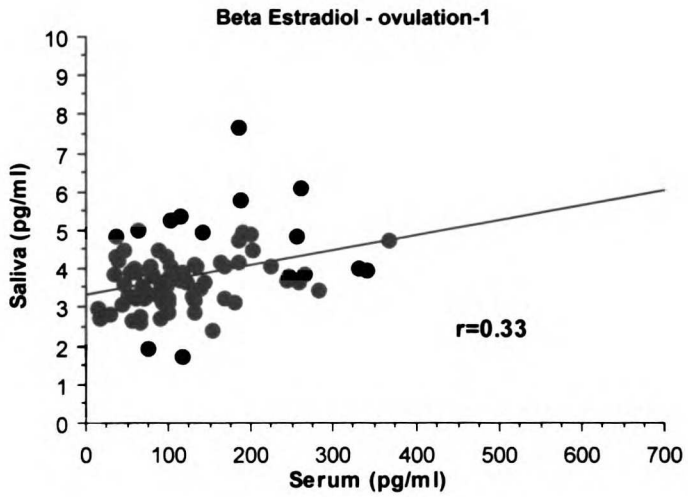

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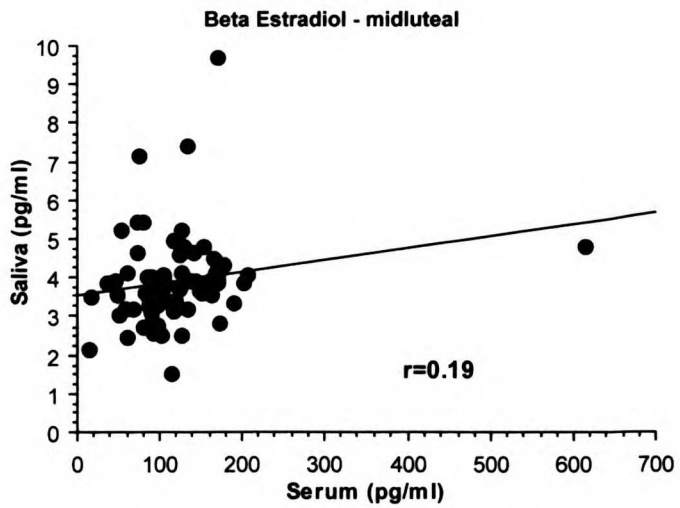
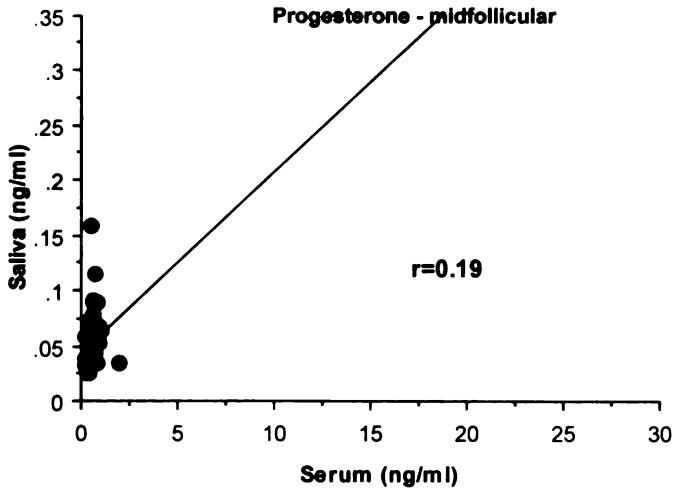


Figure 13a-c - Scatterplot showing the relationship between serum and salivary β-estradiol at midfollicular, ovulation-1, and midluteal (N=77).

a.



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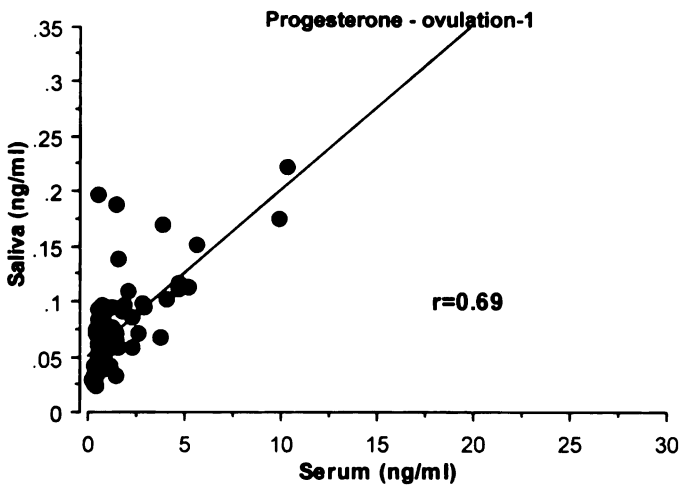


c.

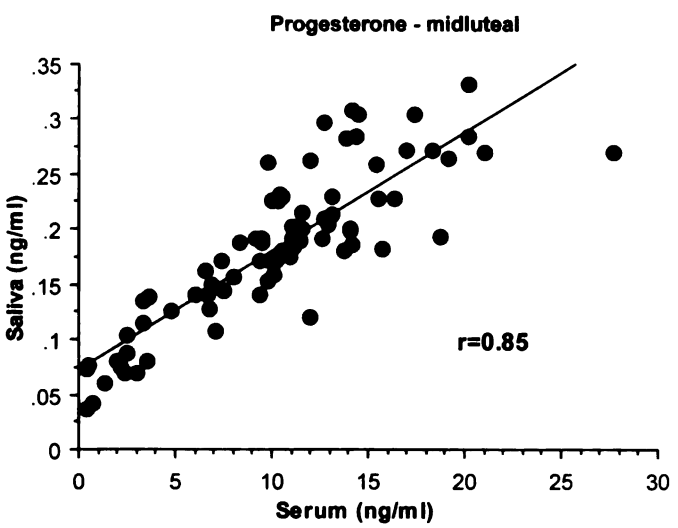
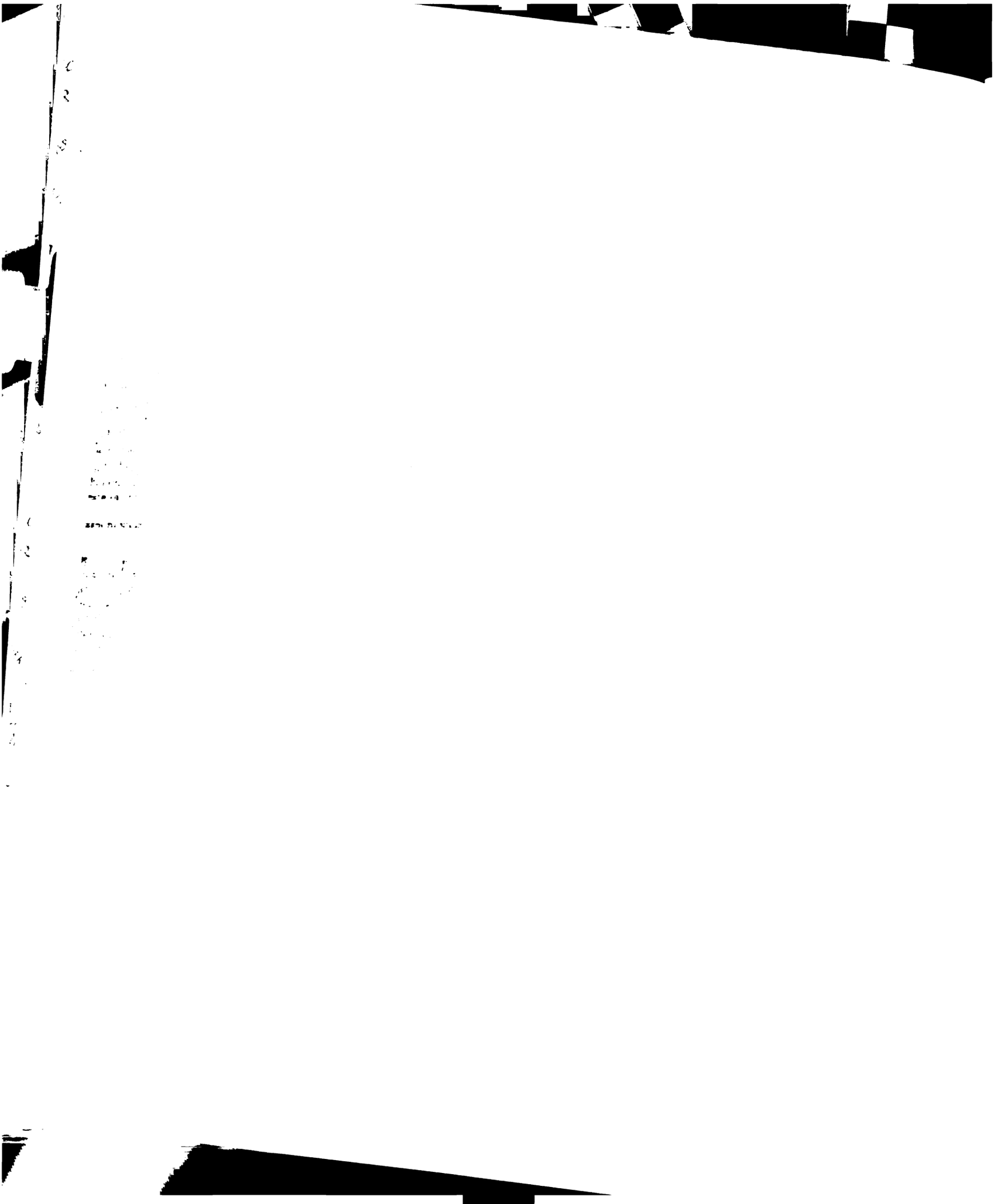


Figure 14a-c - Scatterplot showing the relationship between serum and salivary progesterone at midfollicular, ovulation-1, and midluteal (N=77).



IV. DISCUSSION

This investigation includes 97 subjects, which is one-third of the total desired sample size (290 subjects) of this larger ongoing study. It is one of the first studies to evaluate the relationship between TMJ disease, female reproductive hormones, and systemic joint hypermobility. It is also one of the first studies to determine if serum levels of β-estradiol and progesterone correlate with salivary levels of these hormones.

Division of the subjects into the disease or non-disease categories was a difficult process in the analysis phase of this study. Many subjects were described with multiple signs/symptoms of TMJ disease, which often made it difficult to place them in one of two groups only. Once the sample size of this study is expanded beyond 97, it may be possible to have more than two divisions of subjects. Eventually it may be possible to divide the subjects in the following divisions: 1) clicking, 2) crepitation, 3) TMJ pain, 4) myalgia, and 5) no signs/symptoms of TMJ disease. This way of dividing the subjects may become a more appropriate way of comparing subjects with different signs and symptoms of TMJ disease, and also to determine if hormone levels differ between these categories of TMJ symptoms.

It was also evident, when dividing the subjects (non-disease vs disease), that there was a large number of women who were diagnosed with fine crepitus (N=47). Every subject was evaluated for joint sounds by using a two-way stethoscope. Due to the large number of subjects defined with fine crepitus, it was assumed that the two-way stethoscope may have been instrumental in allowing fine crepitus to be over diagnosed. To verify the diagnosis of crepitus (coarse crepitus being obvious) all subjects diagnosed

with fine crepitus had their tomograms evaluated to support or reject the finding. All subjects with osteophytes, erosion, or moderate to severe TMJ flattening (grade 2 or 3) were considered to have a diagnosis of crepitus and we therefore included them in the disease group. Crepitus has been shown to be indicative of structural changes in the boney surfaces of the joint (DeBont et al., 1985; McNeill, 1993).

Upon evaluation of linear and lateral jaw opening, a significant difference was discovered in the non-diseased subjects who had larger maximum jaw openings. Our initial studies (Peikoff, 1996; Reed, 2000) did not find significant differences in jaw movements, but the trends are the same. In these previous studies (Peikoff, 1996; Reed, 2000) non-diseased females had an increased linear range of TMJ motion. Our current results may show increased significance due to our larger sample size (N=97). It was initially assumed that women with greater joint laxity would demonstrate an increased linear range of TMJ motion. It could be proposed that laxity in the TMJ ligament may predispose an individual to dislocation, which may result in inflammation, and subsequent limitation of jaw opening. It is also possible that presence of pain and changes in hard/soft tissue of the TMJ may limit movement in the joint as well. Although women with muscular pain (with no other signs/symptoms of TMD) were excluded from our study, 36.2% of the diseased subjects were experiencing myalgia, as well as arthralgia, upon exam. Such muscle pain may also result in reduced jaw movement. Therefore, TMJ mobility testing may not provide an accurate assessment of TMJ mobility in diseased subjects due to limited TMJ function as a result of pain, internal derangement, or muscle splinting (Westling, 1990).

A joint sound assessment found that a high percentage (70.2%) of diseased subjects displayed coarse/fine crepitus. This amount is increased compared to our previous

studies (Peikoff, 1996; Reed, 2000) which displayed a prevalence of crepitus at 57.7% and 55.0% respectively. In our study, crepitus may have been overdiagnosed due to artifact noises (movement of the ear rods in the ears) heard during movement when using the two-way stethoscope. Crepitus is demonstrative of structural boney changes of the joint (McNeill, 1993) which coincides with the radiographic findings in our study. The majority (78.7%) of diseased subjects were diagnosed with osteoarthritis or osteoarthrosis and exhibited flattening, erosion, or osteophyte formation of the condyle. These radiographic changes are considered to be abnormal and may tend to progress until the surface area of the bones within the joint increases, thereby being able to dissipate the increased forces (Ogus and Toller, 1986).

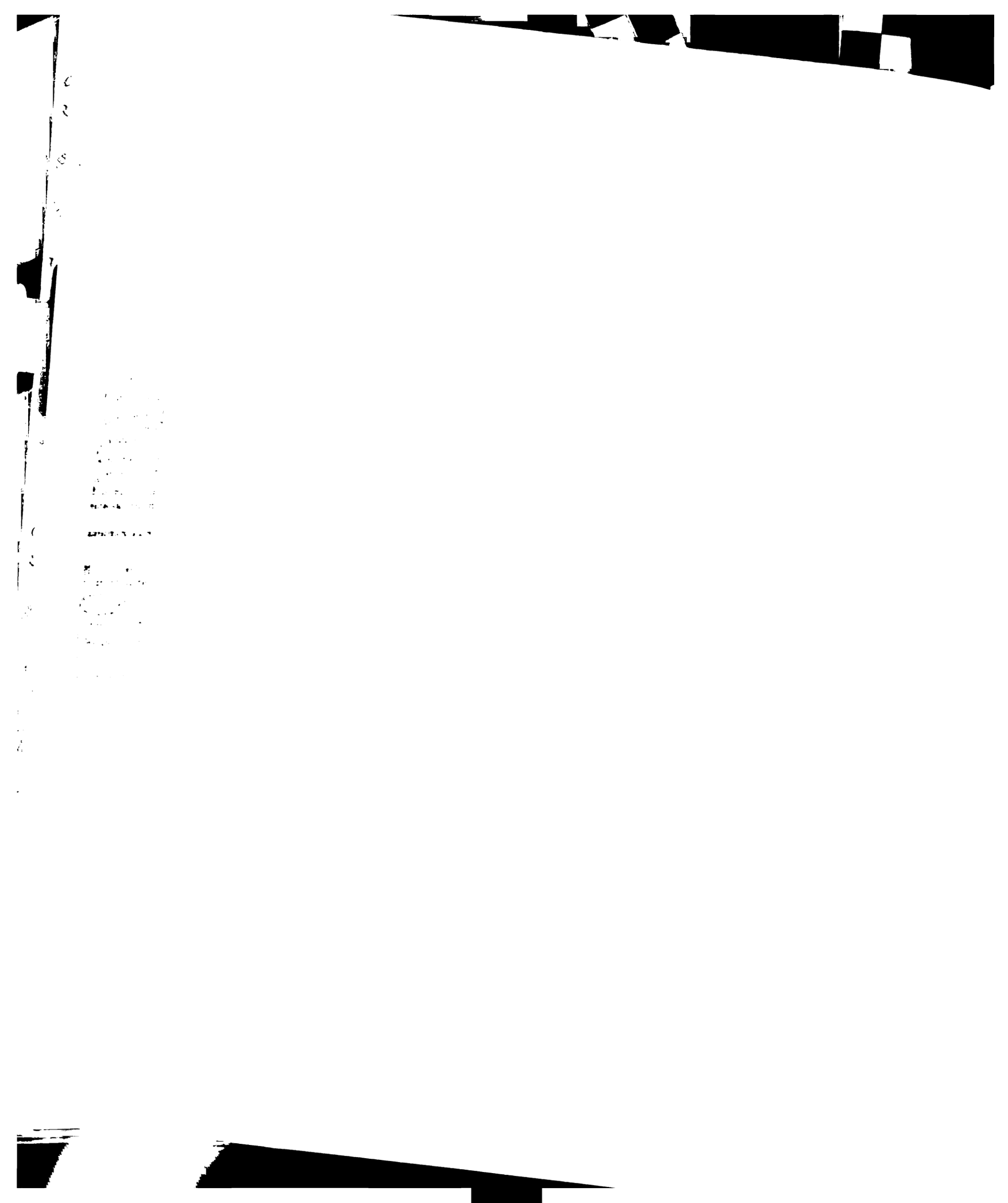
31.3% of the non-diseased subjects displayed no joint sounds, but a larger proportion (45.8%) displayed fine crepitus (with mild boney changes) and 18.7% displayed opening or closing clicks. Our finding of 18.7% clicking for non-diseased subjects is much lower than other studies. It was reported that nearly 60% of the population have some form of simple click, without the presence of other signs or symptoms (Rugh, 1985; Bell, 1992; Tallents, 1993). The majority of women in the non-diseased group demonstrated flattening (60.4%) and sclerosis (33.3%). Over half of the non-diseased subjects displayed flattening, but the median score was a 1 (mild flattening), compared to the flattening group of diseased subjects who had a median score of 2 (moderate flattening). It would be understandable that one might assume that the diseased group would show significantly greater radiographic evidence of boney remodeling than the non-diseased group. However, both cadaver and radiographic studies show that boney changes occur in a large percentage of the non-diseased population as well (Lubsen et al, 1985; Solberg et al., 1985). Our study confirms this finding.

Several factors may restrict the systemic laxity measured in a joint. The dominant side of an individual is one factor that demonstrates more muscular tone and less laxity as a result (Westling, 1993). History of trauma to a particular joint may also affect its flexibility. It is also thought that laxity in a joint may be dependent upon the time of day. Unfortunately it was not possible to control the time of day in which the TMJ/hypermobility exams were going to take place. The issue with injury and dominant side usage was dealt with by taking all joint hypermobility measurements bilaterally. The value of the most flexible joint was used for the analysis. Our study found no significant differences when comparing the flexibility of the disease and non-disease subjects. Our study showed different trends compared with our previous studies (Peikoff, 1996; Reed, 2000). These previous studies found that the diseased women demonstrated greater flexibility in all 4 joints with the thumb being statistically significant (Peikoff, 1996; Reed, 2000). Our study found the diseased subjects to be more hypermobile in the knee and the fifth digit, with neither results being significant. The non-diseased subjects were actually found to have greater flexibility, but not statistically significant, in the thumb and elbow. The diseased group had a higher total composite score, but this was not statistically significant either.

Upon further analysis of the subjects in this study it was noted that only 9 (5=disease, and 4=non-disease) subjects of the 97 had a total composite score for hypermobility of 5 or greater. The total hypermobility score is usually described in the literature as being mild (0-2), moderate (3-4), and severe (5-8) hypermobility (Grahame et al., 1981; Finsterbush and Pogrund, 1982). Our study had a small number of subjects with severe systemic hypermobility (9 of 97). Other studies have described being able to acquire 40% subjects with severe (score 5-8) hypermobility for comparison (Grahame et al.,

1981). The small number of subjects with this excessive generalized laxity could be one explanation for our differing and non-significant results.

Two ovulation kits were used by each subject to determine the ovulation day minus 24 hours in the first cycle and to confirm the consistency of this day, and therefore the accuracy of sample collections in their second cycle. The three visits for serum and saliva collection (second cycle) were calculated from the length of the subject's cycle, as well as from the information acquired (ov-1) from use of the first kit. Poor compliance with the ovulation kit, or inaccurate recording/recall of the first, ovulation-1, and last day of the subject's menstrual cycle could easily result in the improper prediction of days for serum/saliva collections, leading to variations in hormone levels. By using the second ovulation kit it was easily determined whether the subject had actually had her blood/saliva collected at the correct time in her (second) cycle. The results show that the mean peak in β-estradiol and progesterone for diseased and non-diseased women were similar to what would be expected, based on prior studies that have tracked the levels of these hormones over the course of the average woman's menstrual cycle (Schauf and Moffett, 1990). The mean peak in both of these hormones was also seen to be similar between the current and our initial studies (Reed, 2000). The mean levels of estrogen at the ovulation-1 and midluteal timepoints, although not statistically significant, were observed to be higher in the diseased subjects. The non-diseased subjects had higher mean levels of progesterone at all time points, with the baseline values (mf) being statistically significant, except after Bonferroni adjustments. It could be hypothesized on the basis of our laboratory studies(Kapila and Xie, 1998; Kapila and Gashem; unpublished data) that baseline progesterone levels have a protective effect and potentially minimize the negative affects that estrogen may have on the degradation of



joint tissues. This hypothesis will be tested as the current studies proceed with increasing the sample size.

Correlation of the serum and salivary levels of these hormones was also determined. A strong correlation between saliva and serum would suggest that saliva (less invasive and easier to collect) would be a satisfactory alternative for determining systemic levels of these hormones. The correlations for progesterone were moderately strong for the ovulation-1 and midluteal time points (r=.69 and .85). There was no correlation between serum and salivary levels of estrogen at all time points (mf, ov-1, and ml). This finding is almost identical with the findings from our initial studies (Reed, 2000). Other studies have shown salivary and serum estrogen and progesterone to correlate significantly with each other (Ellison, 1991; Hardiman and Thomas, 1990). The lack of correlation in estrogen saliva and serum may have resulted from the methods used to process the saliva in which the mucoid portion with any bound estrogen is discarded.

An interesting and statistically significant finding in this study was that women who reported having at least one previous pregnancy were about 4 times as likely to be diseased than non-diseased. These findings are in agreement with our previous studies (Peikoff, 1996). Throughout pregnancy, plasma concentrations of estrogen and progesterone remain high (Vander et al., 1994). It may be speculated that a woman who experiences these high levels of hormones may be at a greater risk for TMJ disease in the future.

There are several interesting trends that may be investigated with the continuation of this study. In addition to expanding upon our current findings, we will continue to analyze the associations among systemic joint hypermobility, range of motion, joint sounds,

radiographic findings and hormone levels. We also intend to analyze whether specific symptoms of TMJ disease show variations in hormone levels when compared to each other and to non-disease controls.

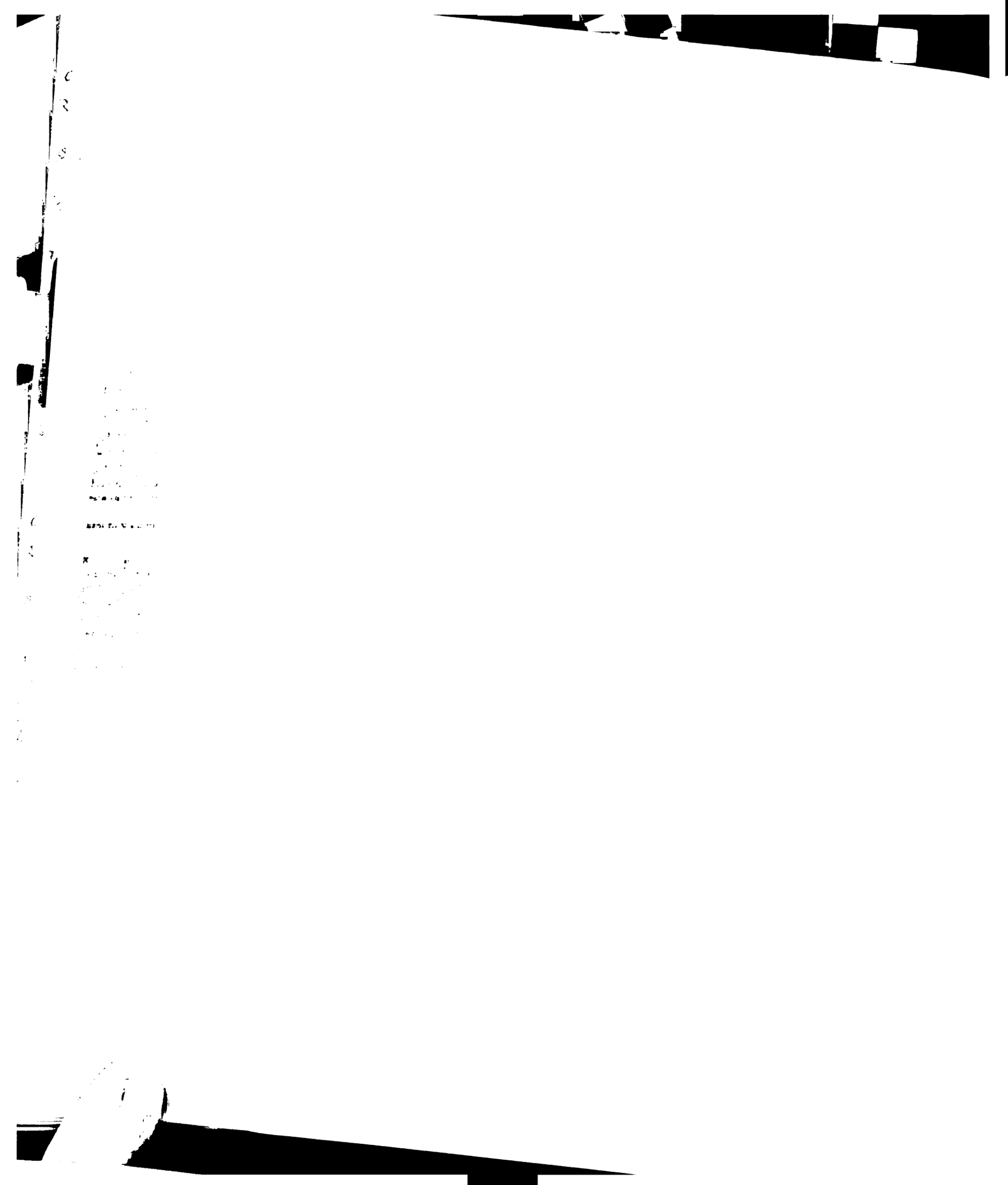
V. CONCLUSIONS

This investigation is the initial component of an ongoing study that will eventually recruit approximately 290 subjects. Despite the various limitations of a study of this nature, several conclusions were drawn from this investigation:

1) The non-diseased subjects had significantly larger maximum jaw opening than the diseased group.
2) There was no difference in systemic joint hypermobility between the disease and non-disease groups.
3) The non-diseased subjects had statistically significant higher levels of baseline (mf) progesterone. This statistical significance was lost after Bonferroni adjustments. Although not statistically significant, the diseased subjects demonstrated higher levels of β-estradiol at ovulation-1 and midluteal time points. Also, the E/P ratio was higher, although no statistically, in disease vs non-disease subjects.
4) Correlations between serum and salivary hormone levels were moderately strong for progesterone at the ovulation-1 and midluteal time points.
5) Women who reported a previous pregnancy were about 4 times more likely to have TMJ disease than women with no TMJ disease.

The findings gathered from this study will be expanded upon as the study continues. We anticipate that one day this information may shed more definitive light on the etiology and pathogenesis of some types of TMJ diseases, thus enabling clinicians to provide

more accurate diagnoses, treatments, and even preventive measures for people suffering from or predisposed to these diseases.



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 of the baboon: An autoradiographic study. Oral Surg Oral Med Oral Pathol 61:307-14, 1986.

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

Bates, R.E. Jr., Stewart, C., Atkinson, W.: The relationship between internal derangements of the temporomandibular joint and systemic joint laxity. J Am Dent Assoc 109:446-7, 1984.

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.

Bird, H.A., Tribe, C.R., Bacon, P.A.: Joint hypermobility leading to osteoarthrosis and chondrocalcinosis. Ann Rheum Dis 37:203-11, 1978.

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.: Human relaxins: chemistry and biology. Endocrine Reviews 15:5-26, 1994.

Burman, M., Sinberg, S.E.: Condylar movement in the study of internal derangement of the temporomandibular joint. J Bone Joint Surg 28:351-373, 1946.

Cachiotti, D., Plesh, O., Bianchi, P., McNeill, C.: Signs and symptoms in samples with and without temporomandibular disorders. J Craniomand Disord Facial Oral Pain 5:167-172, 1991.

Calguneri, M.: Changes in joint laxity occurring during pregnancy. Ann Rheum Dis 41: 126-8, 1982.

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.

Carraro, J.J., Caffesse, R.G., Albano, E.A.: Temporomandibular joint syndrome. Oral Surg Oral Med Oral Pathol 28:54-62, 1969.

Castanada, R., McNeill, C.: Biomechanics in TMJ osteoarthritis. Part II. J Dent Res 68(special issue): abstr 620, 1989.

Chander, C.L., Spector, T.D.: Oestrogens, joint disease and cartilage. Ann Rheum Dis 50:139-40, 1991.

Cohen, S.: Follow-up evaluation of 105 patients wit 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, one 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.

Dijkstra, P.U., Kropmans, T.J.B., Stegenga, B.: The Association between Generalized Joint Hypermobility and Temporomandibular Joint Disorders: A Systematic Review. Journal of Dental Research 81(3):158-163, 2002.

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., 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.

Ericson, S., Lundberg, M.: Structural changes in the finger, wrist and temporomandibular joint. A comparative radiologic study. Acta Odontolog Scand 26:111-126, 1968.

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.

Finsterbush, A., Pogrund, H.: The hypermobility syndrome: Musculoskeletal complaints in 100 consecutive cases of generalized joint hypermobility. Clin Orthop 168:124-7, 1982.

Foged, J.: Temporomandibular arthrosis. Lancet 2:1209-11, 1949.

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

Franks, A.S.T.: The social character of temporomandibular joint dysfunction. Dent Pract 15:94-100, 1964.

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.

Grahame, R.: A clinical and echocardiographic study of patients with the hypermobility syndrome. Ann Rheum Dis 40:541-546, 1981.

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

Hatch, J.P., Rugh, J.D., Sakai, S., Saunders, M.S.: Is use of exogenous estrogen associated with TMD signs and symptoms? J Am Dent Assoc 132(3):319-26, 2001.

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.

Isaacson, G., Linde, C., Isberg, A.: Subjective symptoms in patients with temporomandibular joint disc displacement versus patients with myogenic craniomandibular disorders. J Prosthet Dent 61:70-77, 1989.

Isberg-Holm, A.M., Westesson, P.: Movement of the disc 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.

Kirk, J.A.: The hypermobility syndrome: musculoskeletal complaints associated with generalized joint hypermobility. Ann Rheum Dis 26:419-25, 1967.

Kruse, I.: The jaw dysfunction syndrome. Odontol Tidskr 73:125-7, 1965.

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.

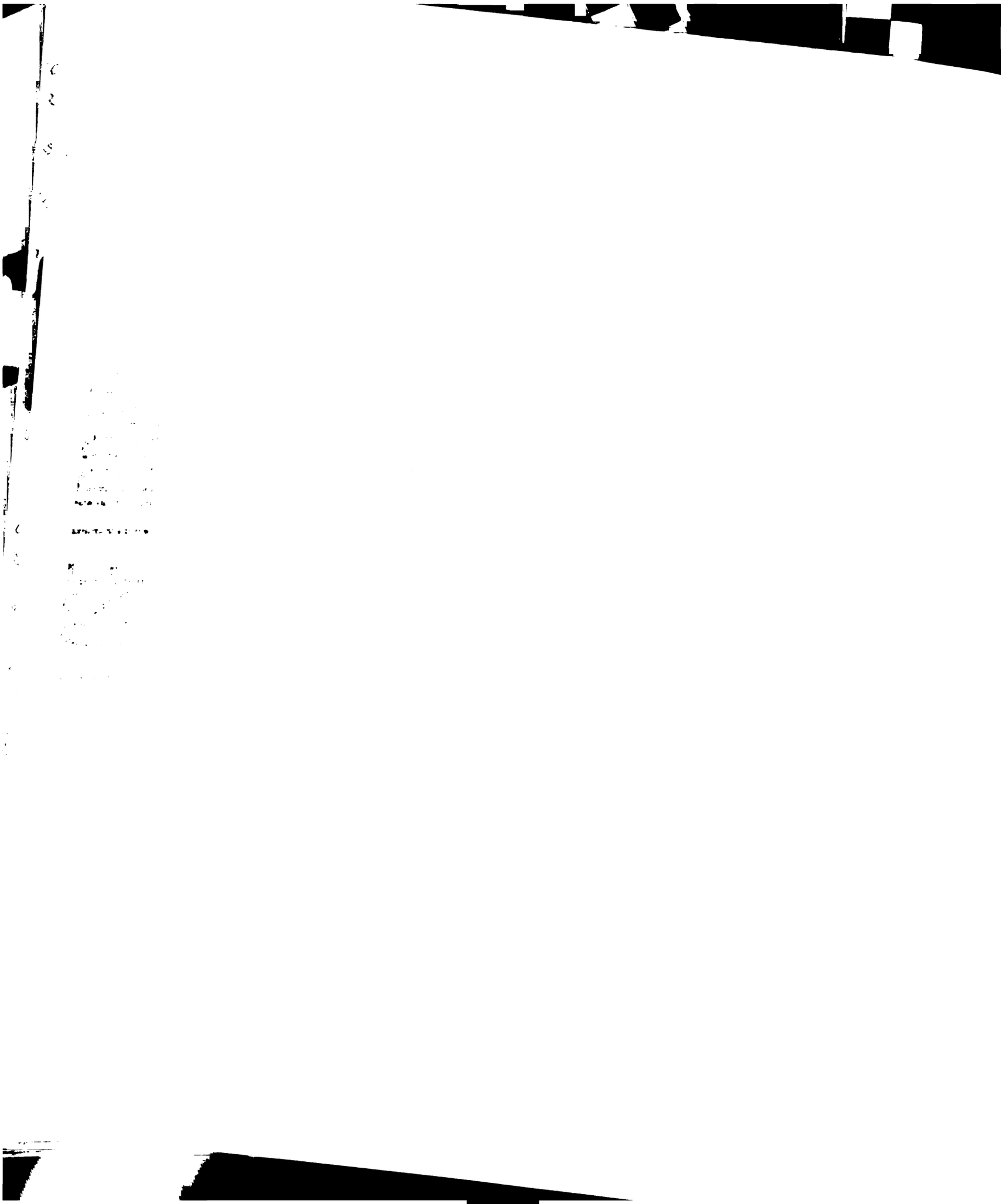
Lieberman, M.A., Gazit, E., Fuchs, C.: Mandibular dysfunction in 10-18 year old school children as related to morphologic malocclusion. J Oral Rehabil 12:209-214, 1985.

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 hormones relaxin in human reproduction and pelvic girdle relaxation. Scand J Rheumatol 88:7-15, 1991.

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

McCreary, C.P.: Psychological distress and diagnostic subgroups of temporomandibular disorder patients. Pain 44:29-34, 1991.



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

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.

Perry, H.T.: The symptomatology of the temporomandibular joint disturbance. J Prosthet Dent 19:288-298, 1968.

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.

Rasmussen, O.C.: Description of population and progress of symptoms in longitudinal study of temporomandibular arthropathy. Scand J Dent Res 89:196-203, 1981.

Raustia, A.M. et al: Temporomandibular joint findings obtained by brain MRI. Cranio 12:28-32, 1994.

Reed, T.: The role of female reproductive hormones in the etiopathogenesis of TMJ disorders. Masters Thesis, 2000.

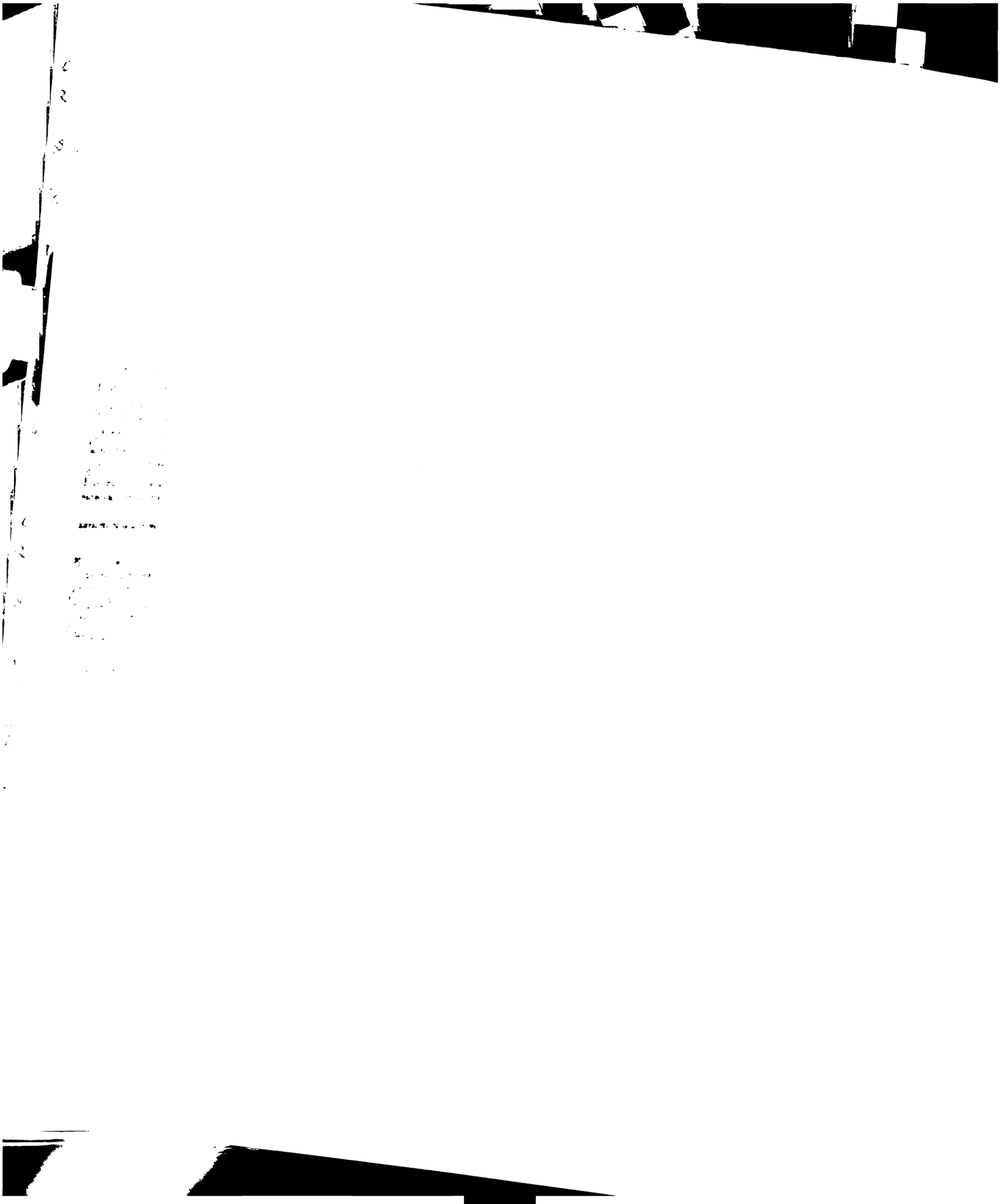
Reider, C.E.: The prevalence of mandibular dysfunction, Part I: Sex and age distribution of related signs and symptoms. J Prosthet Dent 50:81-88, 1983.

Roberts, C.A., Tallents, R.H., Katzberg, R.W.: Comparison of internal derangements of the TMJ with occlusal findings. Oral Surg Oral Med Oral Pathol 60:244-251, 1985.

Roth, R.: Temporomandibular pain dysfunction and occlusal relationships. Angle Orthod 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.

Runge, M.E., Sadowsky, C., Sakols, E.L.: The relationship between temporomandibular joint sounds and malocclusion. Am J Orthod 96:36-42, 1989.



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.

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

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.

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

Schwartz, L., Cobin, H.P.: Symptoms associated with the temporomandibular joint. Oral Surg Oral Med Oral Pathol 10:339-44, 1957.

Scott, D., Bird, H., Wright, V.: Joint laxity leading to osteoarthrosis. Rheum Rehabil 18:167-9, 1979.

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.

Solberg, W.K.: Temporomandibular disorders: Clinical significance of TMJ changes. Br Dent Jr 160:231-236.

Stegenga, B., de Bont, L.G.M., Boering, G.: A proposed classification of temporomandibular disorders based on synovial joint pathology. J Craniomand Pract 7:107-118, 1989.

Stegenga, B., de Bont, L.G.M., Boering, G.: Tissue responses to degenerative changes in the temporomandibular joint: A review. J Oral Maxillofac Surg 49:1079-1088, 1991.

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

Vander, A.J., Sherman, J.H., Luciano, D.S.: Human Physiology; The Mechanisms of Body Function. McGraw-Hill, Inc. 1994.

Westling, L.: Background factors in craniomandibular disorders: With special reference To general joint hypermobility, parafunction and trauma. J Craniomand Disord 4:89-98, 1990.

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

Westling, L.: Temporomandibular joint dysfunction and systemic joint laxity. Swed Dent J (Suppl) 81:1-79, 1993.

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

Wilkes, C.H.: Internal derangements of the temporomandibular joint. Arch Otolargngol Head Neck Surg 115:469-77, 1989.

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

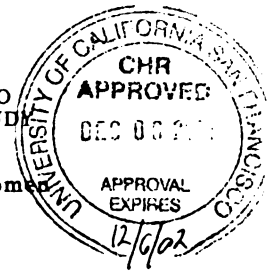
$100

FOR PARTICIPATION IN THE STUDY

If interested please contact Dr. Kristen Miller at [redacted]

APPENDIX 2 – CONSENT FORM

APPENDIX D



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., Dr. Kristen Miller, D.D.S. and Dr. Kristy Chung, D.M.D., 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. I will be required to time the beginning of my second and third menstrual cycles and repeat the ovulation test again in the following month. If the timing of my first and second cycles do not match, I may be asked to maintain records of my ovulation date and first day of the cycle for an additional month. 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, and estradiol) which are suspected of being involved in the cause of temporomandibular joint disease.

6. I may be withdrawn from the study without my consent if the researchers believe it is in my best interest, or if I fail to follow study procedures.

Participation in this study will take a total of about 4 hours 45 minutes over a period of 8 weeks. In instances with mismatched first and second cycles the period of the study will be approximately 12 weeks. All study procedures will be done at the Medical Center at U.C. San Francisco.

C. RISKS/DISCOMFORTS

1. Venipuncture: The risks of drawing blood include temporary discomfort from the needle stick, bruising and rarely, infection.

2. Discomfort/ pain: Subjects with TMJ diseases may experience some pain or discomfort during and following their TMJ examination.

3. Confidentiality: Participation in research may involve a loss of privacy, but information about me will be handled as confidentially as possible. My name will not be used in any published reports about this study.

4. X-rays: The jaw joint X-rays have minimal radiation exposure with no known adverse effects.

APPENDIX D

Treatment and Compensation for Injury:

If I am injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, I may call the office of the Committee on Human Research at (415) 476-1814.

D. BENEFITS

I will not directly benefit from participating in this study. It is hoped that the information gained from this study will help the investigators learn more about the cause of jaw joint problems in women, which may improve the treatments available.

E. COSTS

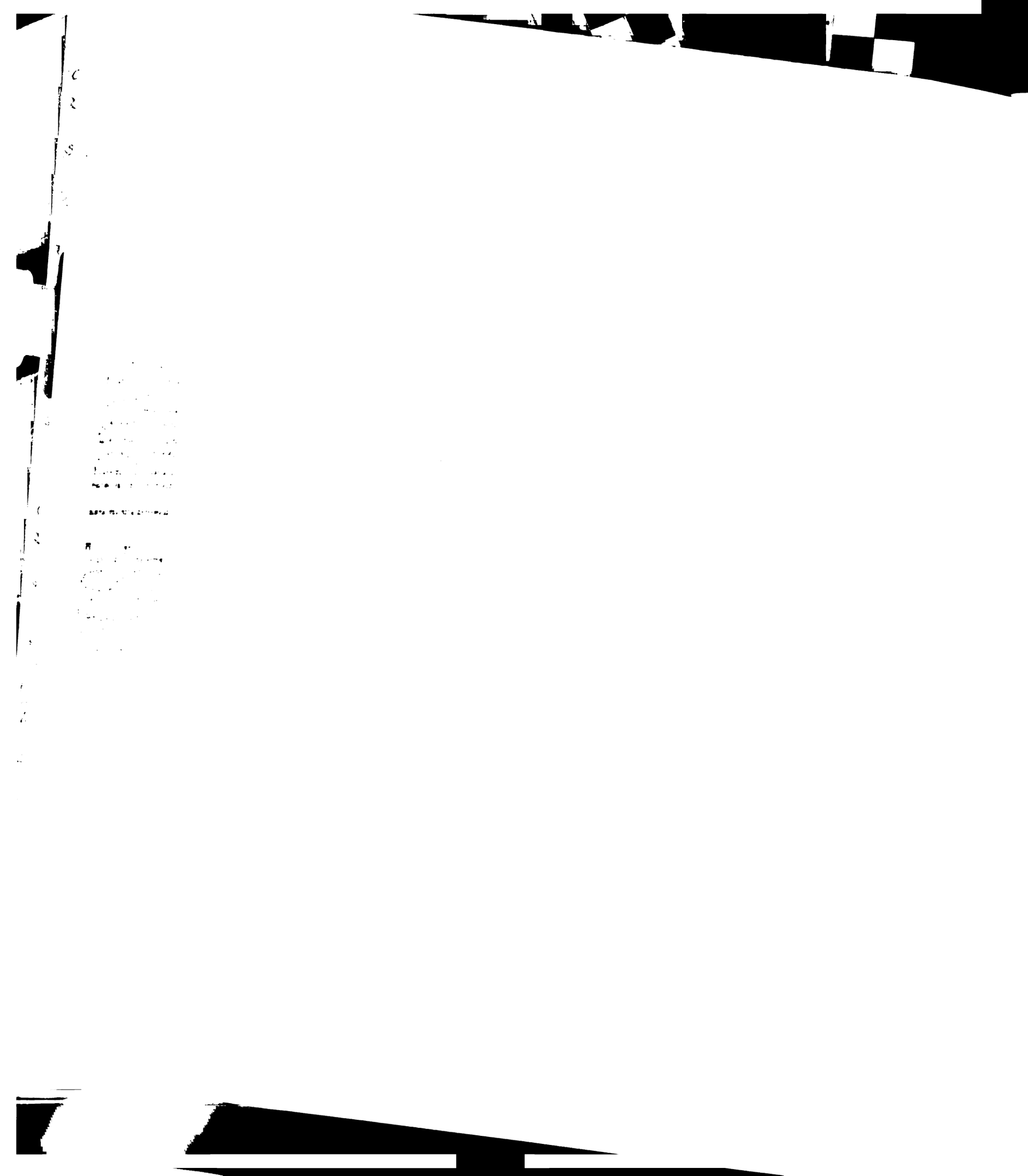
I will not be charged for any of the study treatments or procedures.

F. REIMBURSEMENT

I will be reimbursed for $100.00 for participating in this study. If I am required to maintain an additional month of timing of my cycle, I will be reimbursed $130.00. However, I will be reimbursed the appropriate 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. Miller or Dr. Chung and my questions were answered. If I have any other questions about the study, I may call Dr. Miller at (415) 476-6100 (ext: 53587) or Dr. Chung at (415) 476-6100 (ext: 50876) or Dr. Kapila at (415) 476-8401.



APPENDIX D

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 disease?
   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? ___ ___ ___ ___ ___

32. List the total number of pregnancies you have had_____

33. How many successful pregnancies have you had?_____

34. If you have or have had TMJ symptoms (joint pain, clicking, popping, or jaw locking), when did you first notice these symptoms?_____

a. Did you have TMJ symptoms **before** any pregnancy ?(yes/no)_____ If yes, which pregnancy?_____
b. Did you have TMJ symptoms **during** any pregnancy ?(yes/no)_____ If yes, during which pregnancy?_____
c. Did you have TMJ symptoms **after** any pregnancy ?(yes/no)_____ If yes, after which pregnancy?_____

35. Have you ever used birth control pills?_____

36. When was your last cycle of birth control pills (month/year)?_____ _____
month year

37. How many days is your typical menstrual cycle?_____

APPENDIX 4 – TMJ EXAM 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
   None 0
   Jaw Joint 1
   Muscles 2
   Both 3

   Left
   None 0
   Jaw Joint 1
   Muscles 2
   Both 3

[Examiner feels area 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/stethoscope)

		Right	Left
a. Opening	None	0	0
	Click	1	1
	Coarse Crepitus	2	2
	Fine Crepitus	3	3
Measurement of Opening Click — mm — mm			
b. Closing	None	0	0
	Click	1	1
	Coarse Crepitus	2	2
	Fine Crepitus	3	3
Measurement of Closing Click — mm — mm			
c. Reciprocal click eliminated on protrusive excursion	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

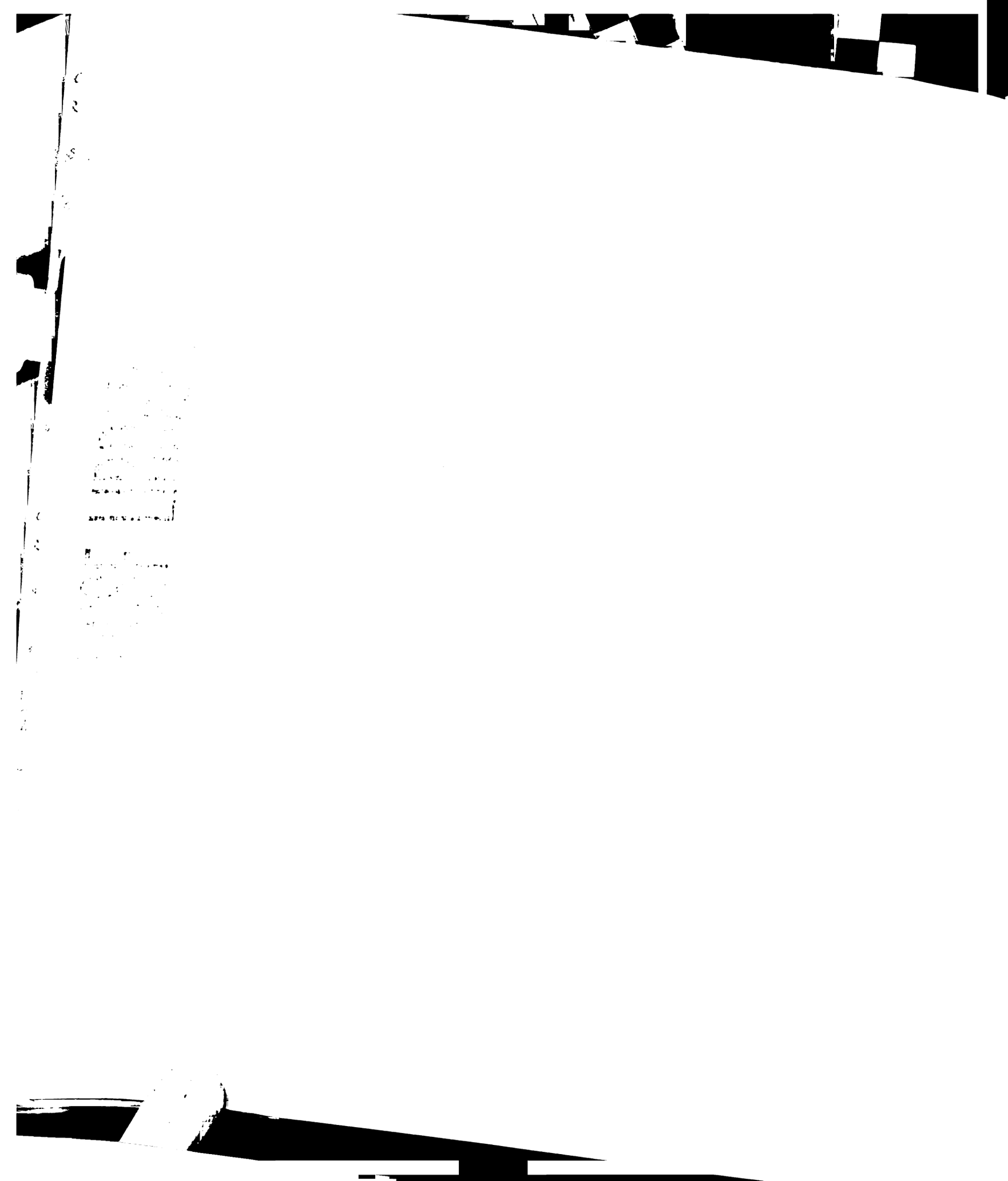
Left Sounds:	None	Click	Coarse crepitus	Fine crepitus
Excursion Right	0	1	2	3
Excursion Left	0	1	2	3
Protrusion	0	1	2	3

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)



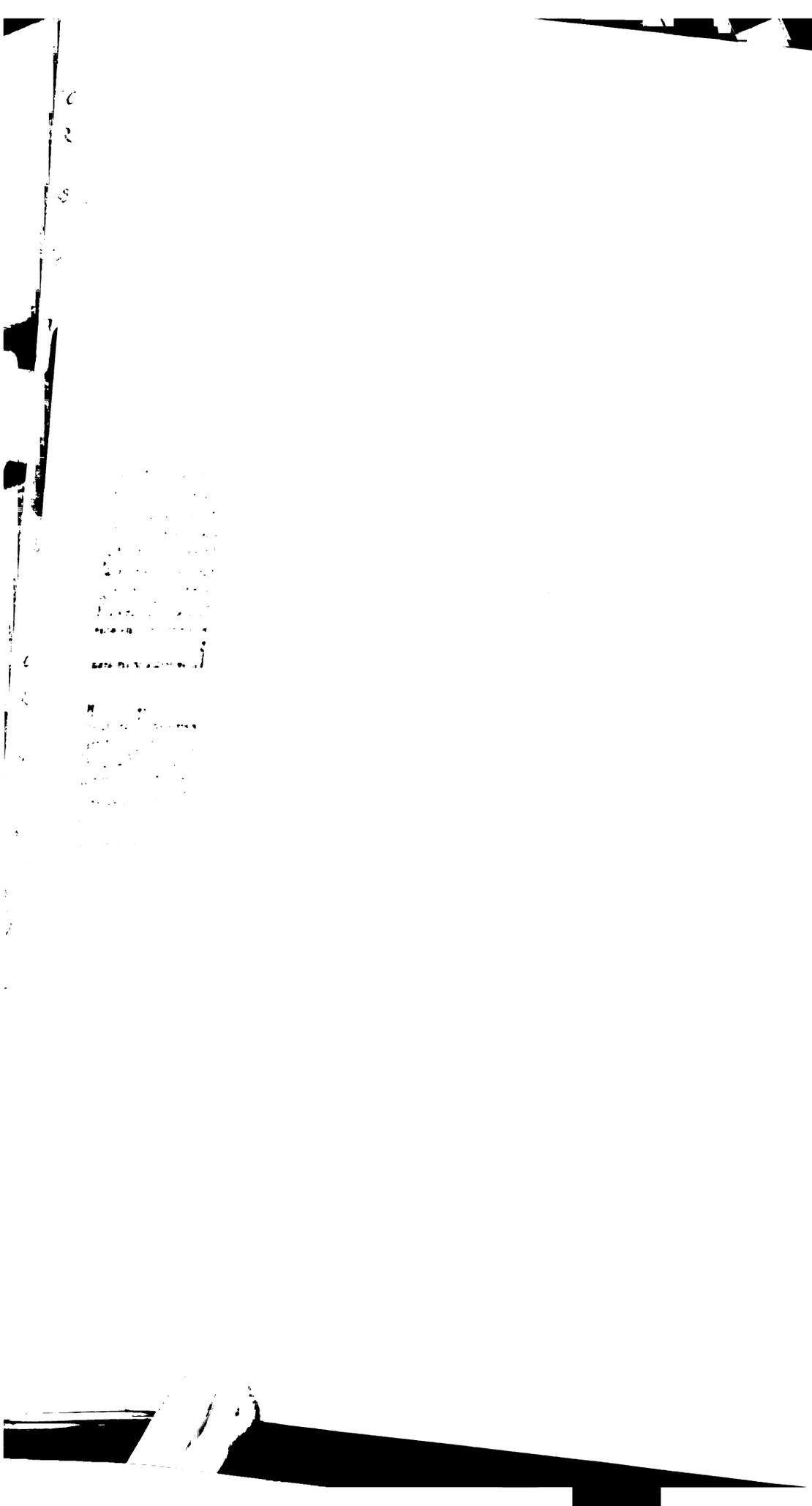
g. Posterior Mandibular Region (stylohyoid/posterior digastric region) "Jaw/throat region"	0 1 2 3	0 1 2 3
h. **Submandibular Region (medial pterygoid/suprahyoid/ anterior digastric region) "Under chin"**	0 1 2 3	0 1 2 3

9. Joint Pain With Palpation

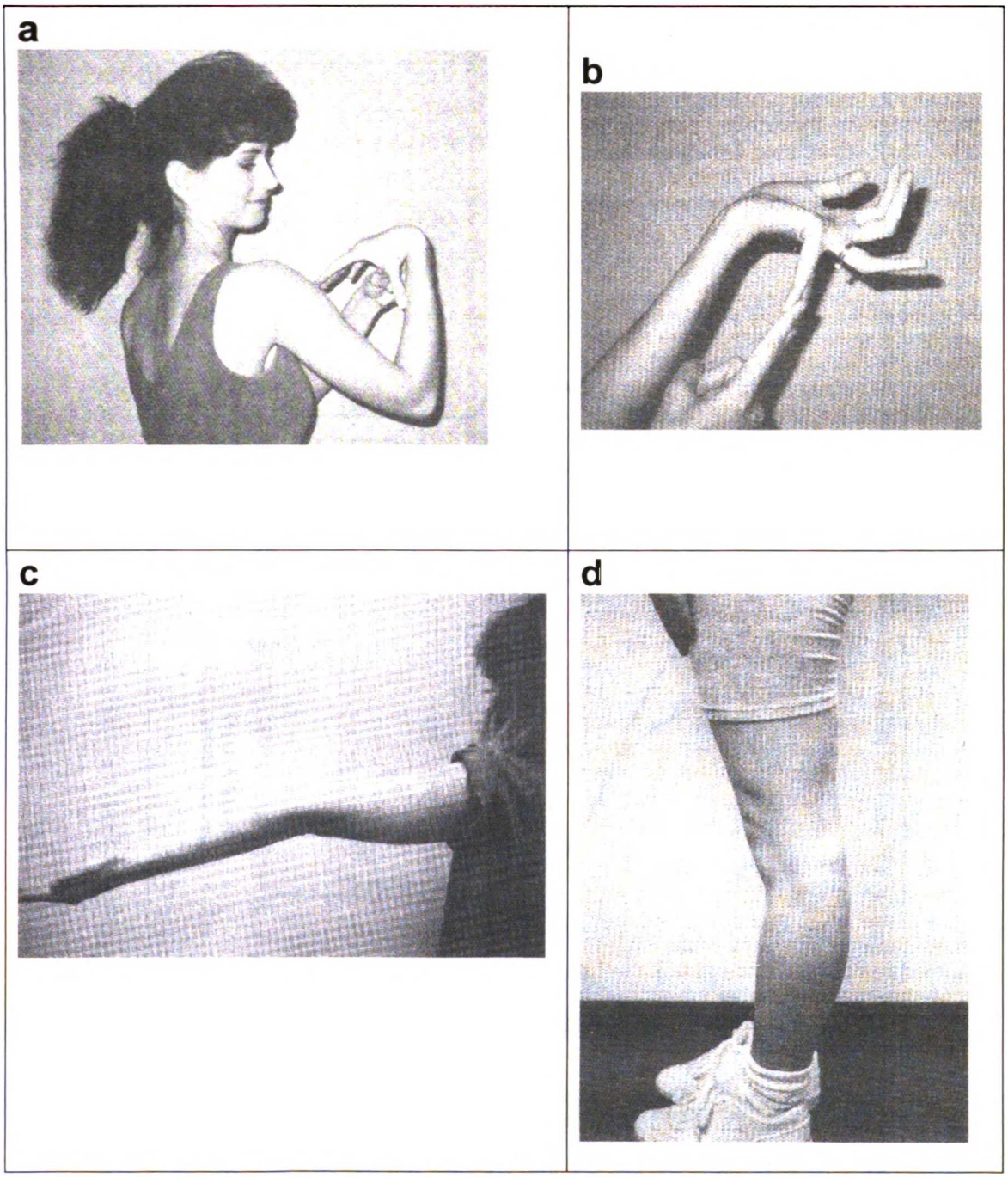
	Right	Left
a. Lateral Pole "Outside"	0 1 2 3	0 1 2 3
b. Posterior Attachment "Inside ear"	0 1 2 3	0 1 2 3

10. **Intraoral Muscle Pain With Palpation:**

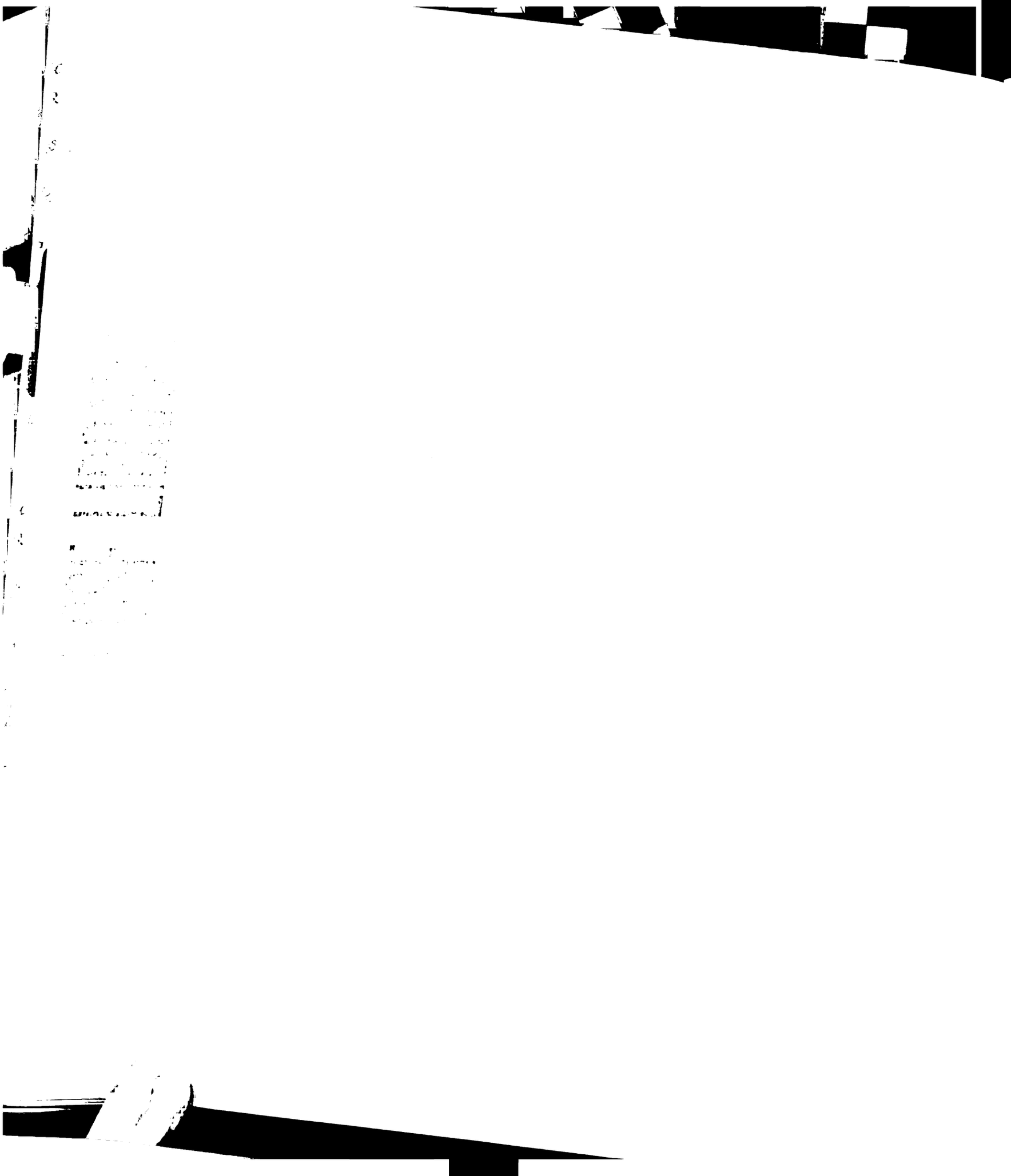
	Right	Left
a. **Lateral Pterygoid Area "Behind upper molars"**	0 1 2 3	0 1 2 3
b. **Tendon of Temporalis "Tendon"**	0 1 2 3	0 1 2 3



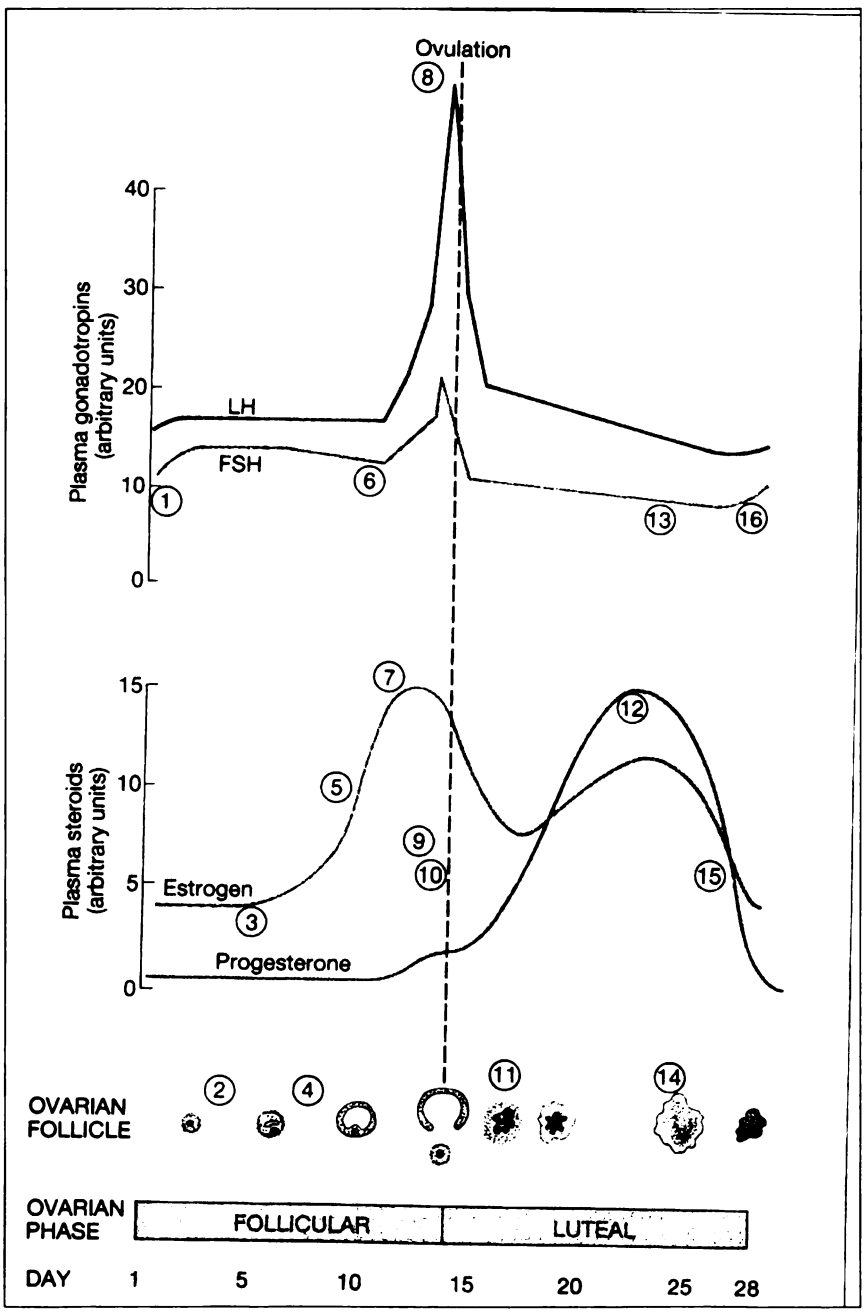
APPENDIX 5 – DEMONSTRATIONS OF JOINT HYPERMOBILITY TESTS



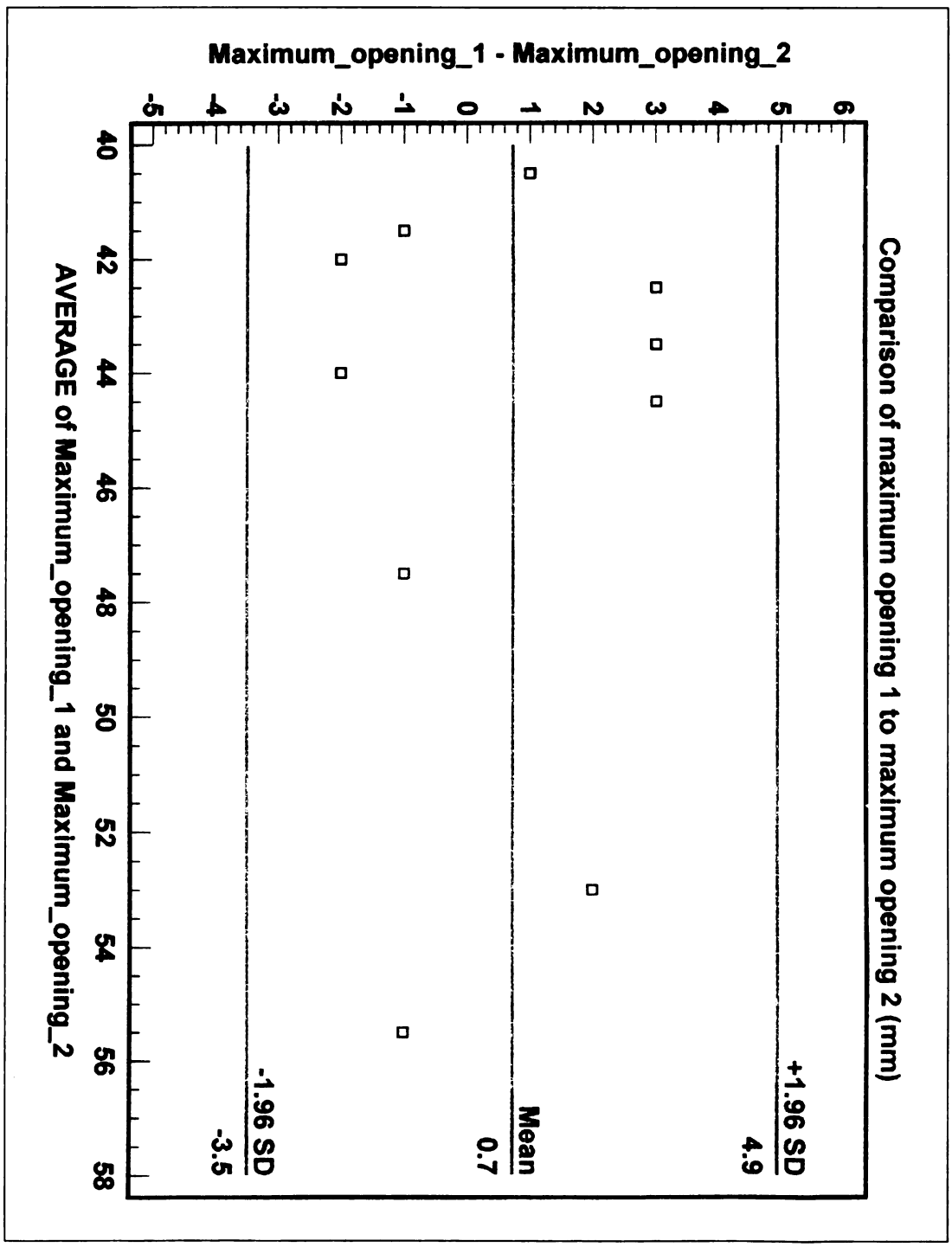
Modified from: Exercise and Total Well Being for Vertebral and Craniomandibular Disorders (Antonioth; T.; 1990; I for C Publications, pp. 16-17)

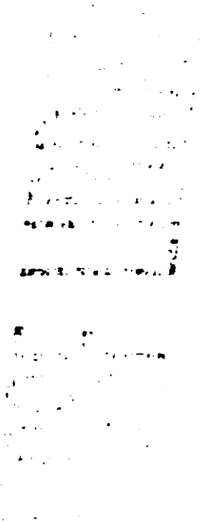


APPENDIX 6 – HORMONE PEAK LEVELS

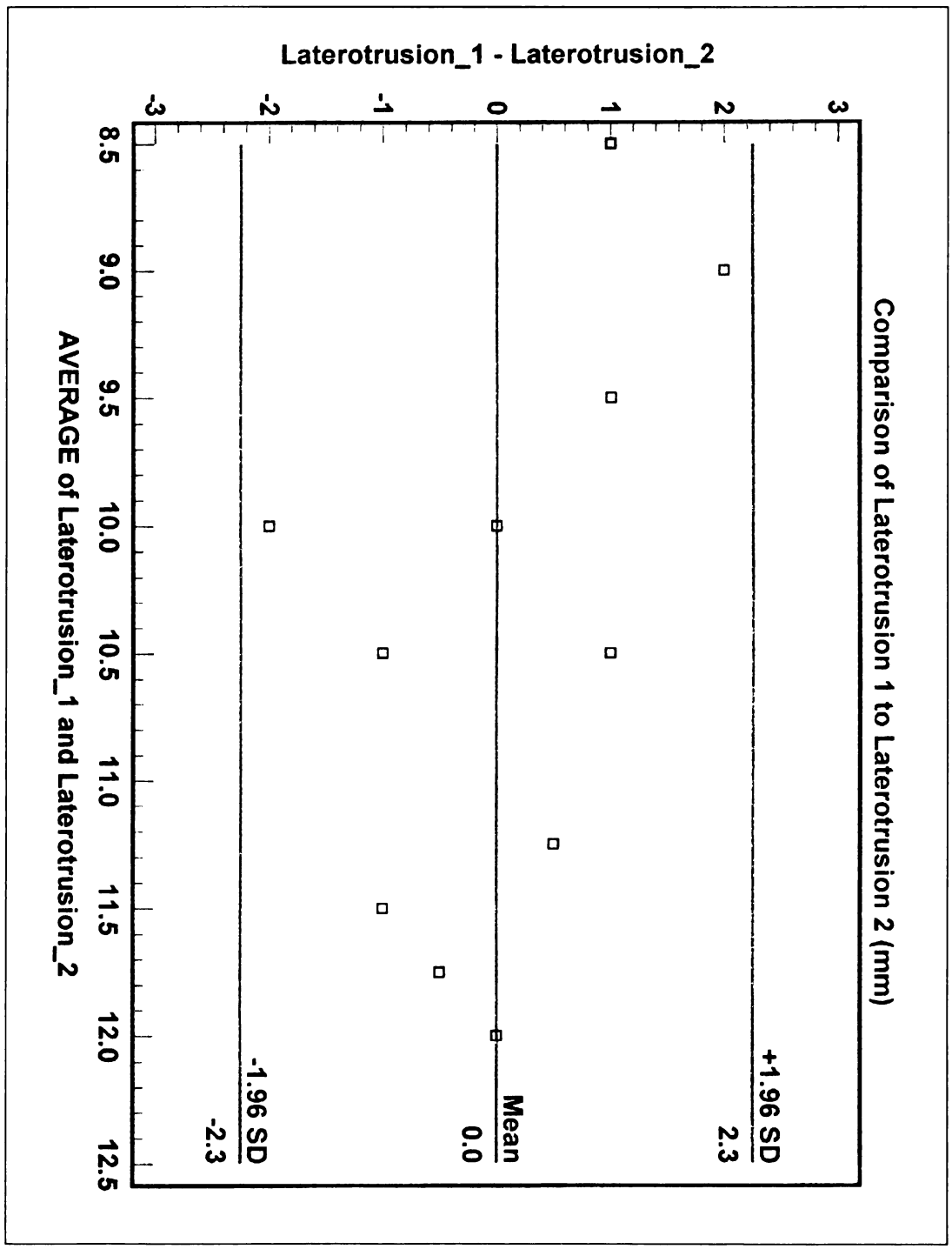


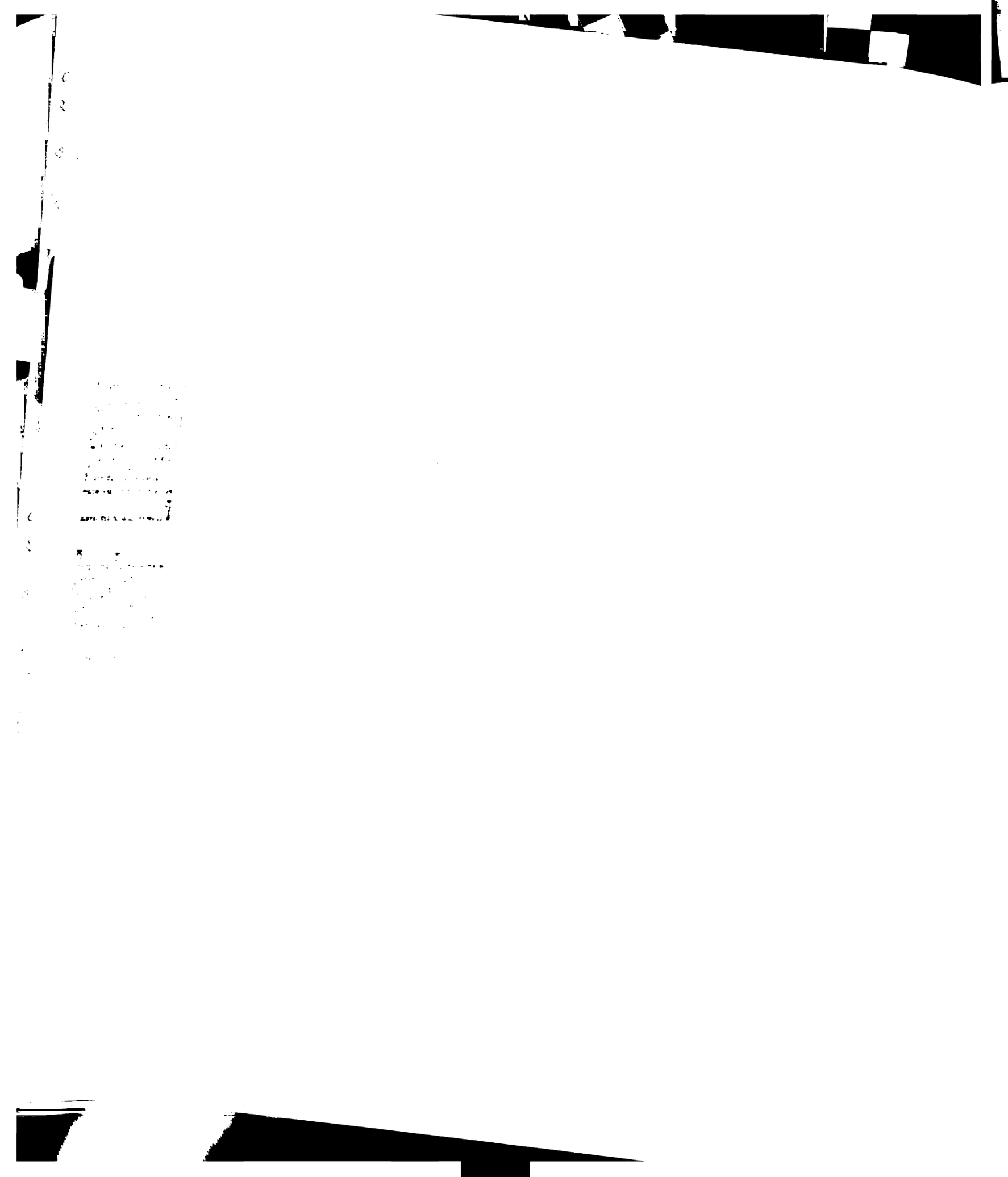
APPENDIX 7 – MAXIMUM TMJ OPENING (Bland-Altman)



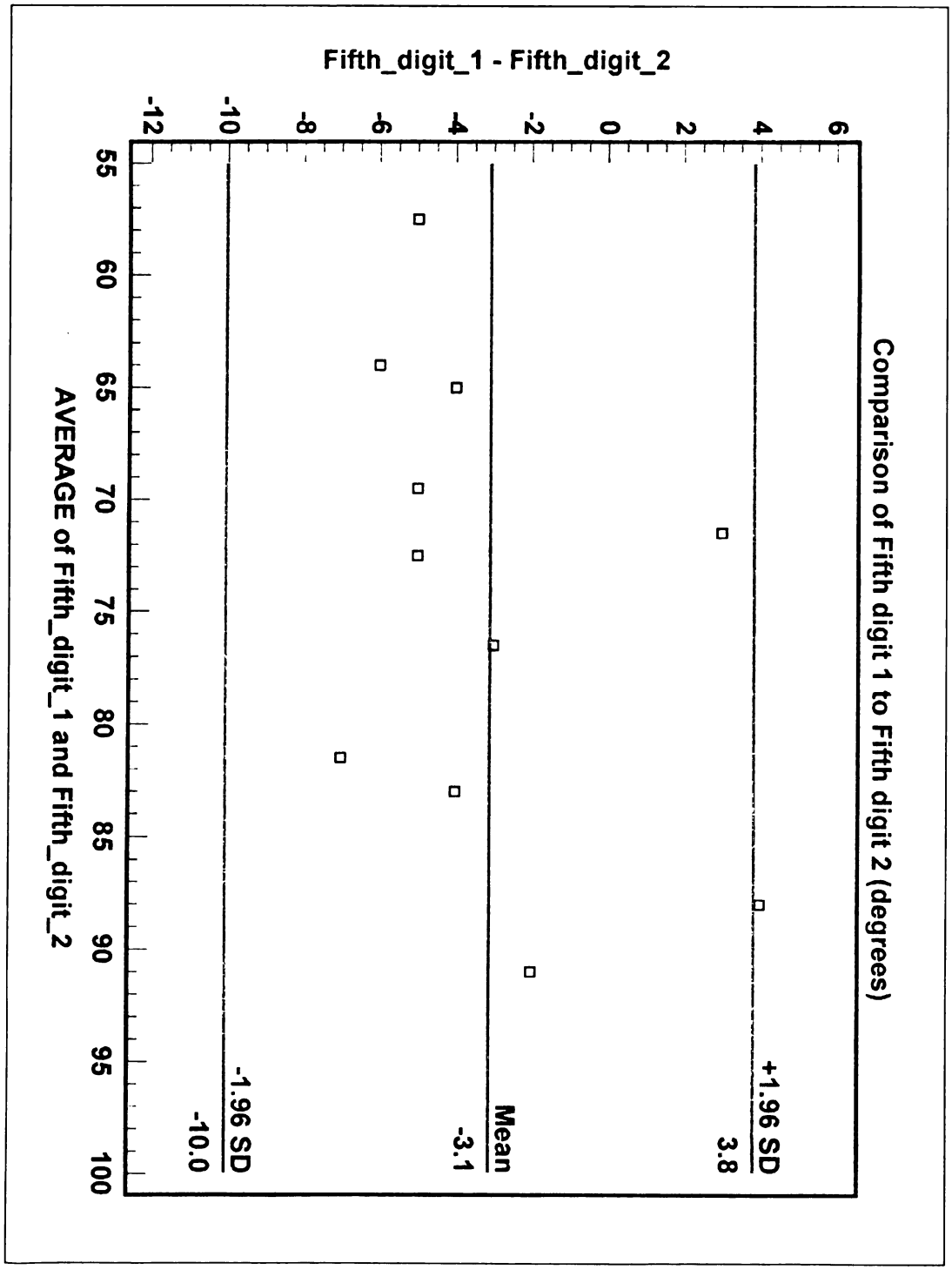


APPENDIX 8 – LATEROTRUSION (Bland-Altman)

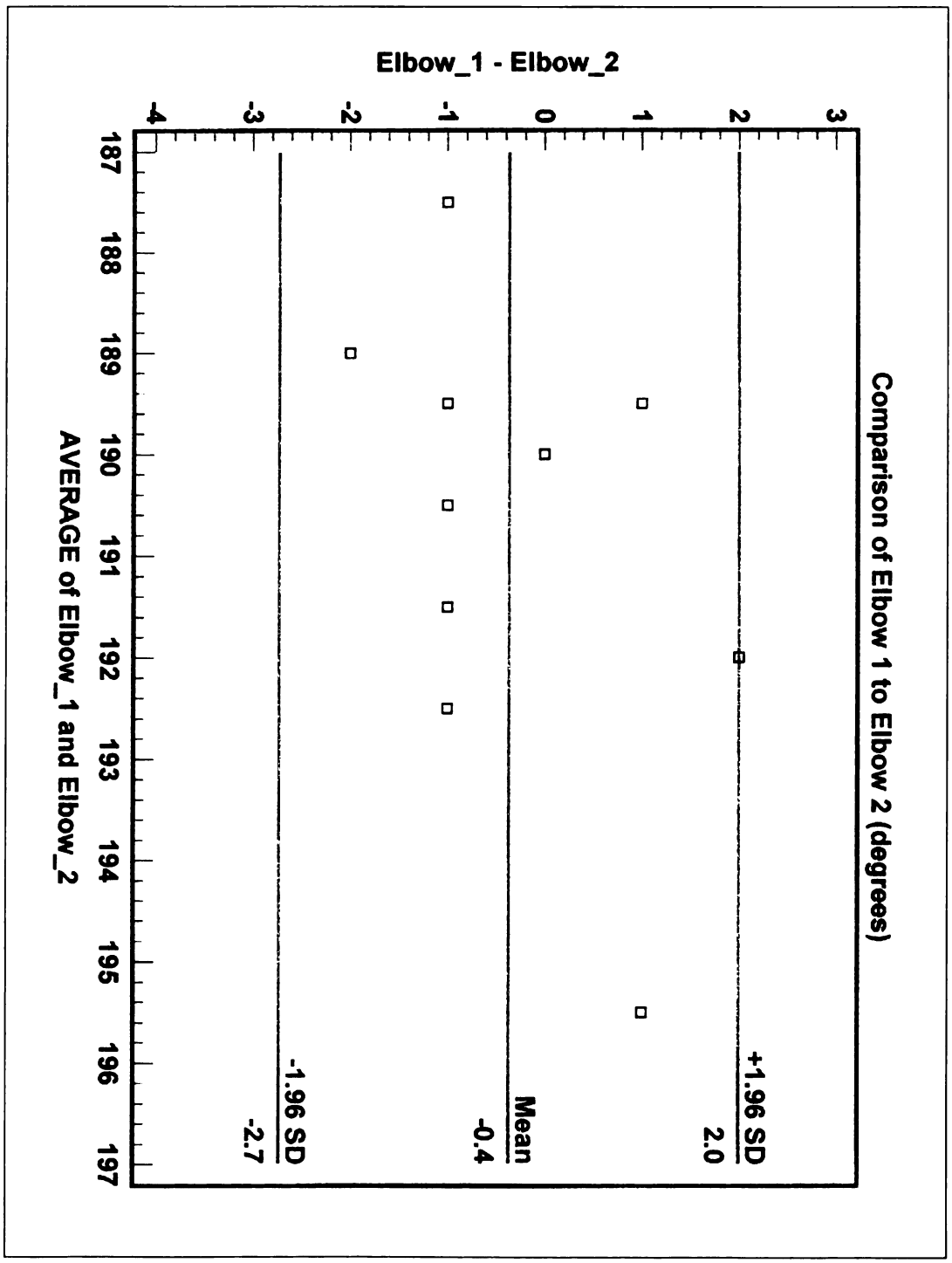




APPENDIX 9 – FIFTH DIGIT (Bland-Altman)



APPENDIX 10 – ELBOW (Bland-Altman)



APPENDIX 11 – KNEE (Bland-Altman)