THE ROLE OF ESTROGEN AND ESTROGEN RECEPTORS IN KNEE OSTEOARTHRITIS

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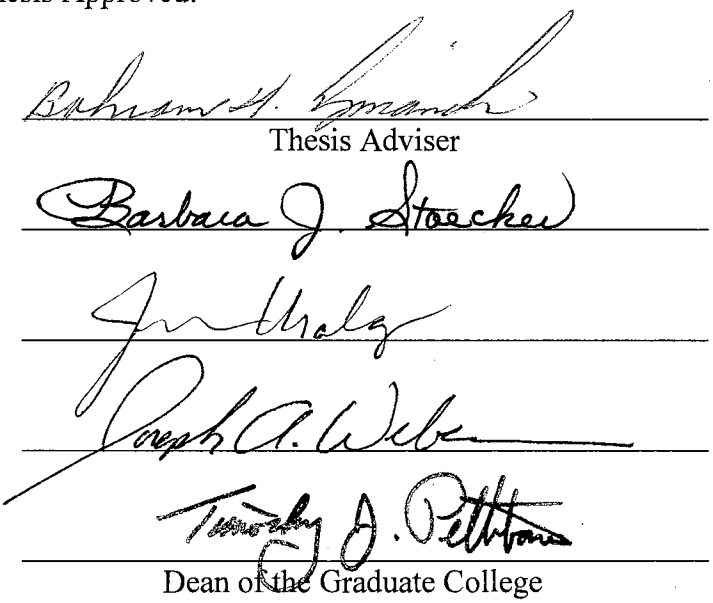

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To my dearest wife, Amynah and my parents,
Shabanali and Habibe Juma.
Without their love, support,
encouragement, and most
of all patience it would
have been impossible
to accomplish

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LIST OF ABBREVIATIONS

BMD	Bone Mineral Density
COMP	Cartilage Oligomeric Protein
CRP	C-Reactive Protein
ELISA	Enzyme Linked Immunosorbent Assay
ER	Estrogen Receptor
IL	Interleukin
IGF-I	Insulin-like Growth Factor I
IGFBP-3	Insulin-like Growth Factor Binding Protein-3
LIF	Leukemia Inhibitory Factor
NHANES I	First National Health and Nutrition Examination Survey
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
RA	Rheumatoid Arthritis
TGF-β	Transforming Growth Factor β
TNF	Tumor Necrosis Factor
YKL-40	Human Cartilage Glycoprotein 39

CHAPTER I

INTRODUCTION

Background

Osteoarthritis (OA) is the most common joint disorder and its etiology is relatively unknown. It currently affects more than 20 million Americans. Osteoarthritis begins with the breakdown of cartilage of the joints, resulting in joint pain and stiffness. OA commonly affects the joints of fingers, knees, hips, and spine. After age 45, women experience a more dramatic increase in the incidence of OA than men. The gender difference in prevalence of OA has been linked to menopause and ovarian hormone deficiency. However, an alarming number of both men and women are afflicted with this joint disorder. The incidence of OA is greatest in the knee joint in comparison to the other joints involved. Although its link to sex hormones is yet undetermined, findings from animal studies imply that OA can be provoked by estrogen administration. Furthermore, recent reports indicate that estrogen is causatively related to OA in both men and women. The fact that OA is not always symmetrical in a given individual indicates that variations within the joints may be involved.

Research Objectives

The principal objective of this research is to elucidate if estrogen receptors (ER) exist in articular cartilage of male and female with and without OA. Furthermore, this study will determine if osteoarthritic symptoms are related to the abundance of these receptors and the concentration of 17β-estradiol in the synovial fluid of individuals with

OA in comparison to individuals without OA.

The specific objectives of this research are as follows:

1. To examine the presence of estrogen receptors in the knee cartilage of individuals with and without knee OA.
2. To compare and contrast the estrogen levels in the knee synovial fluid of individuals with and without knee OA.
3. To compare and contrast selected anabolic and catabolic markers of OA in synovial fluid of individuals with and without knee OA.

Hypotheses

The hypothesis of this study is that there is a relationship between synovial fluid levels of 17β-estradiol and knee OA and that cartilage from afflicted joints has higher ER levels than cartilage from non-afflicted joints.

Format of Dissertation

The research work is organized in two individual manuscript for publication. Chapter III and IV were written in a journal article format using the journal guidelines for Arthritis and Rheumatism. The other chapters follow the CBE form.

CHAPTER II

REVIEW OF THE LITERATURE

Osteoarthritis

Epidemiology and Prevalence

Osteoarthritis (OA) is the leading cause of disability in the elderly population. In the United States, it afflicts 13% of individuals between the ages of 55 and 64 and 17% of those 65 to 74 years old (Maurer, 1979). Increasing prevalence of OA with age is further aggravated by the lack of treatment to reduce the overall cause of OA. It is the most common joint disorder, both in the U.S. and throughout the world, with relatively unknown etiology. Exhausting a major proportion of health care dollars, OA ranks among the top three health care problems of the developed world. Currently 20 million people in the U.S. are affected by OA and this number is predicted to exceed 59 million by the year 2020 (March, 1997). The relative economic impact of musculoskeletal conditions is growing rapidly with the passage of time, having reached 2.5% of the gross national product in 1992 (March, 1997). The prevalence is higher in men than in women up to approximately age 45, however, after age 45 the reverse is true. In addition, after age 45, women experience a more dramatic increase in the incidence of OA in comparison with men (March, 1997). The epidemiologic definition of OA encompasses symptoms, disability, and structural changes associated with this disorder. Epidemiological data focus not only on the structural disease but also characterizes the radiographical definition with the presence and absence of symptoms. The most widely used system for grading the severity of the radiograph is known as the Kellgren-

Lawrence grading system (Kellgren and Lawrence, 1963). This grading defines the degree of osteophyte formation from 0 to 4 but is unclear on the issue of grading the knee with joint space narrowing without the presence of osteophytes.

Incidence of Osteoarthritis

Reports on the percentage of people who have been diagnosed with osteoarthritis based on medical history, examination, or radiographic evaluation confirm a striking increase in the prevalence of OA of the hand, foot, knee, and hip joints with increasing age (Felson et al., 1995; Lawrence et al., 1998). In the Framingham study population, it was found that the percentage of people who have mild, moderate, or severe radiographic changes indicative of osteoarthritis in at least one joint increases progressively from less than 5% in individuals younger than 25 years of age to more than 80% in individuals more than 75 years of age (Felson et al., 1987; Anderson and Felson, 1988). More specifically, those with moderate or severe radiographic changes in relation to OA in at least one joint increases dramatically from 5% in individuals younger than 45 years of age to 40% in those 75 years and older (Lawrence et al., 1996). Regardless of the relationship between age and OA, the widespread view is that osteoarthritis results from normal wear and tear. The resulting stiffness of joints of almost everyone who lives past the age of 65 and the association between joint use, aging, and joint destruction remains uncertain (Cooper et al., 1996; Lawrence et al., 1998).

Unlike the joint destruction seen in other rheumatic joint diseases with major systemic inflammatory components such as rheumatoid arthritis (RA), OA consists of a regressive sequence of changes in the cells and matrix that results in the loss of structure and

function of articular cartilage accompanied by attempts at cartilage repair and the remodeling of underlying bones (Grynpas et al., 1991; Dequeker et al., 1997). Because of these repair and remodeling processes, the degeneration of the articular surface in OA is not uniformly progressive, and the rate of degeneration varies among individuals and among joints (Buckwalter and Lane, 1996; Buckwalter and Lane, 1997). On occasions, this degeneration may occur rapidly, but in most joints it progresses slowly over many years. However, in some cases, this process may stabilize or even decrease spontaneously with partial restoration of the articular surface and a resulting decrease in symptoms.

Primary and Secondary Osteoarthritis

Primary or idiopathic osteoarthritis is the degeneration of articular cartilage and alterations in single or multiple joints due primarily to aging and wear and tear of the tissue. This results in the loss of structure and function of the articular cartilage causing pain and loss of motion (Buckwalter and Martin, 1995). In an increasing number of individuals, OA is classified as secondary on the basis that the joint degeneration is mainly as a result of traumatic joint injury or from developmental, metabolic, and systemic disorders which destroy the articular surface (Buckwalter and Martin, 1995). This generally progressive loss of articular cartilage is accompanied by attempted repair of the cartilage, remodeling and sclerosis of the underlying subchondral bone, and in many cases the formation of bone cysts and marginal osteophytes. The diagnosis of OA requires the presence of symptoms and signs that may include; joint pain, restriction of motion, crepitus with motion, joint effusions, and deformity (Kuettner and Goldberg, 1995). Although OA is most prevalent in the foot, knee, hip, spine, and hand joints (Altman et al., 1986; Altman et al., 1990; Altman et al., 1991), it

can affect any synovial joint as well.

Osteoarthritis develops most frequently in the absence of a known cause (primary OA) and less often it develops as a result of a joint injury, infection or one of a variety of hereditary, developmental, metabolic and neurologic disorders (secondary OA). The age of onset associated with secondary OA depends on the underlying cause, thus, it may develop in young adults and even children as well as the elderly (Mitchell and Curress, 1977). In contrast, there is a strong association between the prevalence of primary osteoarthritis and increasing age. The tools to assess the prevalence of osteoarthritis have numerous limitations, including difficulty in defining and establishing the diagnosis and in evaluating more than a few synovial joints in each individual (Altman et al., 1986; Altman et al., 1990; Altman et al., 1991).

Biology of Articular Cartilage

Composition of Cartilage

Articular cartilage appears histologically to be a simple inert tissue and has a firm slick surface that resists deformation (Buckwalter et al., 1988). Under the light microscope, the articular cartilage consists primarily of extracellular matrix, with only one type of cell, known as chondrocytes. Cartilage is a non-vascular tissue as it lacks blood vessels, lymphatic vessels, and nerves. In comparison with tissues such as muscle and bone, it has a low level of metabolic activity and appears to be less responsive to changes in loading or to injury (Buckwalter et al., 1988; Buckwalter et al., 1990). The morphology of articular cartilage shows that it has a highly intricate and ordered structure and that a variety of complex interactions between the chondrocytes and the protein matrix actively maintain the

integrity of the tissue. The articular cartilage is composed of cells, water, and a matrix macromolecular framework, and it is this framework that provides the cartilage its mechanical properties (Buckwalter et al., 1990). The cells of the cartilage make up the smallest part of the total volume of cartilage (Stockwell, 1967).

Chondrocytes

Within the articular cartilage, there is only one specialized type of cell known as chondrocyte (Stockwell, 1978; Buckwalter et al., 1988). Chondrocytes from different locations of the articular cartilage differ in size, shape and activity (Aydelotte et al., 1992). These cells contain organelles necessary for matrix synthesis and in some cases have short cilia extensions, which may play a role in sensing mechanical changes in the matrix. Chondrocytes are surrounded by extracellular matrix and do not form cell-to-cell contacts. These cells in their mature stage are spheroidal in shape and synthesize type II collagen, large aggregrating proteoglycan, and specific non-collagenous proteins to form cartilaginous matrix (Aydelotte et al., 1992; Aydelotte et al., 1996). Individual chondrocytes are quite active metabolically but the total activity of the cartilage tissue is quite low due to the low cell density (Stockwell, 1978; Aydelotte et al., 1996).

In relation to the mechano-structural properties of the articular cartilage, chondrocytes appear to remain unchanged in location, appearance, and activity. The type of macromolecules that form the matrix and the concentration of water are important to the structural response of this tissue (Mow and Rosenwasser, 1988). Maintenance of the articular surface requires turnover of the matrix macromolecules, which involves the continuous replacement of degraded matrix components and alteration in the macromolecular

framework of the matrix in response to the use of the joint (Mow and Rosenwasser, 1988). To achieve these activities, the chondrocytes must sense the changes in the matrix due to degradation as well as changes in macromolecules in relation to the demands placed on the articular surface (Athanasiou et al., 1991). The cells respond by synthesizing appropriate types and amounts of the macromolecules (Buckwalter et al., 1990).

Cartilage Metabolism

The chondrocytes derive their nutrients from the synovial fluid through diffusion via a double barrier, first in the synovial tissue and then the cartilage matrix. The matrix barrier is restrictive in respect to not only the size of the materials but also with respect to their charge (Mourades, 1973). The barrier system results in low concentration of oxygen for the chondrocytes in relation to most other tissue, therefore, these cells depend primarily on anaerobic metabolism (Mourades and Schneiderman, 1987; Fischer et al., 1995). The activity of the chondrocytes varies during different stages of skeletal growth. In growing individuals they produce new tissue to expand and model the articular cartilage surface (Fischer et al., 1995). In skeletal maturity, the chondrocytes do little to change the volume of the tissue, but mainly replenish the degraded macromolecules and minimal remodeling of the articular surface (Buckwalter et al., 1994; Buckwalter and Lane, 1996). Also, the rate of metabolic activity and matrix synthesis declines. After completion of skeletal growth, chondrocytes do not undergo further cell division, but rather continue to synthesize collagens, proteoglycans and non-collagenous proteins (Buckwalter et al., 1993; Buckwalter and Lane, 1996).

Enzymes produced by the chondrocytes are responsible for degradation of the matrix macromolecules. The presence of the fragmented matrix molecules increases the synthesis

activity of chondrocytes to replace the matrix framework (Buckwalter, 1995; Guerne et al., 1995). Other factors, which may influence the balance in the synthetic and degradative activities, include frequency and intensity of the joint loading. Immobilization of the joint or a marked decrease in joint loading alters chondrocyte activity so that degradation of cartilage exceeds synthesis (Buckwalter, 1995). With aging, the capacity of the cells to synthesize the proteoglycans and their response to other stimuli such as growth factors decreases (Guerne et al., 1995; Martin and Buckwalter, 1996). These age-related changes may limit the ability of the cells to maintain the tissue and thereby contribute to the degeneration of the articular cartilage (Martin and Buckwalter, 1996).

Matrix and Collagen Framework

The matrix of the articular cartilage is made up of tissue fluid and the framework of structural macromolecules, which includes proteoglycans, collagens and non-collagenous proteins, that give the tissue its form and stability. The interaction of these two components gives the tissue its mechanical properties of stiffness and resilience (Buckwalter and Mow, 2001). Water contributes as much as 80% of the wet weight of articular cartilage, and the interaction of the water with the matrix macromolecules substantially influences the mechanical properties of the tissue (Mankin, 1978; Maroudas and Schneiderman, 1987; Lai et al., 1981). The tissue fluid contains small proteins, metabolites, and a high concentration of cations to balance the negatively charged proteoglycans. The structural macromolecules of the cartilage, collagens, proteoglycans, and non-collagenous proteins, contribute 20% to 40% of the wet weight of the tissue (Buckwalter et al., 1990; Martin and Buckwalter, 2000). These individual components which make up the macromolecule framework differ in their

concentrations within the tissue and in their contributions to the tissue properties. Collagen makes up about 60% of the dry weight, proteoglycans contribute about 25 to 35%, and non-collagenous proteins and glycoproteins 15% to 20%. Collagen is distributed relatively uniformly throughout the depth of the cartilage, and its meshwork gives cartilage its form and tensile strength (Buckwalter and Mow, 2001). Proteoglycans and non-collagenous proteins bind to the collagen meshwork and become mechanically entrapped within it. Some of the non-collagenous proteins assist in organization and stabilization of the macromolecular framework while others help the chondrocytes to bind to the macromolecules of the matrix.

Articular cartilage contains multiple genetically distinct collagen types and the organization of these throughout the tissue provides tensile stiffness and strength (Bruckner et al., 1988; Hardingham et al., 1992). These fibrils also contribute to the cohesiveness of the tissue by mechanically entrapping the large proteoglycans. The principal collagen, Type II, accounts for 90 to 95% of the collagen in articular cartilage and is the primary component of the cross-banded fibrils strength (Bruckner et al., 1988; Hardingham et al., 1992; Hagiwara et al., 1993). Proteoglycans consist of a protein core and one or more glycosaminoglycan chains (long-unbranched polysaccharide) (Hardingham et al., 1992; Roughley and Lee, 1994). Glycosaminoglycans found in cartilage include hyaluronic acid, chondroitin sulfate, keratan sulfate, and dermatan sulfate. The concentration of these molecules varies among sites within the articular cartilage and also with age, injury to the cartilage, and disease. Articular cartilage contains two major types of proteoglycans known as large aggregating monomers or aggrecans and small proteoglycans including decorin, biglycan, and fibromodulin (Hardingham et al., 1992; Roughley and Lee, 1994). Link proteins stabilize

the association between the monomers and hyaluronic acid and appear to have a role in directing the assembly of aggregates (Hardingham et al., 1992). The formation of aggregates helps to anchor the proteoglycans within the matrix and prevent their displacement during the deformation of the tissue (Hardingham et al., 1992; Roughley and Lee, 1994). This helps to stabilize and organize the relationship between proteoglycans and the collagen meshwork. The non-collagenous proteins and glycoproteins are not very well studied for their role in relation to proteoglycans and collagen (Roughley and Lee, 1994). Anchorin CII, a collagen binding chondrocyte surface protein helps in anchoring chondrocytes to the collagen fibers of the matrix (Rosenberg, 1992). Cartilage oligomeric protein (COMP), an acidic protein, is concentrated primarily within the matrix of the chondrocyte and allows for binding between the cells and the matrix (Rosenberg, 1992; Marcelino and McDevitt, 1995). This molecule also serves as a good marker of cartilage turnover in progression of cartilage degeneration in patients with osteoarthritis (OA). Other non-collagenous matrix proteins such as fibronectin and tenascin also function in matrix organization and cell-matrix interactions. The response of these molecules is also altered in conditions such as OA (Rosenberg and Buckwalter, 1986).

Structural Organization of Cartilage

The primary function of the chondrocytes is to synthesize and organize collagens, proteoglycans and the non-collagenous proteins into unique and highly ordered matrix structure to form articular cartilage (Buckwalter et al., 1990; Mankin et al., 1994). The composition, organization, and mechanical properties of this matrix as well as the chondrocyte morphology and function vary according to the depth of the articular surface.

These changes are identified in layers called zones and there are four zones known as superficial, transitional, middle (radial), and a zone of calcified cartilage (Buckwalter et al., 1988; Poole, 1997). The zones differ in respect to their concentration of water, proteoglycan, and collagen including the size of the aggregates. Cells in these zones also differ in size, shape, and orientation relative to the articular surface (Buckwalter et al., 1988). This also causes a variation in the degree of metabolic activity between the zones. Chondrocytes respond differently to mechanical loading which suggests that the development and maintenance of articular cartilage under normal conditions depends partly on the differentiation of the distinct populations of these cells across the zones (Buckwalter et al., 1990; Guilak et al., 1997).

Matrix Compartments

The variations in the matrix within the structural zones are distinctly categorized into three compartments or regions called pericellular, territorial, and interterritorial (Buckwalter et al., 1988; Poole, 1997). The pericellular and territorial regions serve the needs of the chondrocytes in relation to their binding of the matrix macromolecules and protecting the cells from damage during the loading and deformation of the tissue (Guilak et al., 1997). These regions also serve to transmit mechanical signals to the chondrocytes when the matrix deforms during joint loading. The interterritorial matrix provides the mechanical properties of the tissue (Buckwalter et al., 1988; Poole, 1997).

The interdependence of the chondrocytes and the matrix makes the lifetime maintenance of the cartilage tissue possible and this does not end when the cells secrete the matrix macromolecules (Mankin et al., 1994; Simkin and Bassett, 1995). The matrix protects

the chondrocytes from mechanical damage during normal use of the joint by helping to maintain their shape and phenotype (Treadwell and Mankin, 1986; Keuttner et al., 1991). Nutrients, substrates for the synthesis of matrix molecules, degraded matrix molecules, newly synthesized molecules, metabolic waste products, and molecules that help regulate cell function all pass through the matrix and in some instances are stored within the matrix (Levick and McDonald, 1995). The type of molecules and the rate at which they can pass through the matrix depends on the composition and the organization of the matrix, especially that of the large proteoglycan molecules (Levick and McDonald, 1995).

Changes in the Diseased Joint

Morphological Changes

Osteoarthritis involves all of the tissues that form the synovial joint, including articular cartilage, subchondral and metaphyseal bone, synovial tissue and ligaments, joint capsule, and muscles that surround the joint (Sokoloff, 1987). However, the primary changes consist of loss of articular cartilage, remodeling of subchondral bone, and formation of osteophytes (Mitrovic and Riera, 1992; Poole et al., 1995). The earliest histological changes in OA include the superficial zone of the articular cartilage and extend into the transitional zone (Radin and Rose, 1986; Clark and Huber, 1990). There is decreased staining for proteoglycans in these regions and protrusion of blood vessels from subchondral bone as a result of increased remodeling (Heingegard et al., 1987). Investigators suggest that the stiffening of subchondral bone due to this remodeling causes the degeneration of articular cartilage (Radin and Rose, 1986). Alternatively, the loss of articular cartilage could increase the level of mechanical stress on the underlying bone, causing aggressive bone remodeling to

occur (Radin and Rose, 1986). However, it is not certain as to what is the correct order of these events. But in most instances, the degeneration of articular cartilage and the remodeling of subchondral bone are both present when symptoms develop and it is this loss of cartilage that leads to the direct loss of joint function.

Stages of Joint Degradation

Many of the mechanisms responsible for the progressive loss of cartilage in degenerative joint disease remain unknown. However, this process can be divided into three overlapping stages which include disruption or alteration of the cartilage matrix, the chondrocyte response to tissue damage, and the decline of the chondrocytic response to restore or maintain tissue leading to loss of articular cartilage (Martin and Buckwalter, 1996; Poole, 1997). The first stage is when there is an overall disruption of the matrix framework and an associated increase in the concentration of water (Poole, 1997). The collagen meshwork may also be damaged and the degree of proteoglycan aggregation decreases (Martin and Buckwalter, 1996). These changes proceed to the response of the chondrocytes in the second stage by increasing synthesis and decreasing the degradation of the matrix. Rate of degradation is controlled by the balance in activity between both anabolic and catabolic growth factors and cytokines (Poole et al., 1995).

In this second stage of the development of OA, the repair mechanisms may increase the synthesis of matrix macromolecules and to a lesser extent cell proliferation which can counter the catabolic effects and stabilize or in some instances restore the tissue (Martin and Buckwalter, 1996). This repair response may continue for years and in some individuals reverse the course of osteoarthritis at least temporarily. The failure to stabilize or restore the

tissue leads to the third stage in the development of OA (Poole, 1997). In the third stage, there is a progressive loss of articular cartilage as well as a notable decline in the anabolic response of the chondrocytes (Setton et al., 1999). This decline can result from mechanical insults and chondrocyte death which no longer can be supported by a stable and functional matrix (Martin and Buckwalter, 1996). There is also a down-regulation of the chondrocytic response to anabolic growth factors and cytokines and an increase in synthesis and accumulation of molecules in the matrix such as catabolic cytokines and growth factor-binding proteins which decrease the overall anabolic effect (Setton et al., 1999). The loss of articular cartilage leads to the symptoms of pain and joint dysfunction associated with OA. This loss occurs most frequently with increasing age, possibly because age related changes in the cartilage matrix and the decrease in the chondrocytic anabolic response compromise the ability of the tissue to maintain and restore itself (Martin and Buckwalter, 1996).

Osteoarthritic Bone

The alteration in the subchondral bone that accompanies the degeneration of articular cartilage includes increase in bone density of the subchondral bone (Milgram, 1983; Dequeker et al., 1997). This includes the formation of cyst-like bone cavities containing fibrous and cartilaginous tissue and the appearance of regenerating cartilage within and on the subchondral bone surface (Amir et al., 1992; Oegema et al., 1997). This occurs more frequently on the periphery of the joint, where osseous and cartilaginous bulges known as osteophytes can sometimes form (Altman et al., 1986; Pottenger et al., 1990). The increase in bone density of the subchondral bone results from the formation of abnormal bone on existing trabeculae and is the usual first sign of the degenerative joint disease (Milgram,

1983). At the end stage of the disease, the articular cartilage has been completely lost, leaving thickened dense subchondral bone articulated with a denuded osseous surface (Migram, 1983; Kamibayashi et al., 1995). The bone remodeling combined with the loss of articular cartilage changes the shape of the joint and can lead to shortening of the involved limb, deformity, and instability.

Formation of Osteophytes

In most synovial joints, growth of osteophytes accompanies the changes in articular cartilage and in the subchondral and metaphyseal bone. Osteophytes have a cartilaginous surface that closely resembles normal articular cartilage and that makes them appear to be an extension of the joint surface (Pottenger et al., 1990). They are usually palpable and may appear to be tender and in all joints they can restrict motion and contribute to pain with motion. Osteophytes represent a response to the degeneration of articular cartilage and the remodeling of subchondral bone, including the release of anabolic cytokines that stimulate cell proliferation and the formation of osseous and cartilaginous matrices (Poole et al., 1993; Matyas et al., 1997). The loss of articular cartilage also leads to secondary changes in the synovial tissue where the synovial membrane often has a mild-to-moderate inflammation reaction and may contain fragments of articular cartilage (Poole et al., 1993). With time, the ligaments and the muscles become contracted causing decreased use of the joint and a decreased range of motion leading to muscle atrophy. These changes often contribute to the stiffness and weakness associated with OA (Poole et al., 1993).

Abnormal Loading Causes Degeneration

Despite the importance of regular activity for maintenance of the joint, some types of repetitive joint use can accelerate the development of degenerative joint disease (Buckwalter, 1995). Studies of individuals who have physically demanding occupations have shown that repetitive, intense joint loading may lead to early joint degradation (Buckwalter et al., 1993; Buckwalter and Lane, 1997). Specific activities that have been associated with osteoarthritis include repetitive lifting or carrying of heavy objects, awkward work postures and continuously repeated movements. Participation in sports or other activities that repeatedly expose joints to high levels of impact also may increase the probability of joint destruction (Lane, 1995; Buckwalter and Lane, 1996).

Individuals who have an abnormal anatomy or function of the joint including dysplasia, malalignment, instability, disturbances in the innervation of the joint muscles, and inadequate muscle strength or endurance, probably have a greater risk of degenerative joint disease (Buckwalter, 1995; Fisher and Pendergast, 1997; Hurley, 1999). Subjecting the joints to loading greater than those that result from normal activities of daily living can presumably increase the risk even further. These individuals as well as those who have early OA require regular exercise, but should have a detailed evaluation of their joint structure and function before initiating such an exercise regimen. In most instances, exercise programs that maintain joint motion and muscle strength with minimal loading should be selected (Leviseth et al., 1989; Minor et al., 1989; Schilke et al., 1996; Suomi and Lindauer, 1997).

Osteoarthritis of the Knee

Incidence and Gender Differences

Epidemiologic surveys have suggested sex-associated differences related to age and disease severity (Wilson et al., 1990; Oliveria et al., 1995; Nevitt et al., 1998). Among individuals older than 50 years of age, women appear to be affected with greater severity, and with more joints involved per female patient in comparison with men. The knee joint is one of the more commonly afflicted joints in OA (Felson, 1988). Of all of the joints involved, there is definitive evidence of knee OA present in 60% to 70% of the afflicted individuals in their seventh and eight decade of life (Bagge et al., 1992a; Bagge et al., 1992b). Sex differences have consistently been observed in knee OA, with an excess prevalence in women with age. Kellgren and Lawrence (Kellgren and Lawrence, 1957) found that persons with generalized OA often had involvement of the knee joint as well. In the Framingham study, 2% of the women versus 1.4% of the men developed radiographic knee OA per year (Felson et al., 1995). Another study which evaluated the incidence of symptomatic OA of the hand, hip and knee reported higher rates in women in comparision to men (Oliveria et al., 1995). A study by Wilson and colleagues in a Northern European county indicated that the incidence of symptomatic OA was equal in women and men but the age-adjusted rates between the gender varied where the incidence is greater in women compared to men (Wilson et al., 1990).

Pathogenesis, Clinical Features, and Risk Factors

Osteoarthritis is generally viewed as a degenerative disorder involving cartilage degradation in aging (Rosenberg et al., 1992). Typically, the degenerative changes are

accompanied by a local inflammatory component that may accelerate joint destruction (Ismaiel et al., 1992). Hyaline articular cartilage is made up of a matrix of type II collagen fibers and proteoglycans, as well as the chondrocytes that produce the matrix, and water (Kraus, 1997). In OA the proteoglycan content of the cartilage is gradually depleted, leading to an initial increase in water content and a loss of compressibility and shock absorption, and culminating in fibrillation and breakdown (Kraus, 1997).

The main clinical features of OA are pain, stiffness, loss of function, and ultimately joint deformity (March, 1997). Any of the joints may be affected in isolation or as part of primary generalized OA. The most common joints involved in OA include those of the knee, hands, feet, hips, and the apophyseal joints of the spine. OA is primarily a disorder of the hyaline articular cartilage that coats the bony surface of all synovial joints and the meniscus (Dequeker, 1985).

In addition to age, a number of other factors have been suggested as risk factors for the development of OA including: genetics, obesity, joint hypermobility, joint trauma, immobilization, peripheral neuropathy, crystals in joint fluid or cartilage, and repetitive joint overuse (Dequeker, 1985). The pathological processes and the radiological features vary to some degree across the different joints. Therefore, OA is generally considered to be a heterogeneous disorder and no two joints may be afflicted equally in the same individual. Predominance of generalized OA in women suggests that changes in production and nature of estrogens may be risk factors in the development of OA.

Osteoarthritis and Bone Fractures

There is an inverse relationship between the incidence of osteoarthritis and

osteoporosis. This suggests that high periarticular bone mass may be detrimental to cartilage integrity and increase the risk of OA (Radin et al., 1972). Cross sectional studies (Hannan et al., 1993, Hart et al., 1994, and Nevitt et al., 1995) have established an increased prevalence of radiographic knee and hip OA in the presence of osteophytes and increased bone mineral density (BMD). Prospective study found a positive relationship between higher bone densities and increased risk of knee OA in older women (Zhang et al., 2000). However amongst women in this study who already had knee OA, those that had a low BMD and were losing bone faster had a more rapid progression of knee OA compared to those who had high BMD and were losing bone more slowly (Zhang et al., 2000). Overall, individuals with knee and hip OA appear to have an increased rate of bone loss (Jones et al., 1995; Burger et al., 1996; Arden et al., 1999) which can contribute to increased risk of fracture. However, not all of the increased fractures can be attributed to bone loss because individuals with joint dysfunction and hypermobility have a greater risk of experiencing a fall.

In a cohort of white women, Hart and colleagues (2002) found that lumbar spine and hip BMD measured at baseline were significantly higher in women who subsequently developed knee osteophytes. The higher BMD was only associated with knee osteophytes and not with joint space narrowing (Hart et al., 2002). During the follow-up period, subjects from this study who sustained a fracture had a 70% lower risk of developing knee osteophytes. From the clinical perspective, knee osteophytes are an important radiographic measure of early knee OA and are strongly suggestive of knee pain and progression of the degenerative condition (Hart et al., 2002; Lane and Nevitt, 2002). Lack of measurement techniques has limited the data on the relationship between the bone changes associated with OA and the incidence of fracture.

Proposed Role of Estrogen in Osteoarthritis

Epidemiologic studies have indicated that women over the age of 50 are the most vulnerable to joint disorders, especially OA. Sex hormones may play a role in the development of OA in women (Spector and Campion, 1989; Felson and Nevitt, 1998). The prevalence of OA increases in women following menopause and rises faster with age in women then in men (Van Saase et al., 1989; Oliveria et al., 1995). Tsai and colleagues (Tsai et al., 1992a) have suggested that excessive levels of synovial fluid estrogen are responsible for the development of OA in humans. In some animal studies, when estrogen was administered directly to the knee joint, it resulted in an increased frequency and severity of OA (Rosner et al., 1986, Tsai and Lui, 1992b). Furthermore, Tsai and Lui (1992b) have shown that intraarticular injections of estradiol to ovariectomized rabbits induced an up-regulation of estrogen receptors in condylar cartilage at an early stage of OA and cartilage degeneration and erosion at the late stage. In OA, looser matrix might be synthesized in the medial compartment, allowing molecules in the synovial fluid, including estrogens to be taken up by the chondrocytes, and therefore resulting in cell death and cartilage erosion (Tsai and Lui; 1992b). Estrogen may affect articular cartilage by modulating the synthesis of the cartilage matrix and the production of matrix enzymes (Rosner et al., 1982). Estrogen receptors are found in articular cartilage (Ushiyama et al., 1999), but estrogen may also influence cartilage metabolism via cytokines (Pelletier et al., 1993). Estrogen has also been suggested to influence the development of OA through its effects on bone metabolism (Healey et al., 1985; Burger et al., 1996; Dequeker et al., 1996).

In partially menisectomized rabbits, estrogen accentuates the frequency and severity of OA. Estradiol also modulated articular cartilage metabolism by suppression of DNA and

proteoglycan synthesis (Rosner et al., 1982). Furthermore, intraarticular injection of estradiol to the knee joint impaired lactate dehydrogenase resulting in an overall disruption of matrix collagen (Rosner et al., 1982; Rosner et al., 1992) in cartilage tissue. These observations imply that the estrogen can affect chondrocyte metabolism and proliferation, possibly through a receptor-mediated mechanism (Rosner et al., 1992).

There are a number of epidemiological studies that have examined the possible relationship between estrogen replacement therapy (ERT) and risk of OA. Several investigators reported that women who at one time have been on ERT have a lower than expected risk of knee or hip OA (Hannan et al., 1990; Samanta et al., 1993; Nevitt et al., 1996; Spector et al., 1997; Vingard et al., 1997). Prospective cohort investigation of the Framingham study indicated that the use of ERT had a moderate protective effect against worsening of radiographic knee OA among elderly women (Zhang et al., 1998). Although evidence from these studies may suggest that estrogen protects against OA, there are important limitations to be considered. Women on ERT tend to be generally healthier, thinner and more active, as well as better educated than non-users. These characteristics as well others may benefit the estrogen users and make them less prediposed to have OA than the non-users. Currently there are no published clinical trials on ERT in OA. Longitudinal studies are needed in which estrogen users and non-users can be followed and carefully characterized for OA.

Estrogen may also play an important role in male OA. Higher synovial estradiol and higher estrogen receptor levels in cartilage of individuals with OA compared to non-OA may be primary reasons for the incidence of OA in males as well (Tsai et al., 1992a; Ushiyama et al., 1999). The negative effects of estradiol in synovial fluid may be counterbalanced by

endogenous testosterone, resulting in a lower estrogen/testosterone ratio or a lower unopposed, free estradiol to interact with cartilage.

Estrogen Receptors and Selective Estrogen Receptor Modulators

Estrogen Receptors

Estrogen has been shown to act genomically by coupling with the estrogen receptor and its coactivators to induce changes in gene expression. 17β-estradiol (E_2) and the estrogen receptors have been shown to form a ligand-estrogen receptor complex. There are two distinct pathways by which E_2 exerts its effects. In the "classical" pathway, the ligand estrogen receptor complex dimerizes and interacts with the estrogen response element (ERE), a specific sequence within the DNA (Paech et al., 1997). The ligand-estrogen receptor complex has two transactivation domains (AF-1 and AF-2) that will bind to various proteins and initiate transcription of the estrogen-dependent gene. In the "nonclassical" pathway, the ligand-estrogen complex does not bind directly to the DNA, but instead, it interacts with an AP-1 response element by binding to coactivator proteins known as fos and jun (Paech et al., 1997). Coactivator proteins will either recruit proteins that are directly involved in transcription or that will facilitate the process of RNA transcription (Paech et al., 1997) Estrogen can also bind to steroid hormone receptors and activates proteins known as corepressors (Shibata et al., 1997). Corepressors are thought to silence transcription, though the mechanisms are not completely understood (Shibata et al., 1997)

The challenge in understanding the mechanisms by which estrogen exerts its effects is due to the existence of its two receptor subtypes, estrogen receptor (ER)-α and

ERβ. The two receptors share considerable homology but differ in the C-terminal ligand binding domain and its N-terminal transactivation domain (Kuiper et al., 1998). Paech and colleagues (1997) have demonstrated that the transcriptional effects elicited by E_2 are determined by whether or not it interacts with ERα or ERβ. Through non-classical binding, transcription is activated by estrogen at ERα and inhibited through ERβ. The two receptors also exhibit different responses to drugs such as tamoxifen and raloxifene which are categorized as either anti-estrogens or selective estrogen receptor modulators (SERM). While estrogen induces negative transcription regulation, SERM induce positive regulatory sequences when interacting with ERβ. This may explain, in part, how SERM are able to induce beneficial estrogenic effects on bone and cardiovascular without dramatically increasing the risk of cancer of the reproductive organs.

Tissue Specific Actions of SERM

The levels of expression for each ER subtype vary from organ to organ, and bind estrogen with varying affinity. The ratio of ERα to ERβ in different vascular beds vary and this may explain the different responses exerted by SERM in various tissues (Kuiper et al., 1997). The two well known SERM, tamoxifen and raloxifene, are non-steroidal phenylethylene derivatives that have been shown to bind to ERβ with higher affinity than ERα (Gustaffson, 1998). In some cases, SERM mimic the effects of estrogen, while in other cases, antiestrogenic effects are observed (Gustaffson, 1998). They were first characterized as antagonists/partial agonists on the basis of their binding to ERα. However since then they have been shown to act as full estrogen agonists in bone through their interaction with ERβ. The differential estrogenic effects of SERM vary, as

tamoxifen has been shown to have estrogenic actions in the uterus, while raloxifene does not (Gustaffson, 1998). Thus the acronym SERM takes into account the selective modulations of the ER in specific tissues (Gustaffson, 1998). The selective action of both natural and synthetic SERM and their interaction with each of the ER subtype across different tissues needs to be further investigated.

Role of Cytokines and Growth Factors in OA

Function

Cytokines and growth factors are released by cells in response to specific signals and influence the function of target cells by exerting a positive or negative effect on gene expression (Nathan and Sporn, 1991). Because they have a relatively short half-life, they usually exert their influence on cells in the local environment. Cytokine production and its effects on target cells are regulated in several ways. Homeostasis is maintained as a balance between a particular cytokine and various naturally occurring molecules that function as cytokine inhibitors (Nathan and Sporn, 1991). Cellular mechanisms which influence this regulation include: 1) specific receptor antagonists competing with ligand for receptor binding site to prevent signal transduction; 2) autoantibodies which bind to the ligand and neutralize it before it reaches the receptor; and 3) proteolytic cleavage of the extracellular portion of the receptor (Arend and Dayer, 1993). These control mechanisms are essential for maintaining tissue integrity. Alterations in the balance of any of these mechanisms may contribute to destructive processes within the joint. However the complexity of the interactions within cytokines and growth factors makes the unraveling of pathways involved in joint destruction a formidable task (Stockwell, 1993).

The cartilage tissue is maintained by the equilibrium between the effects of catabolic cytokines (e.g. IL-1α and β, tumor necrosis factor-α) which induce the production of specific matrix degrading metalloproteases, and anabolic growth factors such as insulin-like growth factor and transforming growth factor-β which induce the production of building blocks of cartilage such as collagen and proteoglycans (Dayer and Fenner, 1992).

In osteoarthritis, which is the most common type of arthritis, the breakdown of the joint tissue occurs in several phases (Ismaiel et al., 1992). This depletion suggests an overall failure in the cytokine-controlled matrix homeostasis, with a shift in equilibrium between synthesis and degradation favoring catabolic processes. In the earlier stages of OA, the chondrocytes attempt to repair the cartilage by increasing the synthesis of matrix macromolecules (Trippel, 1995). The increased presence of anabolic growth factors presumably activates the chondrocytes to stimulate the matrix synthesis. However, compositional change in the matrix molecules may interfere with this process by reduicng their capacity to aggregrate properly with hyaluronic acid (Mankin et al., 1971). Over time, the matrix losses its resiliency and fails to withstand the mechanical stress placed on the joints. In the later stages of the disease process, enhanced cartilage degradation far exceeds the ability of the chondrocytes to synthesize new matrix. Furthermore, there is increased presence of catabolic cytokines such as IL-1β and TNF-α that potentiate the expression of matrix metalloproteinases causing proteolysis of the cartilage matrix (Trippel, 1995). These factors can also inhibit cartilage matrix biosynthesis (Goldring et al., 1986).

Evidence is accruing to implicate cytokines in the pathogenesis of joint diseases with major inflammatory and autoimmune components (Brennan et al., 1989; Dayer et al., 1992). However the mechanisms that initiate and cause cartilage destruction in osteoarthritis remain

unclear. The profile for cytokines and growth factors in based on quantitative rather than qualitative differences as indicated by fewer cells being recruited for their production in osteoarthritis. This may suggest that other processes are involved in cartilage destruction, and that cytokines are merely indicators of the disease activity (Holt et al., 1992). However, the involvement of cytokines and growth factors is a topic of interest in relation to their function in cartilage integrity (Pelletier et al., 1991; Rickard and Gowen, 1993).

Catabolic Cytokines

Interleukin-1 (IL-1β) was first identified as an inflammatory mediator capable of tissue damage. Its importance in cartilage metabolism appears to be a result of its ability to suppress the synthesis of type II collagen, the predominant form of collagen found in articular cartilage (Eastgate et al., 1988). Furthermore, IL-1β is shown to stimulate synthesis of type I collagen, which is predominant in fibroblasts (Smith et al., 1991; Koch et al., 1992). The increased presence of IL-1β and the formation of fibroblasts suppresses the ability of chondrocytes to synthesize new proteoglycan, a building block in cartilage formation (Eastgate et al., 1988). For cartilage degradation to occur, catabolic cytokines such as IL-1β must act at specific receptors on the target cells (Eastgate et al., 1988; Smith et al., 1991). In osteoarthritis, there is increased presence and sensitivity of IL-1β receptors to interact with IL-1β and trigger the joint destruction processes (Warnock et al., 1994).

The potency for cartilage breakdown is 10-fold less in the case of tumor necrosis factor than for IL-1β and the evidence for its existence within osteoarthritic joint is conflicting (Martel-Pelletier et al., 1990; Westcott et al., 1993). Both in rheumatoid arthritis and osteoarthritis, TNFα has been detected at the cartilage-tendon junction by

immunohistological analysis (Miller et al, 1993). Apparently, Both IL-1β and TNFα are produced by the same cells under the same stimuli. However, in osteoarthritis, the two cytokines are not significantly correlated during active disease (Westcott et al., 1993; Westcott et al., 1994).

The multifunctional cytokine, interleukin-6 (IL-6) is often viewed as anti-inflammatory, although recent reports have shown high levels of IL-6 production within the joint in osteoarthritis (Neitfeld et al., 1990). Catabolic cytokines such as IL-1β and TNFα induce the production of IL-6. In articular cartilage, the presence of IL-6 mediates the influence of IL-1β on inhibition of proteoglycan synthesis through increased IL-1 receptor expression (Nietfeld et al., 1990). However, other studies also have suggested a relationship between increased proteoglycan synthesis by chondrocytes, and increased levels of IL-6 in synovial fluid (Holt et al., 1992). The direct role of IL-6 on cartilage metabolism is uncertain and needs to be examined.

Leukemia inhibitory factor (LIF) has been detected in synovial fluid of patients with osteoarthritis in association with the inflammatory conditions (Caroll and Bell, 1993). Local production by cartilage cells in combination with its capability to degrade cartilage suggests that LIF may stimulate the loss of proteoglycan. But studies remain inconclusive in regards to a role of LIF in disease conditions such as osteoarthritis (Osborne et al., 1989; Caroll and Bell, 1993).

Anabolic Growth Factors

The overall destruction of cartilage associated with osteoarthritis is caused by an imbalance between the catabolic cytokines and those that maintain the proliferative

responses of chondrocytes. These cytokines, most commonly termed as growth factors, stimulate matrix synthesis (Mankin et al., 1991; Moskowitz et al., 1991).

Insulin-like growth factor-I (IGF-I) is a potent mediator of cartilage synthesis that reduces degradation and promotes proteoglycan synthesis even in the presence of IL-1β and TNFα (Denko et al., 1990; Moskowitz et al., 1991; Denko and Boja, 1993). Serum IGF-I levels are found to be low in osteoarthritis, however the intracellular levels and mRNA expression are high in chondrocytes (Middleton and Tyler, 1992). The circulating levels of IGF-I are mediated through the presence of its binding proteins (Moskowitz et al., 1991; Denko and Boja, 1993). In early stages of OA, cartilage synthesis is increased to counterbalance the rapid loss and degradation. This increased cartilage activity coincides with increased IGF-I levels in the synovial fluid. However, the role of IGF-I is quite complex in OA and needs further investigation.

Transforming growth factor-β (TGFβ) is produced by almost all cells in the body, including the chondrocytes (Wahl et al., 1990). It is a powerful mitogen for cells such as those in cartilage and bone and reduces inflammation and stimulates cartilage matrix synthesis. TGFβ also inhibits matrix degradation by stimulating protease inhibitor production, blocking collagenase and metalloprotease release, and promoting cartilage repair by production of collagen and fibronectin (Wahl et al., 1990). TGFβ also increased general protein synthesis and is thought to inhibit IL-1β mediated degradation by decreasing the IL-1β receptor expression (Wahl et al, 1990). However other studies have shown that IL-1β production may be stimulated by TGFβ and suggest that stimulation or degradation of cartilage occurs depending on the balance between TGFβ and the presence of other cytokines within the joint (Wahl et al., 1990; Chu et al., 1992).

In Vitro Models Using Cytokines and Growth Factors

Isolated chondrocytes have been utilized in cell culture to elucidate mechanisms by which cytokines and growth factors may influence cartilage metabolism. Chandrasekhar et al. (1994) exposed chondrocytes isolated from rabbit articular cartilage to IL-1β, IL-6, TNFα, TGFβ or IGF-I. IL-1β, but not IL-6 or TNFα inhibited the synthesis and secretion of osteonectin, a protein indicative of cartilage formation. Stimulatory growth factors such as TGFβ and IGF-I increased the synthesis of osteonectin in culture (Chandrasekhar et al., 1994).

Tyler et al. (1989) have used a model system of explanted cartilage to determine the role of IGF-I in promoting matrix formation. In the presence of IL-1 and TNFα, there were no detectable changes in the aggregating proteoglycan synthesized by the cartilage. When labeled proteoglycan was measured in the explant, IGF-1 alone increased the release of labeled proteoglycan by 50% in relation to ones with IL-1 and TNFα present. Thus, the role of growth factors in promoting the synthesis of cartilage proteins in the presence of catabolic cytokines needs to be investigated using in vivo systems.

Using immunohistochemical analysis, Chu et al (1992) investigated the presence and the role of cytokines in the cartilage/pannus junction. Both catabolic (IL-1β, IL-6, TNFα) and anabolic (TGFβ) cytokines were found in both normal and arthritic tissue confirming their important role in cartilage metabolism. Presence of catabolic cytokines was higher in tissues from rheumatoid arthritis (RA) patients in comparison to those with OA and without arthritis. The difference between RA and OA in relation to presence of cytokines is not clearly known.

Release of protein fragments, such as fibronectin, from cartilage indicates increase in its proteoglycan content. The increased synthesis of proteoglycan may be modulated by growth factors. Homandberg et al (1997) investigated the role of IGF-I and TGFβ in cultured cartilage cells. These growth factors enhanced the synthesis rate and content of proteoglycan by 50-100%. However, the increase in fibronectin was accompanied by increases in catabolic cytokine such as IL-1, IL-6 and TNFα. Thus, the steady-state balance of cartilage metabolism is directly influenced by the presence of both anabolic and catabolic cytokines.

Biochemical Markers of Cartilage Metabolism

Serum and Synovial Fluid

Osteoarthritis is currently diagnosed by clinical and/or radiographic criteria which only allow detection in fairly advanced stages of the disease (Dieppe, 1995). The inability for early diagnosis of OA hinders the clinical assessment of the changes crucial for subsequent development of irreversible joint damage. Reliable and sensitive methods for monitoring such changes would facilitate clinical investigations of key events in the pathophysiology of OA (Petersson IF, 1998; Saxne, 1995).

Cartilage and bone macromolecular fragments are released in synovial fluid during normal tissue turnover, but changes in the release of these molecules are likely with the OA. Quantification of such fragments in serum and synovial fluid should provide important information related to disease conditions such as OA (Saxne and Heinegard, 1995). However, there are confounding factors which influence the levels of potential "markers" which need to be considered in interpreting the results of such measurements (Saxne and Heinegard, 1995). For instance, serum levels will not only depend on the release of these

markers from the cartilage and bone, but also on the elimination rate from the joint cavity. Furthermore, the clearance rate from blood in turn depends on uptake and degradation by the liver and the filtration by the kidneys (Heinegard and Saxne, 1991).

C-Reactive Protein (CRP) as a Marker of Inflammation

The inflammatory marker used most commonly to assess the progression of disease such as OA is serum CRP. This marker is increased markedly in arthritic conditions and is reduced by the use of anti-inflammatory drugs (Loose et al., 1993). Serum CRP concentrations have been demonstrated to have a low grade acute phase response in subjects with mild-to-moderate OA and were positively correlated with the progression of the disease (Spector et al., 1997). Another study also confirmed a slight but significant increase in serum CRP levels ranked according to disease severity (Sipe, 1995). Population studies indicated that CRP levels have a stronger correlation with osteophyte grade according to the Kellgren-Lawrence scoring scale then with the narrowing of joint space (Spector et al., 1993; Hart et al., 1995). This lack of association between CRP levels and narrowing of joint space is perhaps because joint space measurement is considered a poor measure of the disease cross-sectionally (Spector et al., 1993; Hart et al., 1995).

Markers of Cartilage Degradation

A matrix protein known as cartilage oligomeric matrix protein (COMP) which was isolated in cartilage and is relatively specific to synovial joint tissue serves as an important marker for cartilage turnover (Hedborn et al., 1992). Sharif et al. (1995), found serum COMP levels to be significantly increased in patients with knee OA who had decreased joint space

or required a knee surgery during a five-year follow-up. In individuals with knee pain, Petersson et al., (1998) had also demonstrated that serum levels of COMP were significantly higher in individuals with radiographic OA during a 3-year follow-up versus those who had normal radiographs over the same period. Conrozier and colleagues (1998) showed that baseline values of serum COMP correlated with joint space narrowing parameters in patients with hip OA over a one-year period. However, no such correlation was found by Georges et al. (1997) in a study when baseline COMP levels were compared arthroscopically to disease progression. In a group of individuals with knee pain, serum COMP levels have correlated positively with the extent of bone scan abnormalities (Petersson et al., 1998). One factor which may influence serum COMP levels is synovitis. Knee synovitis may play a role in progression of OA. Higher levels of serum COMP were observed in patients with faster progressing joint disease (Vilim et al., 2001).

A specific marker for cartilage metabolism that is secreted by cultured chondrocytes is human cartilage glycoprotein 39 (YKL-40). The name is derived from its 40 kilodalton molecular weight and the one-letter code for its 3-N-terminal amino acids; tyrosine, lysine, and leucine (Hakala et al., 1993; Hu et al., 1996). YKL-40 is synthesized by articular chondrocytes (Hakala et al., 1993; Hu et al., 1996) and synovial cells (Nyirkos and Golds, 1990; Kirkpatrick et al., 1997) in patients with both rheumatoid arthritis (RA) and OA. Histological specimens obtained from patients with OA have shown positive staining for YKL-40 in chondrocytes mainly in the superficial and middle zones of the cartilage whereas it was low or undetectable in normal cartilage samples (Volck et al., 1999). Elevated levels of both serum and synovial fluid YKL-40 are seen in patients with active RA or severe knee OA in comparison to normal subjects (Johansen et al., 1993; Johansen et al., 1996; Johansen

et al., 2000). Johansen and colleagues (2001) demonstrated that YKL-40 was produced in response to removal of chondrocytes from their native extracellular matrix environment related to joint injury and disruption of the cartilage framework. YKL-40 production may be mediated by cytokines and growth factors that have a regulatory effect on chondrocyte function, especially in inflammatory conditions of the joints such as OA.

Other approaches in investigating the changes associated with OA, are to look at the synovium and the bone matrix. The synovium serves as a direct reservoir for molecules that are exchanged in the joint and are involved with joint integrity as well as end products that are degraded in cartilage metabolism. The bone matrix can be assessed in conditions such as OA using bone scintigraphy (Thomas et al., 1975). Bone scintigraphy is the use of a radionuclide tracer agent such as technetcium labeled phosphate to obtain an image of the joint. This imaging technique may supplement the physical examination and a simple radiography of the joint in assessing the severity of OA (Arnold et al., 1980; Dye and Chew, 1993).

Regeneration of Articular Cartilage

Process of Cartilage Repair

Physicians and scientists have sought ways to repair and regenerate the articular surface of synovial joints after the loss or degeneration of articular cartilage for more than 200 years (Buckwalter et al., 1990). Little progress has been made during this time, except for the last few decades in which scientific investigations have shown that implantation of artificial matrices, growth factors, and chondrocytes can stimulate the formation of cartilaginous tissue in osteochondral and chondral defects of the synovial joints (Buckwalter,

1992; Buckwalter, 1996a). Mechanical loading of the joints also can influence the healing and repair process.

Several surgical procedures to treat OA including osteotomy, penetration of subchondral bone, and joint distraction and motion have seem to stimulate formation of new cartilage surfaces (Mitchell and Shepard, 1976; Buckwalter and Mankin, 1997). However, inadequate approaches to assessing the results of these surgical procedures have made it difficult to evaluate their relative success in restoring the function of the joint (Hass, 1944; Buckwalter and Lomander, 1994).

A better understanding of the degeneration of the articular cartilage and the recognition of the limitations of current treatments have also contributed to the recent interest in the repair and regeneration of cartilage (Buckwalter and Mankin, 1997). Advances in the imaging and arthroscopic techniques of synovial joints have led to an increased knowledge of frequency and types of defects and the possibility to diagnose and evaluate them with great accuracy (Buckwalter and Lomander, 1994; Buckwalter, 1996a). Ultimately, the regeneration of normal articular cartilage may not be necessary in order for a procedure to beneficial because stimulating the formation of cartilage repair tissue may decrease symptoms and improve joint function (Buckwalter, 1996b). Clinical reports on procedures intended to restore a damaged or degenerated articular cartilage have shown improvements in more than 75% of the patients (Hass, 1944; Bert, 1993; Buckwalter et al., 1994; Buckwalter, 1996a). However, interpretation of these reports is complicated by variability, patient age, and the types of defects.

Current Treatments and Therapies for OA-Surgical

Stimulating New Cartilage Growth

Penetration of subchondral bone is a commonly used technique to stimulate the formation of new articular surface (Engkvist and Johansson, 1980; Homminga et al., 1990). It is a method to disrupt subchondral blood vessels and excise the damaged cartilage along with the underlying subchondral bone. Another surgical method is arthroscopic abrasion which can successfully decrease the symptoms of osteoarthritis of the knee joint by stimulating the formation of a fibrocartilaginous articular surface that varies in composition in relation to its collagen meshwork (Bert and Maschka, 1989; Bert, 1993). Radiographic evidence indicates an increase in joint space after the abrasion technique which presumably means the formation of new articular surface. However, arthroscopic abrasion did little to relieving the symptoms of OA (Bert, 1993).

Osteotomy

Osteotomy has been the method of choice for surgeons in treatment of hip and knee joints with localized loss or degeneration of the articular surface (Coventary, 1979; Noyes et al., 1993; Millis et al., 1995; Trousdale et al., 1995). In rare cases, it is combined with the penetration of the subchondral joint where it may involve another afflicted joint. Osteotomy is used to decrease the load on the most severely damaged regions of the joint surface and to bring regions of the joint surface that have the remaining articular cartilage into opposition with regions that lack the cartilage. Clinical experience with this procedure has been effective in decreasing the symptoms and stimulating the formation of new articular surface (Millis et al., 1995; Trousdale et al., 1995). However, the mechanisms for this improvement

remain poorly understood (Millis et al., 1995). Long-term follow-up of patients with osteotomy for osteoarthritic hip or knee joint have shown that the clinical results deteriorate over time (Trousdale et al., 1995; Iwase et al., 1996; Perlau et al., 1996). Variables that appear to affect the results of knee osteotomy adversely include older age, obesity, severe degeneration, limited motion of the knee, surgical overcorrection or undercorrection, and postoperative loss of correction (Hernigou et al., 1987; Finkelstein et al., 1996). Even patients who appear to be ideal osteotomy candidates and have an initial good outcome, tend to have recurrent pain and evidence of progressive osteoarthritis with time (Finkelstein et al., 1996).

Soft-Tissue Grafts

Treatment of osteoarthritic joints with soft-tissue grafts includes benefits such as new cell populations along with an organic matrix. The success of soft-tissue arthroplasty depends not only on the severity of the abnormalities of the joint and on the type of graft but also on the postoperative motion to facilitate the generation of a new articular surface (O'Driscoll and Salter, 1986;O'Driscoll et al., 1988). Both perichondral and periosteal grafts placed on articular cartilage defects have produced new cartilage in animal models as well as clinical studies (O'Driscoll and Salter, 1986; O'Driscoll et al., 1988).

Age of the patient may also influence the effectiveness of grafts. Best results were produced in younger patients where the ability of undifferentiated cells or chondrocytes to form articular cartilage was greatest. The limited ability of host cells to restore articular surface has made it necessary to seek alternative methods to transplant cells that can form cartilage. Studies have shown that both chondrocytes and undifferentiated mesenchymal

cells placed in articular cartilage defects survive and produce new cartilage matrix (Itay et al., 1987; Wakitani et al., 1989). Other studies have also indicated that the transplantation of chondrocytes combined with the use of a tissue graft can promote the restoration of an articular surface in humans (Itay et al., 1988; Noguchi et al., 1994). Future research is needed to assess the function and durability of the newly formed cartilage tissue, to determine if it improves joint function and delays or prevents joint degeneration, and to be certain that these approaches are beneficial in treating osteoarthritic joints.

Current Treatments and Therapies for OA-Lifestyle

Unloading of the Joint

The relationship between joint use and joint degeneration is a critical part in developing strategies to prevent and treat OA (Hochberg et al., 1995; Minor, 1999). Both animal and human joint studies have shown that moderate and even strenuous regular activity does not cause or accelerate the development of osteoarthritis in normal joints (Buckwalter, 1995; Buckwalter and Lane, 1996). Furthermore, cyclic loading of cartilage stimulates matrix synthesis whereas prolonged static loading or the absence of loading and motion causes degradation of the matrix as well as the joint (Fisher and Pendergast, 1994; Lane, 1995).

Decreased contact pressures of the articular surfaces combined with movement of the joint may stimulate the restoration of an articular surface on osteoarthritic joints. Before the development of artificial joints, it was found that the resection of an osteoarthritic joint surface followed by decreased loading combined with motion of the joint resulted in the formation of fibrocartilaginous tissue over the osseous surfaces (Buckwalter and Lohmander,

1994). It has also been suggested that decreased loading of the joint by releasing the muscles that act across the degenerated hip joint can decrease symptoms and increase the radiographic cartilage space in some patients (Buckwalter and Mow, 2001).

Current Treatments and Therapies for OA-Medications

Classes of Non-Steroidal Anti-Inflammatory Agents

The choice of effective treatments for individuals with OA is quite a challenge. The concensus recommendation for patients with OA has been to use acetaminophen (Bradley et al., 1991) Their studies were also able to compare the effects of various doses of acetaminophen alone and in comparison with doses of ibuprofen (Brandt, 1991; Bradley et al., 1992). The effectiveness of these two agents has been investigated in treating patients with OA of the hip and knee where the outcome measures included the patient's self-assessment functional scores using the Western Ontario and MacMaster Universities Osteoarthritis Index (WOMAC). These studies included the multi-dimensional Health Assessment Questionnaire which measures the quality of life of patients with arthritis (Bellamy et al., 1988; Pincus et al., 1999). Overall, acetaminophen has been shown to be more tolerable with less gastrointestinal distress in comparison to ibuprofen. Acetaminophen is an excellent analgesic but does not possess any anti-inflammatory activity (Towheed and Hochberg, 1997). Its mechanism of action is not clearly known however at high doses it has been shown to inhibit prostaglandin synthesis (Cryer and Feldman, 1998). The adverse effects with the use of acetaminophen have been due to acute overdose which is associated with liver damage. Furthermore, in a case-controlled study, chronic use can lead to kidney failure in some individuals (Hochberg et al., 1995).

Celecoxib and Rofecoxib

The new class of non-steroidal anti-inflammatory drugs (NSAIDs) has shown vastly improved function in OA and rheumatoid arthritis patients with similar efficacy to their predecessors but with significantly decreased gastrointestinal and platelet effects (Simon et al., 1999; Laine et al., 1999). Americans use these agents on a daily basis and according to the projected statistics by the US Center for Disease Control and Prevention, it is likely that a significant increase in the prevalence of painful and degenerative conditions will likely increase the use of NSAIDs (Baum, 1985; Phillips and Simon, 1997). The newer class of NSAIDs selectively inhibit cycloxygenase-2 (COX-2) more so then COX-1. This makes them more potent anti-inflammatory agents for degenerative joint conditions such as OA without causing detrimental effects on the gastrointestinal tract.

The most commonly used therapeutic compounds for OA in this class are called celecoxib and rofecoxib (Lipsky et al., 1998). Both of these compounds are selective COX-2 inhibitors and have been shown to be effective in reducing or alleviating OA pain and inflammation associated with rheumatoid arthritis (Simon et al., 1998; Benson et al., 1999; Ehrich et al., 1999; Hawkey et al., 1999; Laine et al., 1999; Laine et al., 1999; Simon et al., 1999). These studies also presented clear evidence of no endoscopic gastroduodenal damage and no adverse effect on platelet aggregation (Simon et al., 1998; Benson et al., 1999; Ehrich et al., 1999; Hawkey et al., 1999; Laine et al., 1999; Laine et al., 1999; Simon et al., 1999). However, many of these studies have left the renal effects of these potent COX-2 inhibitors unanswered. Several mechanisms being proposed for these anti-inflammatory compounds include their in vitro inhibition of inducible nitric oxide synthase which subsequently

decreases the production of nitrite (Amin et al., 1995; Pelletier, 1999). It has also been suggested that NSAIDs, by inhibiting prostaglandin production could also be responsible in reestablishing a more normal cell cycle response which is inhibited by prostaglandins (Patrignani et al., 1994; Dubois et al., 1998; Crofford et al., 2000). Longer-term effects of these agents are not yet fully understood in context of their toxic load on the liver as well as their adverse effects on other tissues and organ systems.

Current Treatments and Therapies for OA-Nutraceuticals

Biological compounds represent newer potential therapies for OA and these include chondroitin sulfate, glucosamine sulfate, and glucosamine hydrochloride (Lozada and Altman, 1997). These nutritional supplements are being widely used for the treatment of painful OA of the knee, hip and hand but their effectiveness is uncertain (Hauselmann et al., 2001). In earlier studies, the potential effects of these agents were mostly from small and not well designed studies of short duration and mostly conducted overseas in Europe and Asia. Recent studies with better designs and more patients for longer duration have found that glucosamine sulfate and chondrotin sulfate have modest to excellent effects on controlling pain and improving function in comparison to placebo and are comparable to NSAIDs. These studies have also demonstrated fewer adverse side effects related to the gastrointestinal system (McAlindon et al., 2000).

Glucosamine Sulfate

Basic scientific research on the effects of glucosamine sulfate has been supported by recent in vitro studies of cultured human and animal chondrocytes showing that it stimulates

glycosaminoglycan synthesis (Bassleer et al., 1998; Gouze et al., 2001) and reduces catabolism (Jimenez, 1996; Sandy et al., 1998). Its proposed mechanism is a dose-dependent inhibition of pro-inflammatory cytokines such as IL-β. In the clinical setting, the data to support the benefits of glucosamine have been limited by sample size, effect size, and outcome measures. There are three double-blinded and placebo controlled human studies which have assessed the short-term effects of glucosamine sulfate as determined by improvements in pain, joint tenderness, and swelling (Drovanti et al., 1980; Pujalte et al., 1980; Noack et al., 1994). Additionally, the study by Drovanti and colleagues has investigated the cellular morphology of the cartilage tissue using scanning electron microscopy in subjects on both placebo as well as glucosamine. Their findings have shown a healthier cartilage structure and chondrocyte morphology in those individuals treated with glucosamine (Drovanti et al., 1980).

Several studies have also compared the effects of glucosamine sulfate with ibuprofen. In one particular study where patients received glucosamine (500 mg three times per day) or ibuprofen (1,200 mg per day) for a period of 8 weeks, it was shown that the rate of improvement as defined by reduction in pain became progressively pronounced in the glucosamine group than in the ibuprofen group (Vaz, 1982). In a shorter duration study (4 weeks), it was found that the reduction in pain occurred earlier in the study with the ibuprofen group then the glucosamine group, but there were no differences in effect between the two treatment groups from the second week onward (Qui et al., 1998). Both glucosamine and ibuprofen reduced symptoms of OA with a trend showing glucosamine to be more effective. More importantly, the beneficial effects were greater after the treatments were discontinued indicating a greater residual effect of glucosamine (Qui et al., 1998). Other

short-term studies have also confirmed the beneficial residual effect of glucosamine and suggest a favorable influence on the disease process (Rovati et al., 1994). More recent studies with glucosamine have utilized the Western Ontario-McMaster University (WOMAC) pain subscale scores as the primary endpoint to assess the degree of change (Houpt et al., 1999). Changes in the WOMAC pain scores favor glucosamine over placebo, but these differences were not significant. Positive trends were noted for glucosamine for majority of the questions in this pain assessment questionnaire. The only long-term study of glucosamine, as a potential disease-modifying agent, has shown positive results with respect to cartilage preservation (Reginster et al., 2001). However, this claim cannot be established due to a lack of proper and standardized radiographic techniques in measuring the knee joint and assessing the positive changes in cartilage tissue associated with glucosamine.

Chondroitin Sulfate

Chondroitin sulfate is part of the proteoglycan macromolecule which is responsible for giving cartilage its resiliency (Hauselmann et al., 2001). In vitro using human chondrocytes and in vivo animal studies have shown that chondroitin inhibits cartilage loss by causing a dose-dependent decrease in collagenase activity (Bassleer et al., 1998; Uebelhart et al., 1998). Short term studies of oral chondroitin sulfate have shown that individuals with knee OA receiving chondroitin used fewer NSAIDs then those individuals on placebo over the treatment phase as well as during the two-month follow-up (Mazieres et al., 2001). In another 3-month study, patients in the chondroitin group experienced a more prolonged improvement in categorical measurement of pain and a reduction in the use of pain medication (Morreale et al., 1996).

In a one-year study, Uebelhart and colleagues (1998) studied the potential disease modification role of chondrotin sulfate in 42 patients with knee OA. Outcomes measures included measurement of joint space width which the authors reported to be stabilized in a number of patients who received chondroitin sulfate (Uebelhart et al., 1998). Most studies have focused on the effects of chondroitin sulfate or glucosamine sulfate alone, but not on their combination. However, one, 16-week study was conducted to evaluate the combination effect of glucosamine and chondroitin sulfate in men with pain and radiographic evidence of knee OA (Leffler et al., 1999). These patients reported an overall improvement in the visual analog scale of pain, physical functioning of the afflicted knee, and ease in performing activities of daily living. It is necessary that both glucosamine and chondroitin sulfate should be further evaluated in longer duration studies with longer follow-up periods to substantiate their claim that they are disease and structural modifying agents.

CHAPTER III

EVIDENCE FOR THE PRESENCE OF ESTROGEN RECEPTORS IN HUMAN ARTICULAR CARTILAGE

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Summary

Objective: The present study explores the possible connection between synovial fluid concentrations of 17β-estradiol (E_2), the presence of estrogen receptors in articular cartilage, and knee osteoarthritis (OA).

Methods: Knee cartilage and synovial fluid specimens were obtained from a total of thirty-four individuals (21 females and 13 males) with and without OA. Total RNA was isolated from cartilage specimens to determine the presence of ERα, ERβ and aromatase using reverse-transcriptase polymerase chain reaction (RT-PCR). Immunocytochemical analysis was conducted to confirm the presence and location of the estrogen receptors in cartilage tissue. Additionally, western blot analysis was used to examine the relative abundance of ERα and ERβ protein in cartilage tissue. Synovial fluid specimens were

analyzed to determine E_2 concentrations.

Results: The presence of mRNA for both estrogen receptors α and β was detected in human articular cartilage. In women afflicted with OA, ERβ expression was significantly higher ($P < 0.0001$) than non-osteoarthritic women. There were no differences observed in ERα expression in women with or without OA. No differences were observed in ERα and ERβ expressions in men with and without OA. Immunocytochemical staining techniques confirmed the presence of these receptors as well as its localization within the cartilage cells. Western blot analysis confirmed the presence of proteins for these receptors. Synovial fluid concentrations of E_2 were significantly ($P < 0.03$) elevated in women with knee OA compared to their normal counterparts when normalized to total protein. However, E_2 levels of synovial fluid were not affected by OA in men. Additionally, women with OA expressed higher levels of mRNA for aromatase compared to those without OA. Expression of mRNA for aromatase in men with or without OA were not different.

Conclusion: This study provides initial evidence for the higher ERβ expression, but not ERα in women with OA compared to without OA, No such differences in ERs were observed in men. In women, the elevated levels of E_2 in synovial fluid may, in part, be explained by higher aromatase activity as evident by elevated mRNA expressions. The presence of ERs along with increased E_2 concentrations may contribute to the pathophysiology of OA in women. The functional role of the ERs and their subtypes in cartilage metabolism in conditions such as osteoarthritis needs to be further clarified.

Key words: aromatase, cartilage, estrogen receptors, osteoarthritis, synovial fluid

Osteoarthritis (OA) is the most common joint disorder of both men and women and its etiology is relatively unknown (1). The prevalence and severity of OA increases with age with the knee joint being one of the most commonly afflicted joints. OA of the knee involves a narrowing of the joint space that is usually evident by x-ray radiography (2). Epidemiological surveys have suggested sex-associated differences related to age and disease severity (3). The prevalence of OA is higher in men than in women up to approximately age 45, however, after age 45 the reverse is true. The predominance of generalized OA in women suggests that changes in production and nature of estrogens may be risk factors in the development of OA.

Although the incidence of knee OA is greatest in postmenopausal women, its link to sex hormones is undetermined and seems conflicting. For instance, epidemiological studies point to a lower incidence of OA and more knee cartilage in women who are long-term users of estrogen replacement therapy (4, 5). Yet, experimental studies imply a detrimental role of estrogen on cartilage metabolism. For instance, some animal studies have demonstrated that when estrogen is administered directly to the knee joint, it results in an increased frequency and severity of OA (6,7). Estrogen has also been shown to modulate cartilage metabolism, as evident by its suppression of proteoglycan synthesis (8). Additionally, intraarticular injection of 17β-estradiol to the knee joint impairs lactate dehydrogenase in chondrocytes, an event preceding the disruption of matrix collagen (9). These observations imply that estrogen is involved in the etiology of OA and that it could affect chondrocyte metabolism and proliferation, possibly through a receptor-mediated mechanism.

Of significance is that the incidence of OA does not seem to be related to circulating

levels of estrogen (2). Rather, excessive levels of synovial fluid estrogen, without noticeable differences in circulating estradiol levels, may contribute to the development of OA in humans (10). Furthermore, the effects of estrogen on cartilage may not be confined to females but may also apply to males. Higher synovial estradiol and higher estrogen receptor levels in cartilage have been suggested by Rosner et al. (11) two decades ago as possible primary reasons for the incidence of OA in males as well.

If cartilage is to be considered a direct target tissue for estrogens, then estrogen receptors (ERs) are expected to be present in human cartilage. Hence, the current study was conducted to evaluate: 1) whether there are differences in synovial estradiol levels between osteoarthritic and nonosteoarthritic joints in males and females and 2) whether there is a relationship between the presence of ERs and knee OA.

MATERIALS AND METHODS

Subjects and tissue collection. The purpose of this study was to elucidate the presence of ERs in human cartilage and whether there is a relationship between the expression of ERs and E_2 concentrations in synovial fluid of men and women with OA in comparison to individuals without OA. The study protocol and consent form were approved by the Oklahoma State University Institutional Review Board. Knee cartilage and synovial fluid specimens were obtained from a total of thirty-four individuals (Table 1) with and without OA. Based on patient history, the incidence of knee OA in these individuals was unrelated to traumatic injury to the joints. Of these, 4 males and 14 females were afflicted with knee OA and the remaining 9 males and 7 females were controls who either had

recent knee injury or repeated knee injuries but had no evidence of OA as diagnosed by their orthopedic surgeon (Table 1). These subjects were referred to the surgeon for the repair of their knees, and were otherwise considered healthy. On the day of surgery, synovial fluid was collected, aliquoted, and stored in -80°C for analyses. Articular cartilage specimens removed during routine surgical repair were immediately collected and stored in ice, separated, processed for isolation of total RNA and immunocytochemistry and stored at -80°C for receptor analyses. Additionally, uterine and ovarian tissue specimens were obtained from volunteers undergoing hysterectomy and served as positive controls for receptor and reverse-transcriptase polymerase chain reaction (RT-PCR) analyses.

The joints of patients with insignificant levels of synovial fluid were irrigated with 1 to 2 ml of normal saline and subsequent analyses were normalized to the synovial total protein concentration. Total protein concentrations in the synovial fluid were determined using reagents from Roche Diagnostics (Montclair, NJ) and analyzed by the Cobas-Fara II Clinical Analyzer. Briefly, 10 μL of synovial fluid was incubated with the dye reagent concentrate resulting in a color change in response to various concentrations of protein. A standard curve for this assay was simultaneously determined using lyophilized preparations of bovine plasma albumin which was reconstituted with deionized water. The absorbance of this colormetric reaction was measured at 570 nm and the synovial fluid total protein concentrations were calculated using the standard curve. Subsequently the synovial fluid E_2 values were corrected based on total protein levels and reported as pg E_2/mg protein.

RNA isolation and reverse-transcriptase polymerase-chain reaction. Total cellular RNA was extracted from articular cartilage and uterine tissues using the acid-phenol extraction method of Chomczynski and Sacchi (12) and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as described by Malayer and Woods (13). Briefly, for each tissue, two micrograms of total RNA was denatured by heating at 95°C and reverse transcribed in the presence of random hexamers (pdN_6; 100 pmol; Pharmacia, Piscataway, NJ), dATP, dTTP, dCTP, and dGTP (dNTPs; 250μmol; Pharmacia), $MgCl_2$ RNase inhibitor (20 U per reaction; Promega, Madison, WI), and reverse transcriptase (Superscript^{TM} 200 U per reaction; Gibco BRL) at 37°C for 75 min. The reaction was stopped by heating at 95°C. Aliquots of reverse-transcribed cDNA (2µl) are denatured by heating at 95°C and subjected to polymerase chain-reaction in the presence of 75 pmole specific primers, $MgCl_2$, dNTPs (25µmole), and Amplitaq^{TM} DNA polymerase (0.5 U per reaction; Perkin-Elmer, Foster City, CA) in 20 µl reaction. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was amplified separately to confirm the integrity of the isolated RNA and as an internal standard. The primers were prepared according to the published sequence of the human gene for ERα (14), ERβ (15), aromatase (16), and GAPDH (17) and are shown in Table 1. Products of the RT-PCR were resolved on 1.2% agarose-TAE gels [Tris-acetate (40 mmole), EDTA (1 mmole)]. Gels were briefly stained with etidium bromide dye solution for visualization using a UV box. Gene products were quantified using a BIORAD densitometer and Gel Documentation Software (Hercules, CA)

Western blot analysis. The protein levels of ERα and ERβ were detected by the method

of Towbin et al. (18) with some modification. A 25 μg cytosolic fraction of each cartilage sample was mixed with the gel loading buffer, pH 6.8, (0.125 M Tris[hydroxymethyl]aminomethane, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 10% 2-mercaptoethanol, and 0.2% bromophenol blue) in ratio of 1:1 and boiled for 5 minutes. This was then separated on a 8% SDS-polyacrylamide mini-gel at 100 V, and transferred to polyvinylidene fluoride membrane at 15 V for 1 hour using a semi-dry transfer system (BIO-RAD, Hercules, CA). The membrane was immediately placed in a blocking solution (5% non-fat dry milk in TBS-T buffer containing 50 mM Tris, 150 mM NaCl, and 0.1% Tween 20, pH 7.5) at 4°C for overnight incubation. The membrane was then incubated with a mouse monoclonal ERα (diluted 1:200 in TBS-T buffer) and a rabbit polyclonal ERβ (diluted 1:200 in TBS-T buffer) at room temperature for 1 hr. After three consecutive rinses in washing buffer containing 50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.1% NP-40, 0.5% deoxycholic acid, pH 7.5 for 10 minutes per wash, the membrane was incubated with a horseradish peroxidase-conjugated goat anti-mouse antibody (5% non-fat milk, diluted 1:1000 in TBS-T buffer) and goat anti-rabbit secondary antibody (5% non-fat milk, diluted 1:2000 in TBS-T buffer) at room temperature for 45 minutes. Following four rinses with washing buffer, a secondary antibody labeling system was used to detect the enhanced chemiluminescence. The membrane was then exposed to Hyperfilm. Pre-stained blue protein markers was used for the accuracy of molecular weight determination of the protein of interest. Protein levels of ERα and ERβ were quantified using BIORAD densitometer and Gel Documentation Software (Hercules, CA)

Immunocytochemistry analyses. Immunocytochemistry was carried out to determine

whether human cartilage contains immunoreactive estrogen receptor alpha and beta proteins, and if present to examine the abundance of the immunoreactive cells. Since uterine tissue is known to be rich in ERs, it was used as positive control tissue (19). The tissues were cut into small pieces, mounted on tissue molds, frozen with liquid 2-methyl butane and stored at -80°C. Frozen sections, 6 μm thick, were prepared using a cryostat (Bright Clinicut, Huntington, England) and thaw-mounted onto glass slides coated with poly-L-lysine. The sections were fixed for 10 min in 3.7% paraformaldehyde in 0.4 mole/L phosphate buffered-saline (PBS) at a pH of 7.3, and subsequently rinsed for 5 min in PBS. The slides were immersed in cold methanol and acetone and then washed with PBS buffer. The slides were then transferred to fresh stock of PBS buffer and held for no more than an hour until the primary antibody dilutions with and without blocking peptides were prepared. The slides were then incubated overnight in a humidified chamber at 4°C with antibodies against human estrogen receptors alpha or beta. The following day the sections were processed by the avidin-biotin peroxidase complex (ABC; Vector Laboratories, Inc. Burlingame, CA) procedure of Hsu, et al (20) at room temperature using the reagents and protocol supplied in the ABC kit from the manufacturer. The reaction products were immersed in subsequent washes of PBS buffer and visualized with a chromogen, diaminobenzidine (DAB) stain. Specificity of the ER antiserum was determined from control studies. In control studies, the ER antiserum was replaced with either normal serum or antibodies against ERα and ERβ which were pre-incubated with excess of the peptides used to raise these antibodies (21,22).

Synovial fluid estradiol. 17β-Estradiol concentrations were measured using

radioimmunoassay (RIA; Diagnostic Products Corp, CA) techniques. Briefly, aliquoted samples were thawed and centrifuged at 2400 x g for 2 min (23). A 10 μL aliquot of synovial fluid were incubated in antibody-coated tubes in the presence of 100 μL of [^{125}I]-labeled E_2 at room temperature following the manufacturer's protocol. The tubes were decanted and counted in gamma counter. The standards and controls provided in the RIA kit were used simultaneously according to the manufacturer's protocol in determining a log-logit curve (see Appendix A). The estrogen concentrations in the synovial fluid specimens were determined using this log-logit curve. Synovial fluid estradiol values were then corrected based on total protein levels and reported as pg/mg protein.

Statistical analysis. Data analysis involved estimation of means, standard error of mean (SE), and analysis of variance using InStat Software (Version 3.0; San Diego, CA) . When the analysis of variance indicated significant difference, the differences among means were investigated using the Tukey's multiple comparison procedure. $P<0.05$ was considered statistically significant.

RESULTS

In the present study, it should be noted that the volume of synovial fluid was limited which restricted our ability to perform the validation test for the concentrations of E_2. Another limitation is that certain joints had to be lavaged by using 1 to 2 mL of saline, therefore we could no longer assess the estradiol levels per mL synovial fluid. That is the

reason for reporting synovial fluid E_2 values per mg protein. This type of normalization has not been reported in the literature and the reproducibility of our data needs to be confirmed. Another point that should be mentioned is that there are currently no RIA kits specifically designed to assess the concentrations of E_2 in the synovial fluid and hence the reliability, specificity, and validity of this RIA kit for use in synovial fluid need to be addressed.

Amplification of cDNA from articular cartilage tissues using RT-PCR showed the presence of mRNA for ERβ (Figures 1a and 1b, females and males, respectively) and ERα (Figures 2a and 2b, females and males respectively). Integrity of the isolated RNA and equal loading of specimen for PCR amplification was confirmed using GAPDH (Figures 1a , 1b, 2a, and 2b). When PCR products for ERα (Figure 3) and ERβ (Figure 4) were standardized to GAPDH, only ERβ was greater in articular cartilage specimens obtained from women with osteoarthritic joints compared to specimens that were obtained from nonosteoarthritic women. Western blot analyses confirmed the increase in protein levels of ERβ, but not ERα in women with OA in comparison to individuals without OA (Figure 5a and 5b).

Positive nuclei immunostaining confirmed the presence of both ERα (Figure 6a and 6b) and ERβ (Figure 7a and 7b) in the cartilage specimens obtained from patients with OA. Immunostaining technique for these receptors was verified by their presence in positive control tissues (Figure 8a and 8b). Similar tissues when incubated with an excess of peptide used to produce the antibody did not show specific stainings (data not shown).

The mRNA levels of aromatase standardized to GADPH were higher in women with OA than those without OA (Figure 9). No differences between aromatase mRNA

levels were observed among men (Figure 10).

Synovial fluid specimens from female patients with OA had significantly higher concentrations of E_2 in comparison with those of non-osteoarthritic females or males with or without OA (Figure 11). However, there were no statistically significant differences in E_2 levels of synovial fluid between men with or without OA.

DISCUSSION

The findings of this study suggest the presence of both ERα and ERβ in articular cartilage with greater abundance of ERβ compared to ERα, indicating that there may be a functional role for the ERβ-subtype specific to the tissue. ERβ subtype was cloned by Kupier et al. (24) and appears to be distinctively different from the classical ERα in its role in gene regulation. Findings from this study using RT-PCR and immunocytochemical techniques indicated the presence of both ERα and ERβ in articular cartilage specimens obtained from both men and women with OA. According to our RT-PCR and western blot analyses, we have observed that both the mRNA and protein levels of ERβ are greater in women afflicted with OA. This suggests that there may be a cause and effect relationship that exists between the incidence of OA and the presence of ERβ in this tissue. It is possible that this is analogous to the presence of ERα in mammary tissues and its connection with breast cancer (25,26). However, further investigation is needed to confirm whether such a relationship exists.

Our findings of estrogen receptors in cartilage are in agreement with two previously published reports (27,28). Initially, Nilsson and colleagues (27) reported the presence of

ERβ in human growth plate cartilage but were unable to detect the presence of ERα protein using Western blot analysis. A more recent study by Ushiyama et al. (28) indicated the presence of both ERα and ERβ in human articular cartilage obtained from men and women with and without OA using RT-PCR techniques. In this present study, we utilized both techniques used by these investigators and were able to confirm the presence of both ER subtypes.

Our results indicated that the synovial fluid concentrations of E_2 were significantly greater in women with OA as compared to those without OA or men with or without OA. Hence, higher levels of estrogen in synovial fluid may play a role in the etiology of OA in women but not in men. Whether a synonymous relationship exists in men with respect to androgen concentrations has to be investigated. The higher E_2 concentrations in synovial fluid of women with OA leads us to speculate that localized E_2 in the synovial cavity may be involved in the degenerative process of cartilage. The accumulation of estrogen in the synovial fluid of women with OA, in part, may be explained by increased aromatase activity as evidenced by higher mRNA expression for aromatase.

It is important to pointout that our findings are based on a very limited number of subjects and specimens. Therefore, these findings cannot be extrapolated to populations with OA and the data are at best preliminary in nature. Hence, it would be necessary to validate these findings in future studies with larger sample size from both men and women with and without OA.

If elevations in E_2 are implicated in cartilage degeneration leading to OA, then agents such as selective estrogen receptor modulators (SERMs) may be able to compete with estrogens in occupying ERβ present in the knee cartilage and perhaps negate its

deleterious effects. Selective estrogen receptor modulators are defined as a group of compounds behaving as estrogen agonists in certain tissues while acting as antagonists in some other tissues (29). To this date, ERβ has been found in certain tissues including brain, bone, bladder, and vascular epithelial which are responsive to estrogen as well as SERMs (30). Recent investigations on the effects of synthetic and naturally occurring SERMs on various tissues suggest that these diverse molecules may influence target tissues by exerting their effects mainly through ERβ (31). The tissue distribution of these two ERs differs (30) which may explain the agonistic or antagonistic properties of SERMs in various tissues. Therefore, cells that preferentially express ERβ, including cartilage cells, are more likely to respond to SERMs. However, this concept is speculative and requires further investigation. The impact of SERMs such as genistein, ipriflavone, raloxifene on improvement of OA conditions remains to be shown.

REFERENCES

1. Felson DT. Epidemiology of hip and knee osteoarthritis. Epidemiol Rev 1988; 10:1-28.
2. March LM. Osteoarthritis. MJA 1997; 166:98-103.
3. Davis MA. Epidemiology of osteoarthritis. Clin Geriat Med 1988; 4:241-255.
4. Wluska AE, Davis SR, Bailey M, Stuckey SL, and Cicuttini FM. Users of oestrogen replacement therapy have more knee cartilage than non-users. Ann Rheum Dis 2001; 60:332-6.
5. Nevitt MC, Cummings SR, Lane NE, Hochberg MC, Scott JC, Pressman AR, et al. Association of estrogen replacement therapy with the risk of osteoarthritis of the hip in elderly white women. Study of Osteoporotic Fractures Research Group. Arch Intern Med 1996; 156:2073-80
6. Rosner IA, Goldberg VM, and Moskowitz RW. Estrogens and osteoarthritis. Clin Orthop 1986; 213:77-83.
7. Tsai CL and Lui TK. Upregulation of estrogen receptors in rabbit osteoarthritic cartilage. Life Sci 1992; 50:1727-1735.
8. Ng MC, Harper RP, Le CT, and Wong BS. Effects of estrogen on the condylar cartilage of the rat mandible in organ culture. J Oral Maxillofac Surg 1999; 57:818-23.
9. Tsai CL and Liu TK. Inhibition of estradiol-induced early osteoarthritic changes by tamoxifen. Life Sci 1992; 50:1943-51

10. Tsai CL, Liu TK and Chen TJ. Estrogen and osteoarthritis: A study of synovial estradiol and estradiol receptor binding in human osteoarthritic knees. Biochem Biophys Comms 1992; 183:1287-1291.

11. Rosner IA, Manni A, Malemud CJ, Boja B, and Moskowitz RW. Estradiol receptors in articular chondrocytes. Biochemical and Biophysical Research Communications 1982; 106:1378-1382.

12. Chomczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-159

13. Malayer JR and Woods VM. Expression of estrogen receptor and maintenance of hormone responsive phenotype in bovine fetal uterine cells. Dom Anim Endocrinol 1998; 15:141-154.

14. Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, et al. Human oestrogen receptor cDNA: sequence, expression, and homology to v-ER□-A. Nature 1986; 320:134-139.

15. Arts J, Kuiper GGJM, Janssen JMMF, Gustafsson JA, Lowik CWGM, Pols HAP, et al. Differential expression of estrogen receptor α and β mRNA during differentiation of human osteoblast SV-HVO cells. Endocrinology 1997; 138:5067-5070.

16. Le Bail J-C, Liagre B, Vergne P, Bertin P, Beneytout J-L, and Habrioux G. Aromatase in synovial cells from postmenopausal women. Steroids 2001; 66:749-757.

17. Tang P-Z, Gannon MJ, Andrew A, and Miller D. Evidence for oestrogenic regulation of heat shock protein expression in human endometrium and steroid responsive cell lines. Eur J Endocrinol 1995; 133:598-605.

18. Towbin H, Staehelin T, and Gordon J. Electorphoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. PNAS 1979; 76:4340-4354.

19. Salih MA, Arjmandi BH, Sims SH, and Kalu DN. Examination of putative intestinal estrogen receptor. J Bone Min Res, 1993; 8(suppl 1):S264.

20. Hsu SM, Raine L and Fanger H. The use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures J Histochem Cytochem 1981; 29:577-580.

21. Furlow JD, Ahrens H, Mueller GC, and Gorski J. Antisera to a synthetic peptide recognize native and denatured rat estrogen receptors. Endocrinology 1990; 127:1028-1032.

22. Li X, Scwartz PE and Rissman EF. Distribution of estrogen receptor-β like immunoreactivity in rat forebrain Neuroendocrinology 1997; 66:63-67.

23. Geborek P, Saxne T, Heinegard D, and Woilheim FA. Measurement of synovial fluid using albumin dilution upon intraarticular saline injection. J Rheumatol 1988; 15:57-60.

24. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, and Gustafsson JA. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996; 93:5925-5930.

25. Ferguson AT and Davidson NE. Regulation of estrogen receptor alpha function in breast cancer. Crit Rev Oncog 1997; 8:29-46.

26. Lawson JS, Field AS, Tran DD, Killeen J, Maskarenic G, Ishikura H, and Trichopoulos D. Breast cancer incidence and estrogen receptor alpha mammary tissue-an epidemiologic study among Japanese women in Japan and Hawaii. Int J Cancer 2002; 97:685-687.

27. Nilsson LO, Boman A, Savendahl L, Grigelioniene G, Ohlsson C, Ritzen EM, et al. Demonstration of estrogen receptor-β immunoreactivity in human growth plate cartilage. J Clin Endo Metab 1999; 84:370-373.

28. Ushiyama T, Ueyama H, Inoue K, Ohkubo I, and Hukuda S. Expression of genes for estrogen receptor α and β in human articular cartilage. Osteoarthritis Cart 1999; 7:560-566.

29. Cosman F and Lindsay R. Selective estrogen receptor modulators: Clinical spectrum. Endocr Rev. 1999; 20: 418-434.

30. Setchell KDR and Cassidy A. Dietary isoflavones: Biological effects and relevance to human health. J Nutr 1999; 129:758S-767S.

31. Brzezinski A and Debi A. Phytoestrogens: The "natural" selective estrogen receptor modulators? Eur J Obstet Gynecol. 1999; 85: 47-51.

Table I

Description of study participants

Gender	Status of Knee Joint	N=	Mean Age ± SE
Female	N-OA	7	39.7 ± 2.9
	OA	14	57.8 ± 5.5
Male	N-OA	9	42.7 ± 3.9
	OA	4	59.8 ± 10.5

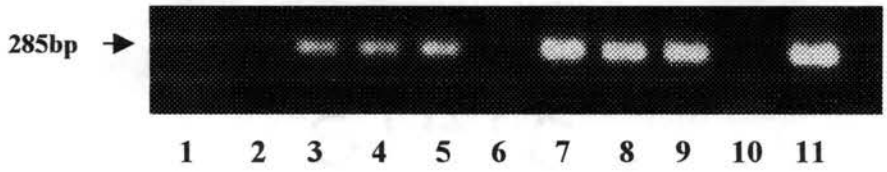
Non-Osteoarthritis (N-OA); Osteoarthritis (OA)

Table II

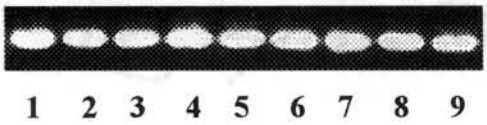
Primer sequences of gene products for RT-PCR

Gene		Sequence	Size (bp)
Aromatase	Sense	5'-TACTACAACCGGGTATATGG-3'	503
	Anti-sense	5'-TGTTAGAGGTGTCCAGCATG-3'	
ERα	Sense	5'-GACCGAAGAGGAGGGAGAA-3	460
	Anti-sense	5'-CCAAGAGCAAGTTAGGAGCAA-3'	
ERβ	Sense	5'-TAGTGGTCCATCGCCAGTTAT-3'	323
	Anti-sense	5'-GGGAGCCACACTTCACCAT-3'	
	Sense (nested)	5'-CGGAACCTCAAAAGAGTCCCTGG-3'	285
	Anti-sense (nested)	5'-CCGAAGTCGGCAGGCCTGGCAGC-3'	
GAPDH	Sense	5'-GAGTCAACGGATTTGGTCGT-3'	156
	Anti-sense	5'-GGTGCCATGGAATTTGCCAT-3'	

Figure 1A

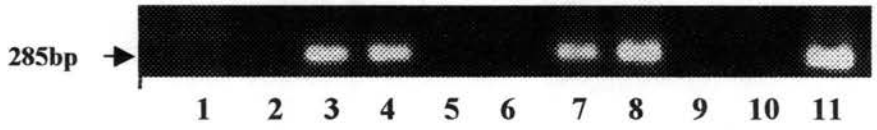


Representative RT-PCR analysis for detection of estrogen receptor (β) in female cartilage specimens.***Lane 1*** represents the 100 bp ladder. ***Lane 2*** represents negative control (water). ***Lanes 3-6*** are amplification of cDNA from female cartilage without OA. ***Lanes 7-10*** are from female cartilage with OA. ***Lane 11*** is amplification of cDNA from female uterine tissue (positive control). Products are expected size of 285bp length resolved on a Tris-actetate-EDTA agarose gel.

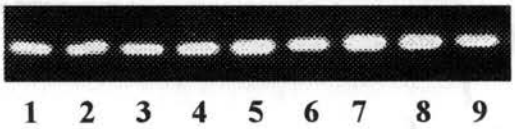


Integrity of the isolated RNA and equal loading of specimen for RT-PCR amplification was assured by assessing glyceraldehyde-3-phosphate dehyrdogenase (GAPDH) gene expression. ***Lanes 1-4*** are amplification of cDNA from female cartilage without OA. ***Lanes 5-8*** are from female cartilage with OA. ***Lane 9*** is amplification of cDNA from female uterus (positive control). Products are expected size of 156bp length resolved on a Tris-acetate-EDTA agarose gel.

Figure 1B



Representative RT-PCR analysis for detection of estrogen receptor (β) in male cartilage specimens***Lane 1*** represents the 100 bp ladder. ***Lane 2*** represents negative control (water). ***Lanes 3-6*** are amplification of cDNA from male cartilage without OA. ***Lanes 7-10*** are from male cartilage with OA. ***Lane 11*** is amplification of cDNA from female uterus (positive control). Products are expected size of 285bp length resolved on a Tris-actetate-EDTA agarose gel.



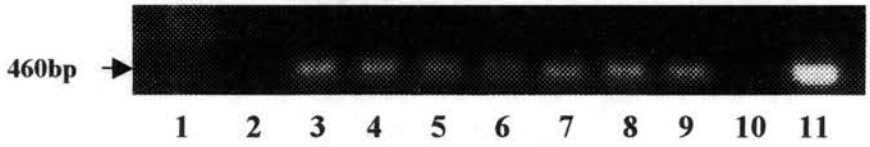
Integrity of the isolated RNA and equal loading of specimen for RT-PCR amplification was assured by assessing by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression. ***Lanes 1-4*** are amplification of cDNA from male cartilage without OA. ***Lanes 5-8*** are from male cartilage with OA. ***Lane 9*** is amplification of cDNA from female uterus (positive control). Products are expected size of 156bp length resolved on a Tris-acetate-EDTA agarose gel.

Figure 2A

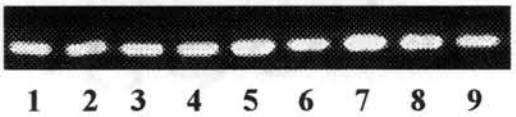
Representative RT-PCR analysis for detection of estrogen receptor (α) in female cartilage specimens. ***Lane 1*** represents the 100 bp ladder. ***Lane 2*** represents negative control (water). ***Lanes 3-6*** are amplification of cDNA from female cartilage without OA. ***Lanes 7-10*** are from female cartilage with OA. ***Lane 11*** is amplification of cDNA from female ovary (positive control). Products are expected size of 460 bp length resolved on a Tris-acetate-EDTA agarose gel.

Integrity of the isolated RNA and equal loading of specimen for RT-PCR amplification was assured by assessing glyceraldehyde-3-phosphate dehyrdogenase (GAPDH) gene expression. ***Lanes 1-4*** are amplification of cDNA from female cartilage without OA. ***Lanes 5-8*** are from female cartilage with OA. ***Lane 9*** is amplification of cDNA from female uterus (positive control). Products are expected size of 156bp length resolved on a Tris-acetate-EDTA agarose gel.

Figure 2B

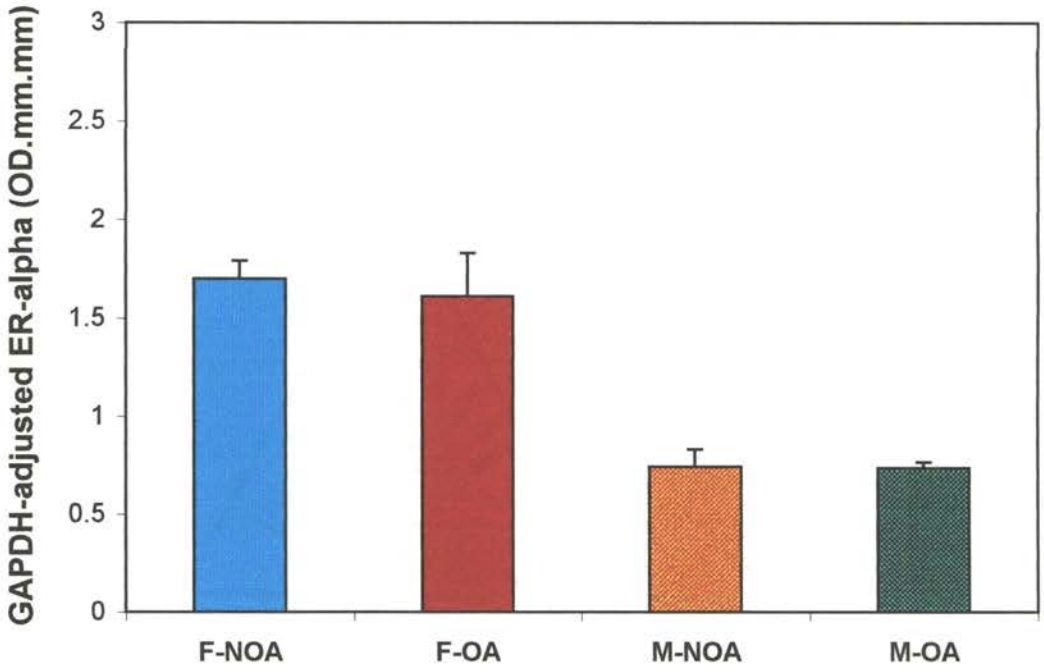


Representative RT-PCR analysis for detection of estrogen receptor (α) in male cartilage specimens. ***Lane 1*** represents the 100 bp ladder. ***Lane 2*** is represent negative control (water). ***Lanes 3-6*** are amplification of cDNA from male cartilage without OA. ***Lanes 7-10*** are from male cartilage with OA. ***Lane 11*** is amplification of cDNA from female ovary (positive control). Products are expected size of 460bp length resolved on a Tris-acetate-EDTA agarose gel.



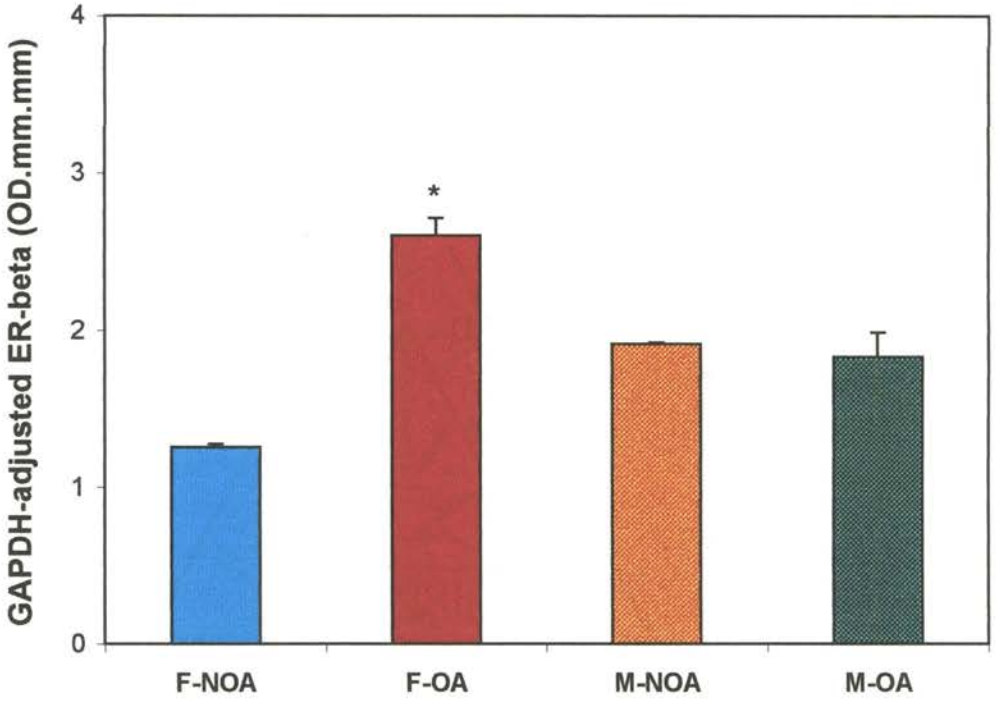
Integrity of the isolated RNA and equal loading of specimen for RT-PCR amplification was assured by assessing GAPDH gene expression ***Lanes 1-4*** are amplification of cDNA from male cartilage without OA. ***Lanes 5-8*** are from male cartilage with OA. ***Lane 9*** is amplification of cDNA from female uterus (positive control). Products are expected size of 156bp length resolved on a Tris-acetate-EDTA agarose gel.

Figure 3



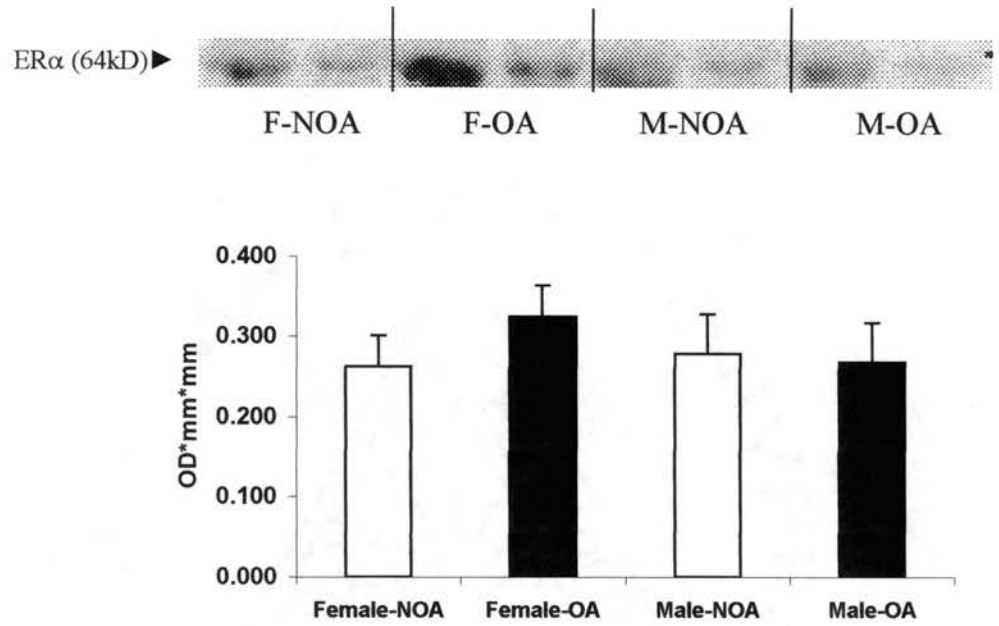
Gene expression for ERα in cartilage specimens standardized by glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) expression from male and female with and without osteoarthritis. Bars represent mean ± SE; n = 3-4. NOA, individuals without osteoarthritis; OA, individuals with osteoarthritis

Figure 4



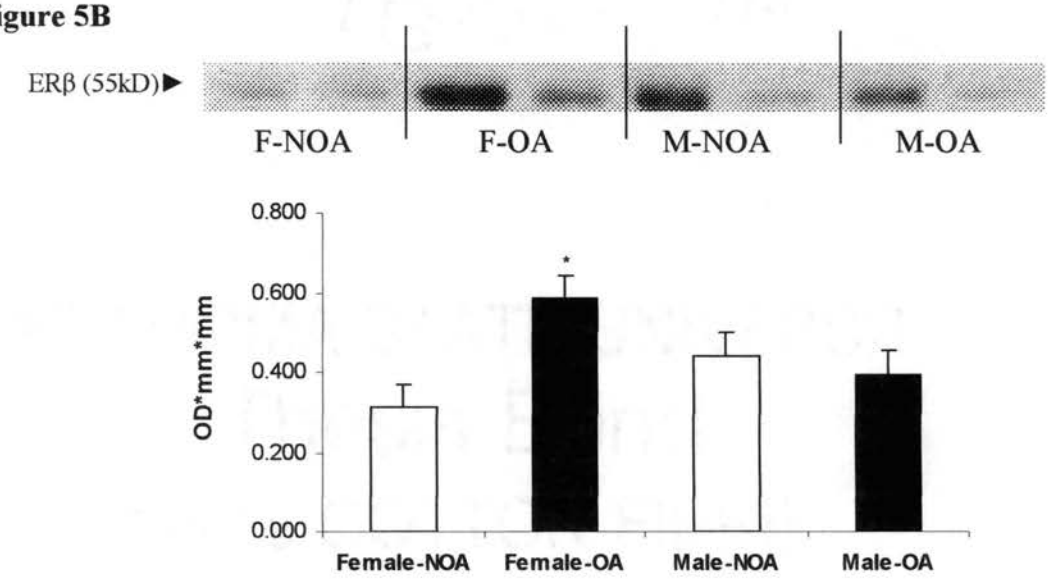
Gene expression for ERβ in cartilage specimens standardized by glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) expression from male and female with and without osteoarthritis. Bars represent mean ± SE; n = 3-4. *Asterisk denotes significantly ($P < 0.0001$) different then others. NOA, individuals without osteoarthritis; OA, individuals with osteoarthritis.

Figure 5A



Representative Western blot analysis of ERα in cartilage specimens from male and female with and without osteoarthritis Bars represent mean ± SE; n = 3-8; NOA (Non-Osteoarthritis); OA (Osteoarthritis)

Figure 5B

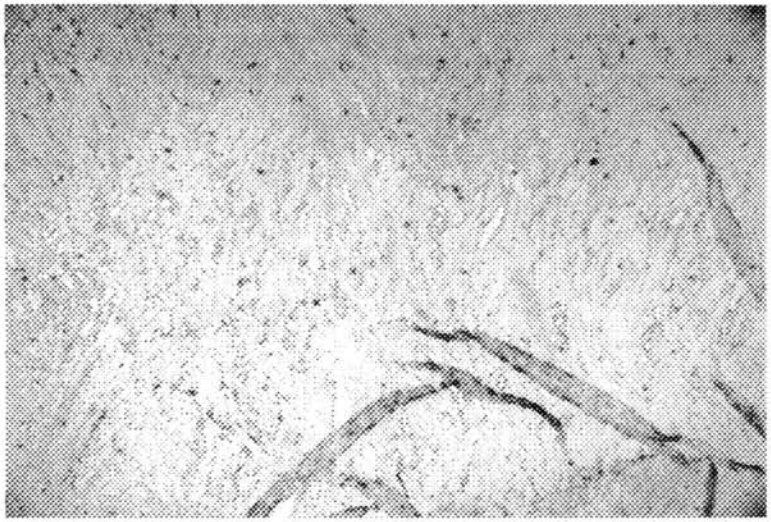


Representative Western blot analysis of ERβ in cartilage specimens from male and female with and without osteoarthritis. Bars represent mean ± SE; n = 3-8.*Asterisk denotes significantly ($P < 0.05$) different then others. NOA (Non-Osteoarthritis); OA (Osteoarthritis)

Figure 6a and 6b

Immunocytochemical localization of ERα in human articular cartilage

A



B

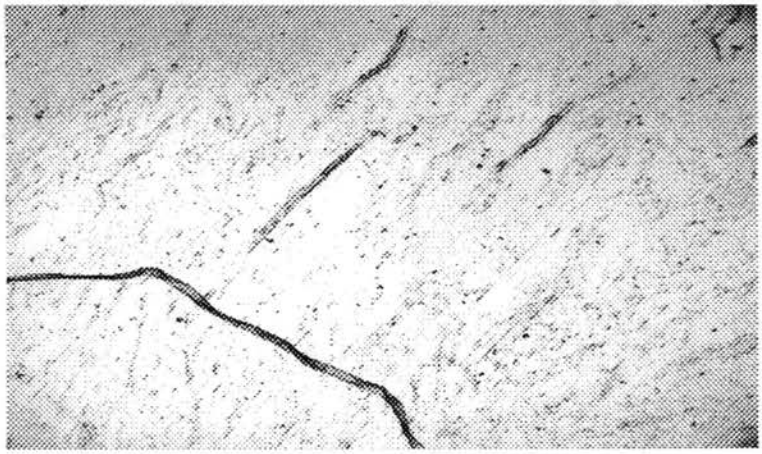


Figure A. An articular cartilage section incubated with polyclonal rabbit ERα antibody against human ERα. ***Figure B.*** A section of articular cartilage specimen incubated with normal rabbit serum (Magnification X 40).

Figure 7a and b

Immunocytochemical localization of ERβ in human articular cartilage

A

B

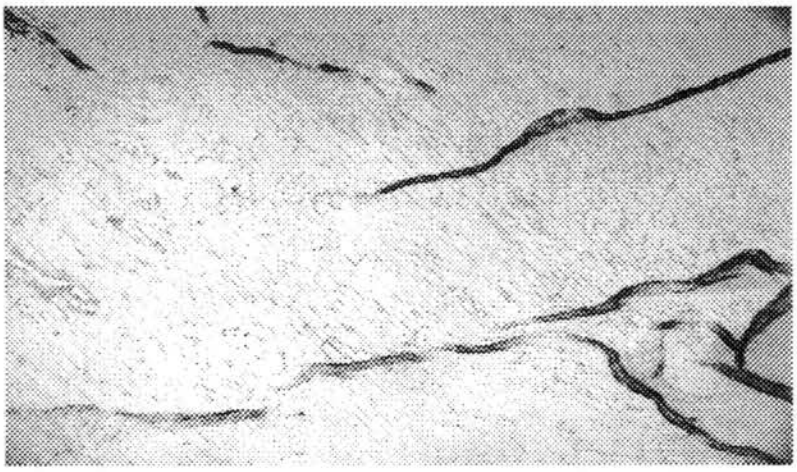


Figure A. An articular cartilage section incubated with polyclonal rabbit ERβ antibody against human ERβ. ***Figure B.*** A section of articular cartilage specimen incubated with normal rabbit serum (Magnification X 40).

Figure 8a and b

Immunocytochemical localization of ERα and ERβ in human ovary and uterine tissue used as positive control.

A

B

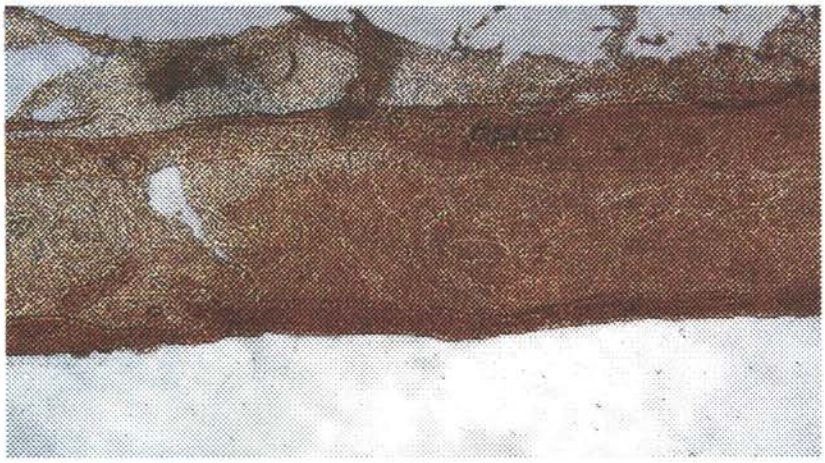
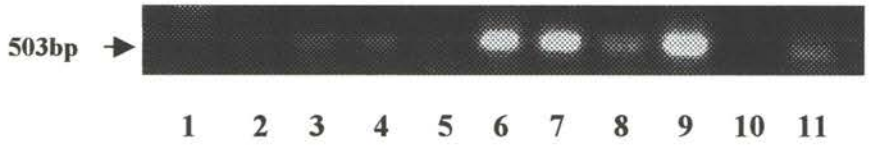
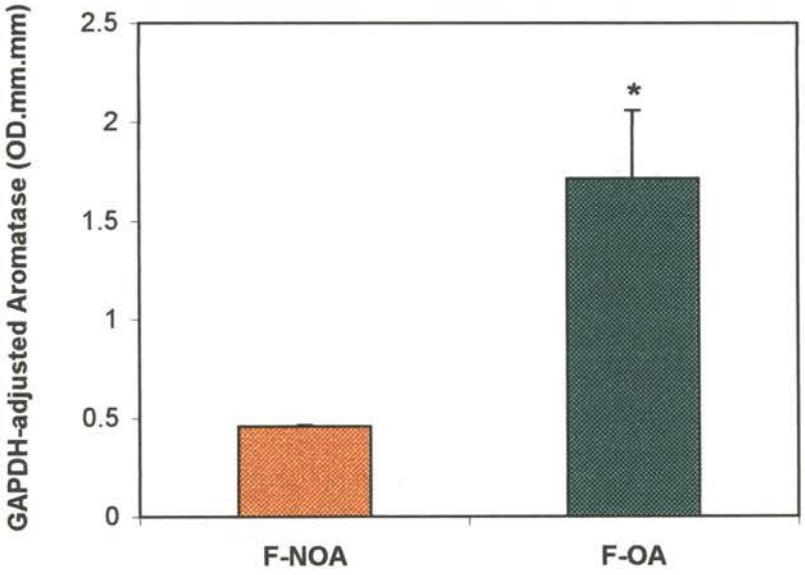


Figure A. A section of ovarian tissue incubated with polyclonal rabbit ERα antibody against ERα. ***Figure B.*** A section from uterine tissue specimen incubated with polyclonal rabbit ERβ antibody against human ERβ (Magnification X 40)

Figure 9

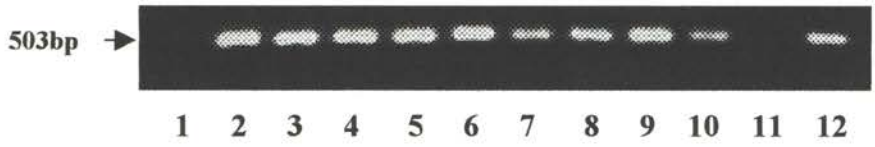


Representative RT-PCR analysis for detection of aromatase in female cartilage specimens ***Lane 1*** represents the 100 bp ladder. ***Lanes 2-5*** are amplification of cDNA from female cartilage without OA. ***Lanes 6-9*** are cartilage specimen from female with OA. ***Lane 10*** represents negative control (water). ***Lane 11*** is amplification of cDNA from female uterus (positive control). Products are expected size of 503bp length resolved on a Tris-acetate-EDTA agarose gel.



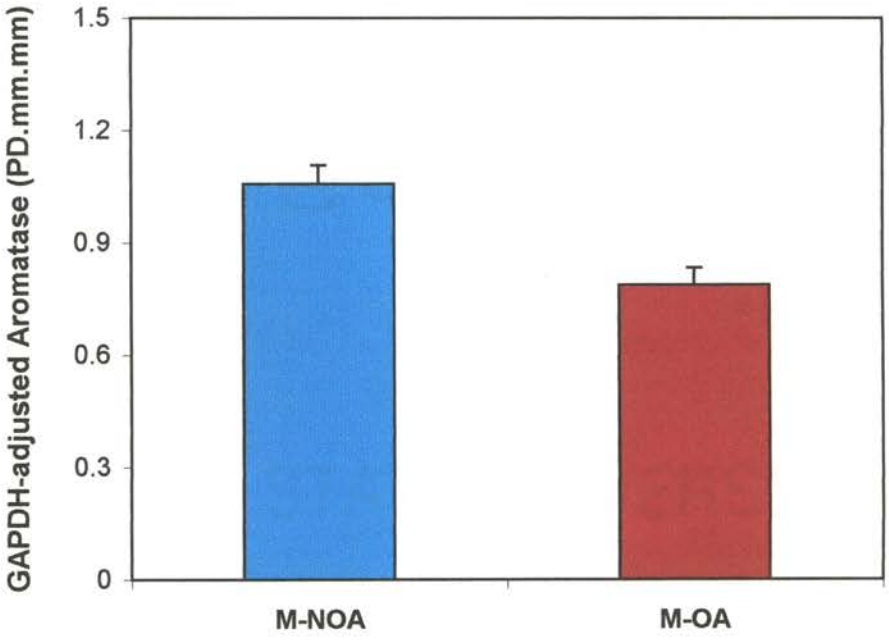
Gene expression for aromatase standardized by glyceraldehyde-3 phosphate dehydrogenase (GAPDH) expression in cartilage from female with (F-OA) and without osteoarthritis (F-NOA). Bars represent mean+SE; n = 4 samples per group. *Asterisk denotes significantly ($P < 0.05$) different then others.

Figure 10



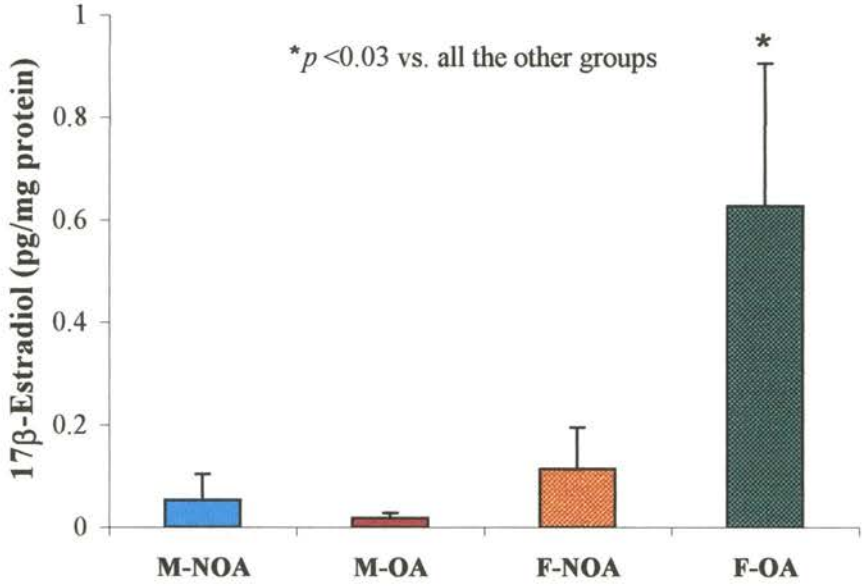
RT-PCR analysis for detection of aromatase in male cartilage specimens

Lane 1 represents the 100 bp ladder. ***Lanes 2-6*** are amplification of cDNA from male cartilage without OA. ***Lanes 7-10*** are from male cartilage with OA. ***Lane 11*** represents negative control (water). ***Lane 12*** is amplification of cDNA from female uterus (positive control). Products are expected size of 503bp length resolved on a Tris-acetate-EDTA agarose gel.



Gene expression for aromatase standardized by glyceraldehyde-3 phosphate dehydrogenase (GAPDH) expression in cartilage from male with (M-OA) and without osteoarthritis (M-NOA). Bars represent mean+SE; n = 4 samples per group.

Figure 11



Protein-normalized 17β-estradiol concentrations in synovial fluid of men and women with and without osteoarthritis. Bars represent mean+SE; n = 5-9 samples per group. *Asterisk denotes significantly ($P < 0.03$) different then others. M, male; F, female; NOA, individuals without osteoarthritis; OA, individuals with osteoarthritis

CHAPTER IV

WOMEN WITH OSTEOARTHRITIS HAVE ELEVATED SYNOVIAL FLUID LEVELS OF INSULIN-LIKE GROWTH FACTOR (IGF)-I AND IGF-BINDING PROTEIN-3

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Summary

Objective: The present study explores the possible connection between synovial fluid concentrations of insulin like growth factor (IGF)-I, IGF-binding protein (IGFBP)-3 and leptin in osteoarthritis (OA).

Methods: Synovial fluid specimens were obtained from a total of thirty-four individuals (21 females and 13 males) with and without OA. Synovial fluid specimens were analyzed to determine IGF-I, IGFBP-3, leptin, and C-reactive protein (CRP)

concentrations.

Results: The presence of IGFBP-3 was detected in synovial fluid of both men and women with and without OA. Protein-normalized measurements of IGF-I, IGFBP-3, and leptin concentrations in synovial fluid showed significantly ($P < 0.05$) elevated levels in women with knee OA but not in men. There were no significant differences in synovial fluid CRP concentrations in either gender with OA.

Conclusion: This study provides initial evidence that protein normalized IGF-I and IGFBP-3 and leptin levels increase in synovial fluid of women but not in men with OA versus those without OA. These findings, if confirmed imply that the etiology of OA differ in men versus women. These molecules though anabolic by nature are perhaps accumulated in the synovial fluid due the loss of their uptake by cartilage tissue.

Key words: cartilage, insulin-like growth factor, leptin, osteoarthritis, synovial fluid

Osteoarthritis (OA) is a common joint disease affecting mainly the hands, spine, hips, and knees. In OA a progressive loss of articular cartilage occurs which results in debilitating pain and disability (1). The etiology of OA is diverse with various predisposing factors rather than a single disease-causing entity (2). However, the distinguishing changes in cartilage leading to its degeneration are not well understood. One of the earliest events in OA is an increase in the water content of cartilage (3). This implies that there is a failure in the elastic restraint of the collagen network due to structural alterations (4), suggesting a shift in the cytokine/growth factor-controlled matrix homeostasis (5). This shift between synthesis and degradation ultimately favors

catabolic processes (5).

One factor important in cartilage synthesis is insulin-like growth factor-I (IGF-I). This growth factor has been shown to stimulate the production of chondrocyte extracellular matrix components and the synthesis of collagen and proteoglycan (6-9).

These anabolic effects help restore disrupted homeostasis even in the presence of the catabolic cytokines such as interleukin-1β (IL-1) and tumor necrosis factor-α (TNF-α) (10,11). Although the levels of IGF-I mRNA expression are high in (12) and in the synovial fluid (13) in OA, the arthritic chondrocytes appear non-responsive to the anabolic effects of IGF-I (14). This lack of responsiveness may be explained by the increased presence of binding proteins in synovial fluid of the afflicted joints (13), thus limiting the availability of IGF-I.

In addition to IGF-I, other factors such as leptin may contribute to the maintenance of cartilage integrity (15,16). Recently, leptin receptors have been reported to be present in human chondrocytes (16) suggesting that cartilage is a leptin responsive tissue. In support of a role for leptin in cartilage, Fingenschau et al. have shown that chondrocytes stimulated with leptin have increased proteoglycan and collagen production (16). These observations served as a basis for examining the relationship between synovial fluid levels of IGF-I and leptin in the present study.

MATERIALS AND METHODS

Subjects and tissue collection. The purpose of this study was to assess markers of cartilage metabolism in synovial fluid of osteoarthritic subjects and to make comparison

with those of non-osteoarthritic individuals. The study protocol and consent form were approved by the Institutional Review Board at Oklahoma State University. Synovial fluid specimens were obtained from a total of thirty-four individuals (21 females and 13 males) with and without OA (Table 1). Based on patient history, the incidence of knee OA in these individuals was unrelated to traumatic injury to the joints. Subjects were referred to the surgeon for the repair of their knees, and were otherwise considered healthy. On the day of surgery, synovial fluid was collected, aliquoted, and stored at -80°C for analyses. The joints of patients with insignificant levels of synovial fluid were irrigated with 1 to 2 ml of normal saline and subsequent analyses were normalized to the synovial total protein concentration. Total protein concentrations in the synovial fluid were determined using reagents from Roche Diagnostics (Montclair, NJ) and analyzed by the Cobas-Fara II Clinical Analyzer. Synovial fluid values were corrected based on total protein levels.

Synovial fluid insulin-like growth factor-I Synovial fluid IGF-I concentrations were determined using radioimmunoassay (RIA; Diagnostic Systems Laboratory; Webster, TX) technique. Briefly, aliquoted synovial fluid samples were thawed and centrifuged at 2400 x g for 2 min (17). A 100 μL aliquot of the specimen was added to a microcentrifuge tube for formic acid-acetone extraction in presence of 0.5% Tween 20. The extracted specimens were centrifuged and 50 μL of the supernatant were incubated in antibody-coated tubes in presence of 100 ul of [^{125}I]-labeled IGF-I at room temperature following the manufacturer's protocol. The tubes were then decanted and counted in a gamma counter. The IGF-I concentrations in the synovial fluid specimens were determined using a log-logit curve. The curve was obtained using appropriate standards

and controls as provided by the manufacturer of the RIA kit.

Synovial fluid IGFBP-3 Levels of IGFBP-3 in synovial fluid were measured using RIA (Diagnostic Systems Laboratories; Webster, TX) technique. Briefly, aliquoted synovial fluid samples were thawed and centrifuged at 2400 x g for 2 min (17). A 100 μL aliquot of the specimen was added to a microcentrifuge tube and diluted 10-fold using a sample diluent as provided by the manufacturer. The diluted specimens were then incubated in antibody-coated tubes in presence of 100 ul of [^{125}I]-labeled IGFBP-3 at room temperature overnight as recommended by the manufacturer. The tubes were then decanted and washed with 3 mL of deionized water. The washings were repeated 2 more times and the tubes were then counted in a gamma counter for 1 minute. The standards and controls provided by the manufacturer were used in plotting a log-logit curve. The IGFBP-3 concentrations in the synovial fluid specimens were multipled by the dilution factor and determining using the log-logit curve.

Leptin concentrations in synovial fluid Synovial fluid leptin levels were measured using a non-competitive RIA (Diagnostic Systems Laboratory; Webster, TX) technique. Briefly, synovial fluid samples were thawed and centrifuged at 2400 x g for 2 min (17). A 100 μL aliquot of the specimen was added to a microcentrifuge tube. The specimens were then incubated in antibody-coated tubes in presence of 100 ul of [^{125}I]-labeled leptin at room temperature overnight as recommended by the manufacturer. The tubes were then decanted and washed with 3 mL of washing solution as provided in the kit. The washings were repeated 2 more times and the tubes were then counted in a gamma counter for 1

minute. Standards and controls provided by the manufacturer were used simultaneously to plot a log-logit curve. This curve was used to calculate the leptin concentrations in the syovial fluid specimens.

Synovial fluid C-reactive protein (CRP) CRP in synovial fluid was measured using the Antibody Reagent Set II obtained from DiaSorin (Stillwater, MN). The test was performed using the Cobas-Fara II Clinical Analyzer (Montclair, NJ). Initially, a standard curve was determined using known amounts of CRP in lyophilized serum specimens. Briefly, antisera was added to aliquoted specimens of synovial fluid and incubated for 1 minute. After which, the absorbance was measured and the CRP concentration was extrapolated from a standard curve.

Statistical analysis Data analysis involved estimation of means, standard error of mean (SE), and analysis of variance using InStat Software (Version 3.0; San Diego, CA) . When the analysis of variance indicated significant difference, the differences among means were investigated using the Tukey's multiple comparison procedure. $P<0.05$ was considered statistically significant.

RESULTS

In the present study, the amounts of synovial fluid specimens were limited. This restricted our ability to perform the validation tests for the concentrations of IGF-I, IGFBP-3, leptin, and CRP. Another limitation is that certain joints had to be lavaged by

using 1 to 2 mL of saline, therefore we could no longer assess these parameters per mL synovial fluid. That is the reason for reporting synovial fluid values of these markers per mg protein. This type of normalization has not been reported in the literature and the reproducibility of our data needs to be confirmed. Another point that should be mentioned is that there are currently no RIA kits specifically designed to assess the concentrations of IGF-I, IGFBP-3, leptin, and CRP in the synovial fluid and hence the reliability, specificity, and validity of these RIA kits for use in synovial fluid need to be addressed.

Results of the synovial fluid analyses are presented in Table 2. Synovial fluid specimens from female patients with OA had significantly higher concentrations of IGF-I, IGFBP-3, and leptin in comparison with females without OA. These significantly higher levels persisted even when values for IGF-I (Figure 1), IGFBP-3 (Figure 2), and leptin (Figure 3) were normalized to total protein in the synovial fluid.

In men, no statistically significant differences in synovial fluid IGF-I, IGFBP-3, and leptin (Table 2) or their protein-normalized concentrations (Figures 1, 2 and 3, respectively) were found in individuals with and without OA.

Synovial fluid CRP and protein normalized CRP levels were not significantly different in OA afflicted individuals compared to individuals without OA in either gender (Table 2).

DISCUSSION

The balance between synthesis and degradation of cartilage matrix in joint tissue

is disrupted under pathological conditions such as ostearthritis, rheumatoid arthritis, and other related disorders (18,19,20). Some of the most common characteristics of arthritic cartilage in the severe stages of the disease are decreased levels of macromolecules and increased degradative products. This study assessed the synovial fluid levels of IGF-I, IGFBP-3, and leptin in individuals with and without OA.

Accumulating evidence suggests that IGF-I has an important role in maintaining one of the principal functions of cartilage cells and that is the production of the matrix proteoglycan molecules (10,21,22). In this study, synovial fluid levels of IGF-I as expressed per mL were close to four-fold higher in women with OA compared to those without OA. The amount of synovial fluid surrounding the afflicted knee varies from one individual to the next. In some cases, to obtain synovial fluid samples from patients it is required that the joints be lavaged with saline resulting in the levels of solutes in these samples to become dilute. Therefore, expressing solute levels per mg total protein may adjust for this variability. In support of this notion, when IGF-I levels were expressed per mg protein, women with OA had around a two-fold increase in synovial fluid IGF-I only when compared to their normal counterparts. In either case, because IGF-I was higher in OA, it is expected that cartilage synthesis should prevail in women with OA. However, as our data and those of others indicate, levels of IGF-binding proteins are also increased in OA (13,23). The bioavailability of the IGF-I is regulated by IGF-binding proteins, of which at least 6 different classes are known, with the most predominant binding protein being IGFBP-3 (24,25). In relation to the higher IGF-I, the levels of protein adjusted IGFBP-3 in synovial fluid were proportionately higher in women with OA compared to those without OA. This suggests that although IGF-I is more abundant in OA, it may not

be bioavailable to exert its beneficial effects on cartilage. Furthermore, increased local production of IGFBP-3 has been suggested in inflamed joints (25). Thus, further limiting the action of IGF-I during the progression of the disease (23). Development of treatment strategies that would either increase the levels of bioavailable IGF-I and/or decrease the local production of IGF- binding proteins may be beneficial to restore homeostasis in OA.

In addition to IGF-I, recent reports have suggested that the cytokine-like hormone, leptin, may play a role in cartilage (16). In order to examine whether synovial fluid levels of leptin are affected in OA, we assessed synovial fluid leptin concentrations in individuals with and those without OA. Our results indicate that, at least in women and similar to IGF-I, both unadjusted as well as protein-normalized leptin levels are significantly higher in OA compared to normal. It is not clear whether the higher levels of leptin in synovial fluid are a result of increased local production of leptin or a reflection of higher circulating leptin levels. Higher circulating leptin levels have been reported in obesity (26,27,28) and being overweight is a predisposing factor in OA (29,30). Unfortunately, anthropometric data could not be obtained from individuals who participated in this study. Nonetheless, increased proteoglycan and collagen synthesis have been observed in *in vitro* studies where normal chondrocytes were stimulated with leptin (16). This suggests that leptin, similar to IGF-I, in the local environment of the chondrocyte stimulates cartilage repair. Whether leptin receptors are present in the cartilage of individuals with OA and whether these receptors are functional leading to higher levels of proteoglycan or collagen production need to be assessed in future studies.

Synovial fluid levels of IGF-I, IGFBP-3, and leptin were not different in men

whether with or without OA, indicating that these factors may not necessarily be involved in the pathophysiology of OA in men. However, since the men in this study may be at different stages of disease progression than the women, it is possible that the abundance and availability of IGF-I may have been influenced by the stage of OA (31). In OA, as the repair process initially keeps pace with the progression of the disease, eventually it is superceded by the degenerative process leading to a more advanced stage of cartilage destruction (22,32). However, the stage of OA progression in our subject was not determined. Additionally, differences between genders in relation to OA have previously been described (33) including age of onset (34), hormonal effects (35,36), and underlying causes (37). Further examinations of synovial fluid as well as cartilage using screening tools for multiple factors such as microarray technology followed by more discriminating tools such as immunohistochemistry may provide basis for identification of distinguishing characteristics in men with OA versus those without OA.

In summary, although the findings of the present study are of interest, these data should be considered preliminary and need to be confirmed in future studies. It would be meritorious to also assess these parameters in serum and investigate whether they correlate with synovial fluid levels.

REFERENCES

1. Jacobsson LTH. Definitions of osteoarthritis in the knee and hand. Ann Rheum Dis 1996; 55:656-658.
2. Felson DT, Zhang Y, and Hannan MT. Risk factors for incident radiographic knee osteoarthritis in the elderly. Arthritis Rheum 1997; 40:728-733.
3. Bagge E, Bjelle A, Eden S, and Svanborg A. Factors associated with radiographic osteoarthritis: results from the population study 70-year old people in Goteberg. J Rheumatol 1991; 18:1218-1222.
4. Chu CQ, Field M, Allard S, Abney E, Feldmann M, and Maini RN. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cyotokines in cartilage destruction and repair. Br J Rheumatol 1992; 31:653-661.
5. Pelletier JP, DiBattista JA, Roughley P, McCollum R, and Martel-Pelletier J. Cytokines and inflammation in cartilage degradation. Rheum Dis Clin N Am 1993; 19:545-568
6. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, and Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986; 240:423-430.
7. Sandell LJ and Dudek EJ. Insulin-like growth factor I stimulates type II collagen gene expression in cultured chondrocytes. Trans Orthop Res Soc 1988; 35:300.
8. Franchimont P and Bassler C. Effects of hormones and local growth factors on articular chondrocyte metabolism. J Rheumatol 1991; 18(Suppl 27):68-70.

9. Tesch GH, Handley CJ, Cornell HJ, and Herrington AC. Effects of free and bound insulin-like growth factors on proteoglycan metabolism in articular cartilage explants. J Orthop Res 1992; 10:14-22.

10. Tyler JA. Insulin-like growth factor-1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cyokines. Biochem J 1989; 260:543-548.

11. Homandberg GA, Hui F, and Wen C. Fibronectin-fragment-induced cartilage chondrolysis is associated with release of catabolic cytokines. Biochem J 1997; 321:751-757.

12 Middleton JFS and Tyler JA. Upregulation of IGF-I gene expression in the lesions of osteoarthritic human cartilage. Ann Rheum Dis 1992; 51:440-447.

13. Tavera C, Abribat T, Reboul P, Dore S, Brazeau P, Pelletier JP, and Pelletier JM. IGF and IGF-binding protein system in the synovial fluid of osteoarthritic and rheumatoid arthritic patients. Osteoarthritis Cart 1996; 4:263-274.

14. Dore S, Pelletier JP, DiBattista JA, Tardif G, Brazeau P, and Martel-Pelletier J. Human osteoarthritic chondrocytes possess an increase number of insulin-like growth factor I binding sites but are unresponsive to its stimulation. Possible role of IGF-binding proteins. Arthritis Rheum 1994; 37:253-263.

15. Aspden RM, Schieven BAA, and Hutchison JD. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. Lancet 2001; 357:1118-1120.

16. Figenschau Y, Knutsen G, Shahzeydi S, Johansen O, and Sveinbjornsson B. Human articular chondrocytes express functional leptin receptors. Biochem Biophys Res Comm 2001; 287:190-197.

17. Pelletier JP, Roughley PJ, DiBattista JA. Are cytokines involved in osteoarthritic pathophysiology. Semin Arthritis Rheum 1991; 20:12-25.

18. Geborek P, Saxne T, Heinegard D, and Woilheim FA. Measurement of synovial fluid using albumin dilution upon intraarticular saline injection. J Rheumatol 1988; 15:57-60.

19. DiCesare PE, Carlson CS, and Stolerman ES. Increased degradation and altered tissue disturbution of cartilage oligomeric protein in human rheumatoid and osteoarthritic cartilage. J Orthop Res 1996; 14:946-955.

20. Nakamura S, Kamihagi K, and Satakeda H. Enhancement of SPARC (osteonectin) synthesis in articular cartilage. Increased levels in synovial fluids from patients with rheumatoid arthritis and regulation by growth factors and cytokines in chondrocyte cultures. Arthritis Rheum 1996; 39:539-551.

21. Schneiderman R, Rosenberg N, and Hiss J. Concentration and size distribution of insulin-like growth factor-1 in human normal and osteoarthritic synovial fluid and cartilage. Arch Biochem Biophys 1995; 324:173-188.

22. Martel-Pelletier J, Di Battista JA, Lajeunesse D, and Pelletier JP. IGF/IGFBP axis in cartilage and bone in osteoarthritis pathogenesis. Inflamm Res 1998; 90-100.

23. Tardif G, Reboul P, Pelletier JP, Geng C, Cloutier JM, and Martel-Pelletier J. Normal expression of type I insulin-like growth factor receptor by human osteoarthritic chondrocytes with increased expression and synthesis of insulin-like growth factor binding proteins. Arthritis Rheum 1996; 39:968-978.

24. Rechler MM. Insulin-like growth factor binding proteins. Vitam Horm 1993; 47:1-14.

25. Neidel J, Blum WF, Schaeffer HJ, Schulze M, Schonau E, Lindschau J, and Foll J. Elevated levels of insulin-like growth factor (IGF) binding protein –3 in rheumatoid arthritis synovial fluid inhibit stimulation by IGF-I of articular chondrocyte proteoglycan synthesis. Rheumatol Int 1997; 17:29-37.

26. Seck T, Englaro P, Blum WF, Scheidt-Nave C, Rascher W, Ziegler R, and Pfeilschifter J. Leptin concentrations in serum from a randomly recruited sample of 50- to 80- year old men and women: positive association with plasma insulin-like growth factors (IGFs) and IGF binding protein-3 in lean, but not in obese individuals. Eur J Endocrinol 1998; 138:70-75.27.

27. Das UN. Is obesity an inflammatory condition? Nutrition 2001; 17:953-966.

28. Ruhl CE and Everhart JE. Leptin concentrations in the United States: relations, demographic, and anthropometric measures. Am J Clin Nutr 2001; 74:295-301.

29. Felson DT, Anderson JJ, Naimark A, Walker AM, and Meenan RF. Obesity and knee osteoarthritis. Ann Intern Med 1988; 109:18-24.

30. Sandmark H, Hogstedt C, Lewold S, and Vingard E. Osteoarthrosis of the knee in men and women in association with overweight, smoking, and hormone therapy. Ann Rheum Dis 1999; 58:151-155.

31. Middleton J, Manthey A, and Tyler J. Insulin-like growth factor (IGF) receptor, IGF-I, interleukin-1 beta (IL-I beta) and IL-6 mRNA expression in osteoarthritic and normal human cartilage. J Histochem Cytochem 1996; 44:131-144.

32. Vignon E, Arlot M, Hartmann D, Moyen B, and Ville G. Hypertropic repair of articular cartilage in experimental osteoarthritis. Ann Rheum Dis 1983; 42:82-88.

33. Hart DJ and Spector TD. The relationship of obesity, fat distribution, and osteoarthritis in women in the general population: the Chingford Study. J Rheumatol 1993; 20:331-335.

34. Pai Y-C, Rymer WZ, Chang RW, and Sharma L. Effect of age and osteoearthritis on knee proprioception. Arthritis Rheum 1997; 40:2260-2265.

35. Cauley JA, Kwoh CK, and Egeland G. Serum sex hormones and severity of osteoarthritis of the hand. J Rheumatol 1993; 20:1170-1175.

36. Zhang Y, McAlindon TE, and Hannan MT. Estrogen replacement therapy and worsening of radiographic knee osteoarthritis: The Framingham Study. Arthritis Rheum 1998; 41:1867-1873.

37. Anderson JJ and Felson DT. Factors associated with osteoarthritis of the knee in the first National Health and Nutrition Examination Survey (HANES I), evidence for an association with overweight, race, and physical demands of work. Am J Epidemiol 1988; 128:179-189.

Table I

Description of study participants

Gender	Status of Knee Joint	N=	Mean Age ± SE
Female	N-OA	7	39.7 ± 2.9
	OA	14	57.8 ± 5.5
Male	N-OA	9	42.7 ± 3.9
	OA	4	59.8 ± 10.5

Non-Osteoarthritis (N-OA); Osteoarthritis (OA)

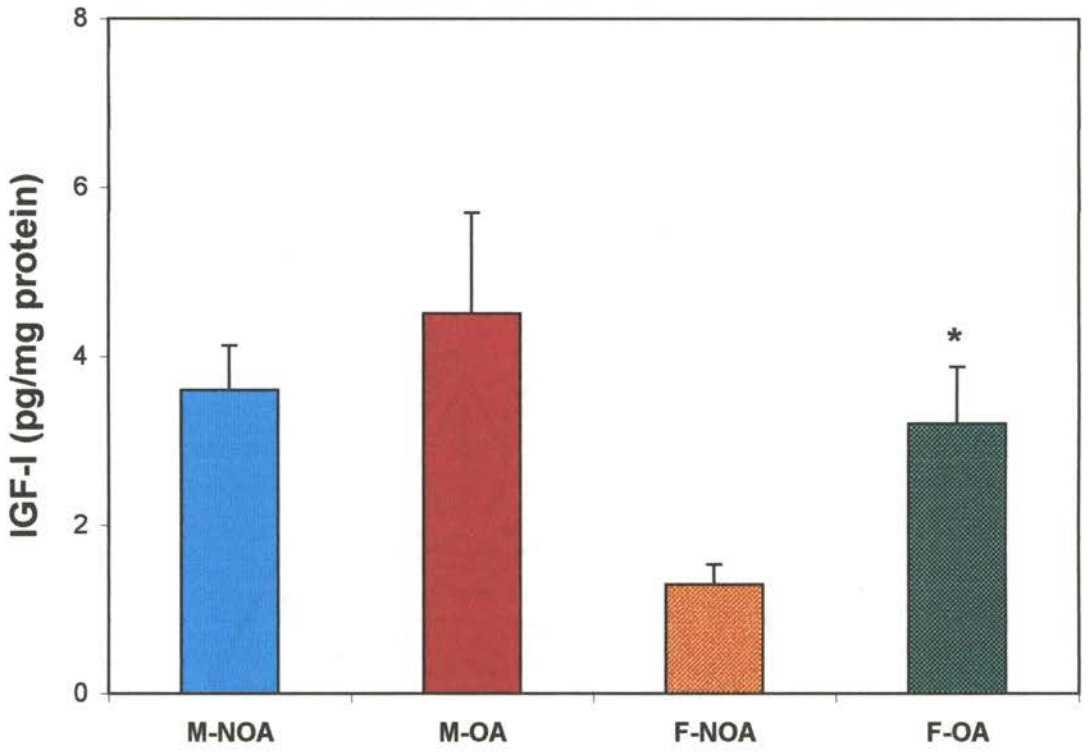
Table II

Synovial fluid analysis from women and men with and without osteoarthritis

Synovial Fluid	**F-NOA**	**F-OA**	**M-NOA**	**M-OA**
Leptin (ng/mL)	7.89 ± 4.75	34.23 ± 5.54*	15.34 ± 4.12	23.95 ± 7.43
IGF-I (pg/mL)	13.2 ± 5.3	52.5 ± 14.2*	51.8 ± 8.8	66.9 ± 10.4
IGFBP-3 (ng/mL)	5.0 ± 1.2	53.4 ± 4.0*	58.4 ± 16.9	74.6 ± 3.7
C-Reactive Protein (CRP; μg/mL)	1.92 ± 0.65	3.62 ± 0.78	2.04 ± 0.32	3.12 ± 1.30
Normalized CRP (μg/mg protein)	0.27 ± 0.07	0.27 ± 0.04	0.21 ± 0.05	0.20 ± 0.09

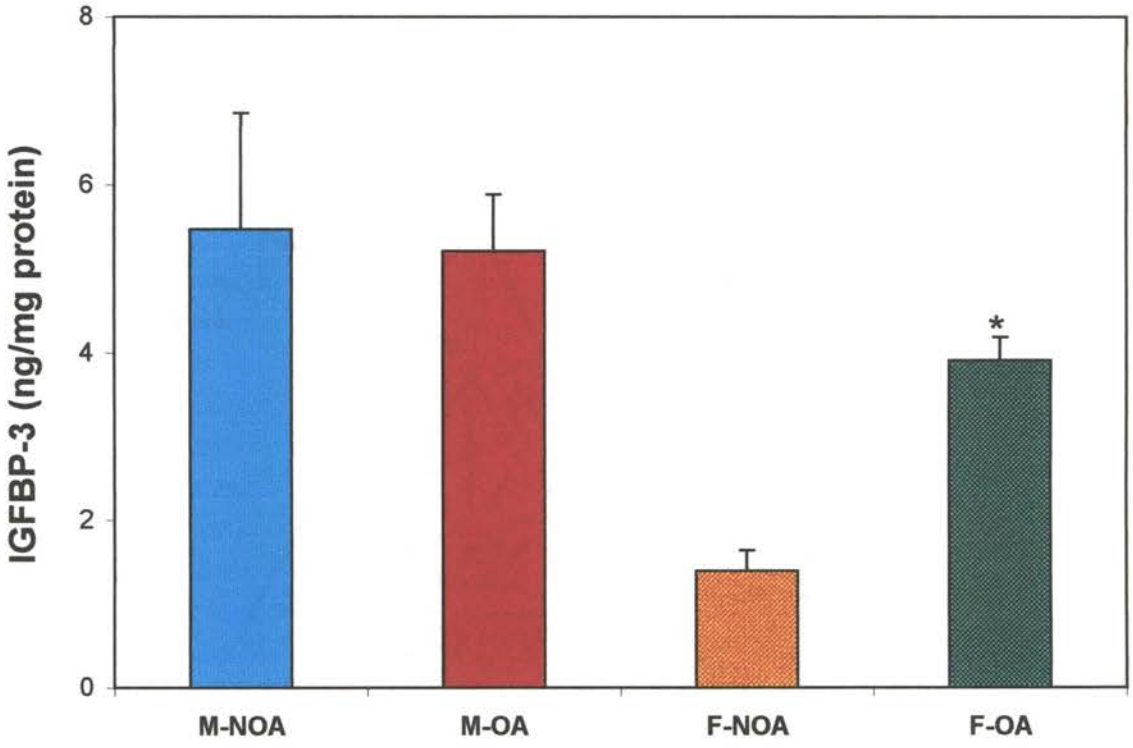
Values are mean ± SE; n = 4-14; * asterisk denotes values that are significantly different ($P < 0.05$) then the non-osteoarthritic counterpart. F-NOA (female non-osteoarthritis); F-OA (female osteoarthritis); M-NOA (male non-osteoarthritis); and M-OA (male osteoarthritis)

Figure 1



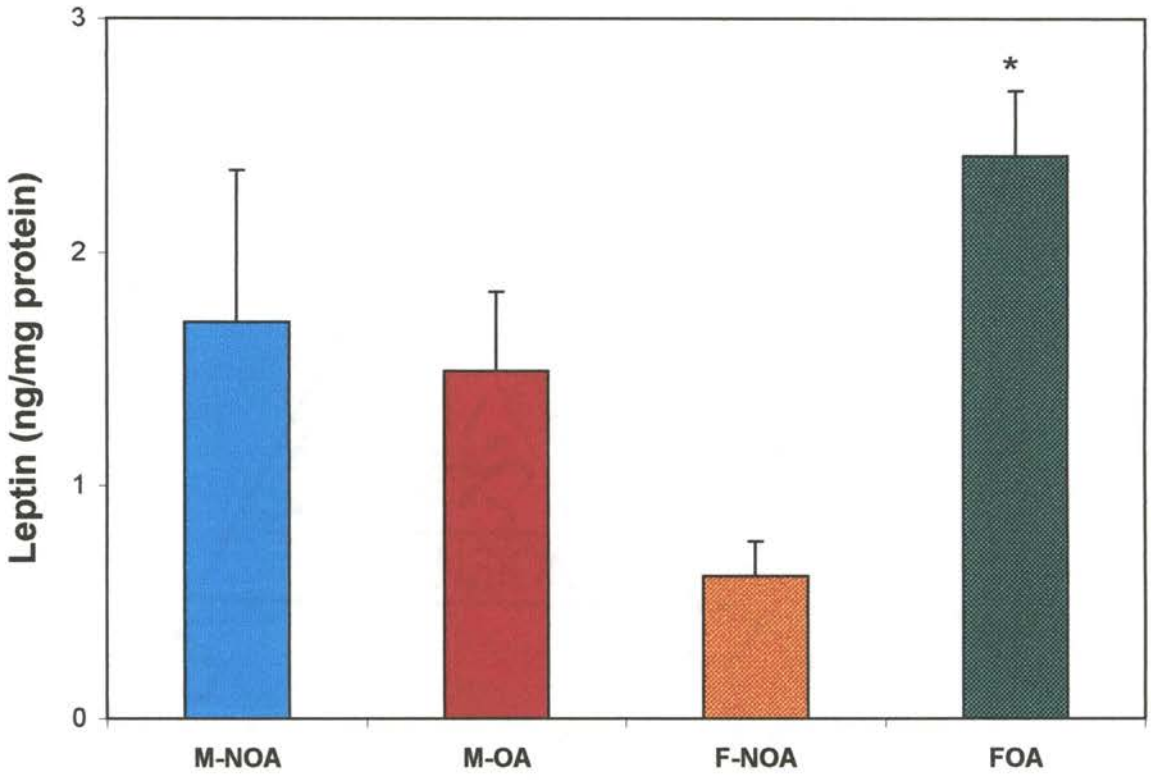
Protein-normalized insulin-like growth factor-I (IGF-I) concentrations in synovial fluid of men and women with and without osteoarthritis. Bars represent mean+SE; n = 4-7 samples per group. *Asterisk denotes that mean protein-normalized IGF-I values in synovial fluid are significantly ($P < 0.05$) different in comparison to those of female without osteoarthritis. M, male; F, female; NOA, without osteoarthritis; OA, with osteoarthritis

Figure 2



Protein-normalized insulin-like growth factor binding protein-3 (IGFBP-3) concentrations in synovial fluid of men and women with and without osteoarthritis. Bars represent mean+SE; n = 4-9 samples per group. *Asterisk denotes that mean protein-normalized IGFBP-3 values in synovial fluid are significantly ($P < 0.05$) different in comparison to those of female without osteoarthritis. M, male; F, female; NOA, individuals without osteoarthritis; OA, individuals with osteoarthritis

Figure 3



Protein-normalized leptin concentrations in synovial fluid of men and women with and without osteoarthritis. Bars represent mean+SE; n = 4-11 samples per group. *Asterisk denotes mean protein-normalized leptin values are significantly ($P < 0.05$) different in comparison to those of female without osteoarthritis. M, male; F, female; NOA, individuals without osteoarthritis; OA, individuals with osteoarthritis

CHAPTER V

SUMMARY, CONCLUSIONS, IMPLICATIONS AND LIMITATIONS

Summary

The principal objective of this research was to elucidate if estrogen receptors (ERs) exist in human cartilage and whether their abundance varied between men and women with and without osteoarthritis (OA). Furthermore, this study was designed to investigate if there was a relationship between the abundance of ERs and higher 17β-estradiol (E_2) levels in the synovial fluid of individuals with OA in comparison to individuals without OA. This study also assessed the selective anabolic and catabolic markers of cartilage metabolism in the synovial fluid of individuals with and without OA.

In Chapter I of this dissertation, three objectives are listed. Chapter III addressed objective 1 and 2, whereas Chapter IV addressed objective 3. The summary of outcomes for each objective will be presented and then general conclusions and recommendations for future research will be discussed.

In Chapter III, amplification of cDNA from articular cartilage tissues using RT-PCR showed the presence of mRNA for ERβ and ERα. Furthermore, mRNA levels for ERβ were greater in articular cartilage specimens obtained from women with osteoarthritic joints compared to specimens obtained from nonosteoarthritic joints. Western blot analysis confirmed the elevated protein levels of ERβ but not ERα in women with OA in comparison to individuals without OA. The mRNA and protein levels for both ERα and ERβ did not differ in men with or without OA. Positive nuclei immunostaining confirmed the presence of both ERα and ERβ in the cartilage specimens

obtained from patients with OA. The mRNA levels of aromatase in articular cartilage of women with OA were found to be higher than those of women without OA. No difference between aromatase mRNA levels was observed among men.

In regards to objective 2, synovial fluid specimens from female patients with OA had significantly ($P<0.03$) higher concentrations of E_2 in comparison with those of non-osteoarthritic females or males with or without OA. However, there were no statistically significant differences in E_2 levels of synovial fluid between men with or without OA.

In Chapter 3, synovial fluid specimens from female patients with OA had significantly higher concentrations of insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3), and leptin ($P < 0.05$, 0.001, 0.01, respectively) in comparison with females without OA. These significantly higher levels persisted even when values for IGF-I, IGFBP-3, and leptin ($P < 0.05$, 0.05, 0.05, respectively) were normalized to total protein in the synovial fluid. In men, no statistically significant differences in synovial fluid IGF-I, IGFBP-3, and leptin or their protein-normalized concentrations were found in individuals with and without OA. Synovial fluid C-reactive protein (CRP) and protein normalized CRP levels were not significantly different in OA afflicted individuals compared to individuals without OA in either gender.

Conclusions

The findings of this study suggest the presence of both ERα and ERβ in articular cartilage with greater abundance of ERβ compared to ERα, indicating that ERs may have a functional role in cartilage metabolism. These findings suggest that there may be a cause and effect relationship that exists between the incidence of OA and the increased

presence of the ERβ sub-type in cartilage. It is possible that this is analogous to the presence of ERα in mammary tissues and its connection with breast cancer. However, this is speculative and further investigation is needed to confirm whether such a relationship exists.

In the present study, synovial fluid concentrations of E_2 were significantly greater in women with OA as compared to those without OA or men with or without OA. Hence, higher levels of estrogen in synovial fluid may play a role in the etiology of OA in women but not in men. Whether a similiar relationship exists in men with respect to androgen concentrations has to be investigated. The higher E_2 concentrations in synovial fluid of women with OA leads us to speculate that localized E_2 in the synovial cavity may be involved in the degenerative process of cartilage. At this time, the pathophysiology of estrogen accumulation in the synovial fluid of osteoarthritic joints is not clear. Since higher expression of mRNA for aromatase was detected only in women with OA, this may partly explain the increased concentration of E_2 in synovial fluid of these women.

Accumulating evidence suggests that IGF-I has an important role in maintaining one of the principal functions of cartilage cells, which is the production of the matrix proteoglycan molecules. This study assessed the synovial fluid levels of IGF-I, IGFBP-3, and leptin in individuals with and without OA. Synovial fluid levels of IGF-I as expressed per mL were close to four-fold higher in women with OA compared to those without OA. The amount of synovial fluid surrounding the afflicted knee varies among individuals and hence this variation affects the levels of solutes present in the specimens. Therefore, by expressing solute levels per mg total protein the variability can be adjusted. In support of this statement, when IGF-I levels were expressed per mg protein, women with OA had around a

two-fold increase in synovial fluid IGF-I only when compared to their normal counterparts. In either case, because IGF-I was higher in OA, it is expected that cartilage synthesis should prevail in women with OA. However, as our data and those of others indicate, levels of IGF-binding proteins are also increased in OA. The bioavailability of the IGF-I is regulated by IGF-binding proteins, of which at least 6 different classes are known, with the most predominant binding protein being IGFBP-3. In relation to the higher IGF-I, the levels of protein adjusted IGFBP-3 in synovial fluid were proportionately higher in women with OA compared to those without OA. This suggests that although IGF-I is more abundant in the synovial fluid of patients with OA, it may not be bioavailable to exert its beneficial effects on cartilage. Development of treatment strategies that would either increase the levels of bioavailable IGF-I and/or decrease the local production of IGF-binding proteins may be beneficial to restore homeostasis in OA.

In addition to the anabolic effect of IGF-I on cartilage, recent reports have suggested that the cytokine-like hormone, leptin, may play a role in cartilage metabolism. In order to examine whether synovial fluid levels of leptin are affected in OA, we assessed synovial fluid leptin concentrations in individuals with and those without OA. Our results indicated that in synovial fluid of women, similar to changes in IGF-I, both unadjusted as well as normalized leptin levels were significantly higher in individuals with OA. It is not clear whether this higher level of leptin in synovial fluid is a result of increased local production of leptin or a reflection of higher circulating leptin levels. Higher circulating leptin levels have been reported in obesity and being overweight is a predisposing factor in OA. Nonetheless, increased proteoglycan and collagen synthesis have been observed in *in vitro* studies where normal chondrocytes were stimulated with

leptin. This observation suggests that leptin, similar to IGF-I, in the local environment of the chondrocytes stimulates cartilage repair. Whether leptin receptors are present in the cartilage of individuals with OA need to be assessed in future studies.

Synovial fluid levels of either IGF-I and IGFBP-3, or leptin were not different in men irrespective of their disease status, indicating that these factors may not necessarily be involved in the pathophysiology of OA in men.

Implications

The findings of this study, at least, in the case of women with knee OA, suggest that women may benefit from compounds that can compete for estrogen receptors and perhaps neutralize the deleterious effects of estrogen on cartilage tissue. Therefore, women with OA may benefit from agents such as selective estrogen receptor modulators (SERMs) that can compete with estrogens in occupying ERs, particularly ERβ present in the knee cartilage. SERMs are defined as a group of compounds behaving as estrogen agonists in certain tissues while acting as antagonists in some other tissues. To this date, ERβ has been found in certain tissues including brain, bone, bladder, and vascular epithelial which are responsive to estrogen as well as SERMs. Recent investigations on the effects of synthetic and naturally occurring SERMs on various tissues suggest that these diverse molecules may influence target tissues by exerting their effects mainly through ERβ. The tissue distribution of these two ERs differs which may explain the agonistic or antiagonistic properties of SERMs in various tissues. Therefore, cells that preferentially express ERβ including chondrocytes, as observed in this study, are more likely to respond to SERMs. However, this concept is speculative and requires further

investigation. The impact of naturally occurring SERMs such as genistein and daizein and synthetic SERMs such as ipriflavone and raloxifene on improvement of OA conditions remains to be shown.

Whether the increased levels of IGF-I and leptin in the synovial fluid of women with OA are due to lack of their diffusion and uptake by cartilage tissue remain to be illustrated. Similar implications may exist in male OA, however, because of the small sample size used in this study no conclusions can be made regarding the role of these anabolic factors. Future studies are needed to explore the pathophysiological pathways which may cause degeneration of cartilage resulting in OA in men and women and to identify whether there are gender differences.

Limitations

In this study, as with any study utilizing fresh operating room specimens the amounts of cartilage and synovial fluid specimens were limited to the samples made available by the orthopedic surgeon. Because the amount of articular cartilage and synovial fluid specimens varied from one subject to the next, it limited our ability to perform the proposed analyses. In order to address the issue of variations in the amount of synovial fluid samples, the orthopedic surgeon lavaged the joint with 1 to 2 mL saline to flush the contents of the joint. Hence, reporting the values of the measured markers based on volume, though reported in the literature, was not appropriate. For these reasons, we had to normalize the concentrations of E_2, IGF-I, IGFBP-3, CRP, and leptin in the synovial fluid to total protein concentration. This type of normalization has never

been done in other laboratories, hence it would be necessary to validate our findings in future studies. Furthermore, the isolation of RNA from articular cartilage was very difficult due to its rubbery texture which further limited the number of assays that we could run which in certain cases reduced the sample size and statistical power of the study.

Another point that should be emphasized is that there are currently no RIA kits specifically designed to assess the concentrations of E_2, IGF-I, IGFBP-3, CRP, and leptin in the synovial fluid. We use kits manufactured for serum analyses which utilized controls and standards provided by the manufacturers. In spite of using serum kits for synovial fluid, the analyses that were performed produced values and curves that were comparable to the manufacturer's recommended levels. Future studies should address the reliability, specificity, and validity of these kits for use in synovial fluid. Unfortunately, we were unable to address these issues in our study due to the small amounts of synovial fluid available. Furthermore, we were unable to look at parallelism or recovery from analyses where the specimens were initially extracted and then diluted to measure the markers such as IGF-I and IGFBP-3. Due to our limited sample size and volume, we were unable to calculate the inter- and intra- assay variability. Therefore, these issues have to be addressed in future studies.

Due to our limitations in the present study, it would be necessary to confirm the findings of this study using a much larger sample size. This larger sample size and tissue availability will allow us to pool specimens within the same gender and joint status group and will provide us with adequate RNA samples for more quantitative analysis of gene expression using Northern blot and Real-Time PCR. With a larger sample size, it would

also make it possible to perform more in-depth immuncytochemistry analysis of the estrogen receptors as well as analysis of other cartilage markers. If a larger pool of subjects were available for studying changes in synovial fluid in OA, it would be feasible to perform validation studies for the immunoassay kits that are designed to be used for serum analyses.

LITERATURE CITED

Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Arthritis Rheum 1986; 29:1039-1049.

Altman R, Alarcon G, Applerouth D, et al. Criteria for classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990; 33:1601-1610.

Altman R, Alarcon G, Appelrouth D, et al. Criteria for classification and reporting of osteoarthritis of the hip. Arthritis Rheum 1991; 34:505-514.

Amin AR, Vyas P, Attur M, et al. The mode of action of aspirin-like drugs: effect on inducible nitric oxide synthase. Proc Natl Acad Sci 1995; 92:7926-7930.

Amir G, Pirie CJ, Rashad S, Revell PA. Remodelling of subchondral bone in osteoarthritis: a histomorphometric study. J Clin Pathol 1992; 45:990-992.

Anderson JJ, Felson DT. Factors associated with osteoarthritis of the knee in the first National Health Nutrition Examination Survey (HANES I), evidence for an association with overweight, race, and physical demands of work. Am J Epidemiol 1988; 128:179-189.

Arden NK, Nevitt MC, Lane NE, et al. The Study of Osteoporotic Fracture Research Group. Osteoarthritis and risk of falls, rate of bone loss, and osteoporotic fractures. Arthritis Rheum 1999; 42:1378-1385.

Arend WP, Dayer JM. Cytokines and growth factors. In: Kelley WN, Harris ED, Ruddy S. (Eds), Textbook of Rheumatology, Vol 1. Saunders, 1993, pp 227-247.

Arnold CC, Djurhuus JC, Heefordt J, et al. Interaosseous phlebography, intraosseous measurements and ^{99m}-Tc-polyphosphate scintigraphy in patients with various painful conditions in the hip and knee. Acta Orthop Scand 1980; 51:19-28.

Athanasiou KA, Rosenwasser MP, Buckwalter JA, et al. Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage. J Orthop Res 1991; 9:330-340

Aydelotte MB, Michal LE, Reid DR, et al. Chondrocytes from the articular surface and deep zone express different, but stable, phenotypes in alginate gel culture. Trans Orthop Res Soc 1996; 21:317.

Aydelotte MB, Schumacher BL, Kuettner KE. Heterogeneity of articular chondrocytes. In. Kuettner KE, Schleyerbach R, Peyron JG, et al. (Eds); Articular cartilage and osteoarthritis. New York, NY, Raven Press, 1992, pp 237-249.

Bagge E, Bjelle A, Svanborg A. Radiographic osteoarthritis in the elderly. A cohort comparison and the longitudinal study of the "70-year old people of Goteborg". Clin Rheum 1992a; 11:486-491.

Bagge E, Bjelle A, Valkenburg HA, et al. Prevalence of radiographic osteoarthritis in two elderly European populations. Rheumatol Int 1992b; 12:33-38.

Bassleer C, Rovati L, Franchimont P. Stimulation of proteoglycan production by glucosamine sulfate in chondrocytes isolated from human osteoarthritic articular cartilage in vitro. Osteoarthritis Cart 1998; 6:427-434.

Baum C, Kennedy DL, Forbes MB. Utilization of nonsteroidal anti-inflammatory drugs. Arthritis Rheum 1985: 28:686-691.

Bellamy N, Buchanan WW, Goldsmith CH, et al. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antiheumatic drug therapy in patients with osteoarthritis of the hip and knee. J Rheumatol 1988; 15:1833-1840.

Bensen WG, Fiechtner JJ, McMillen JI, et al. Treatment of osteoarthritis with celecoxib, a cyclooxygenase-2 inhibitor: a randomized controlled trial. Mayo Clin Proc 1999; 74:1095-1105.

Bert JM. Role of abrasion arthroplasty and debridement in the management of osteoarthritis of the knee. Rheum Dis Clin North Am 1993; 19:725-739.

Bert JM, Maschka K. The arthroscopic treatment of unicompartment gonarthrosis. Arthroscopy 1989; 5:25-32.

Bradley JA, Brandt KD, Katz BP, et al. Comparison of an antiinflammatory dose of ibuprofen, an analgesic dose of ibuprofen, and acetaminphen in the treatment of patients with osteoarthritis of the knee. N Engl J Med 1991; 325:87-91.

Bradley JA, Brandt KD, Katz BP, et al. Treatment of knee osteoarthritis: relationship of clinical features of joint inflammation to the response to a non-steroidal antiinflammatory drug or pure analgesic. J Rheumatol 1992; 19:1950-1954.

Brandt KD. Toward pharmacologic modification of joint damage in osteoarthritis. Ann Intern Med 1995; 122:874-875.

Brennan FM, Chantry D, Jackson A. Inhibitory effect of TNF-alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet 1989; 2:244-247.

Bruckner P, Mendler M, Steinmann B, et al. The structure of human collagen type IX and its organization in fetal and infant cartilage fibrils. J Biol Chem 1988; 263:16911-16917.

Buckwalter JA. Mechanical injuries and articular cartilage. In: Finerman G. (Ed); Biology and Biomechanics of the Traumatized Synovial Joint. Park Ridge, IL, American Academy of Orthopaedic Surgeons, 1992, pp 83-96.

Buckwalter JA. Activity vs rest in the treatment of bone, soft tissue and joint injuries. Iowa Orthop J 1995; 15:29-42.

Buckwalter JA. Cartilage researcher tell progress: technologies hold promise, but caution urged. Am Acad Orthop Surg Bull 1996a; 44:24-26.

Buckwalter JA. Regenerating articular cartilage: why the sudden interest? Orthopaedics Today 1996b; 16:4-5.

Buckwalter JA, Lane NE. Aging, sports, and osteoarthritis. Sports Med Arthrosc Rev 1996; 4:276-287.

Buckwalter JA, Lane NE. Athletics and osteoarthritis. Am J Sports Med 1997; 25:873-883.

Buckwalter JA, Lomander S. Operative treatment of osteoarthrosis: current practice and future development. J Bone Joint Surg Am 1994; 76:1405-1418.

Buckwalter JA, Mankin HJ. Articular cartilage II. Degeneration and osteoarthrosis, repair, regeneration, and transplantation. J Bone Joint Surg Am 1997; 79:612-632.

Buckwalter JA, Martin JA. Degenerative joint disease. Clin Symp 1995; 47:11-32.

Buckwalter JA, Mow VC. Cartilage repair in osteoarthritis. In: Moskowitz RW, Howell DS, Goldberg VM, et al. (Eds); Osteoarthritis, Diagnosis and Medical/Surgical Management, 3rd Ed. Philadelphia, PA, WB Saunders, 2001 pp 71-107.

Buckwalter JA, Rosenberg LC, Hunziker EB, et al. Articular cartilage: composition and structure, In Woo SL-Y, Buckwalter JA, (Eds); Injury and Repair of the Musculoskeletal Soft Tissues. Park Ridge, IL, American Academy of Orthopaedic Surgeons, 1988, pp 405-425.

Buckwalter JA, Rosenberg LC, Hunziker EB. Articular cartilage: composition, structure, response to injury, and methods of facilitating repair, In: Ewing JW, (Ed); Articular Cartilage and Knee Joint Function: Basic Science and Arthroscopy. New York, NY. Raven Press, 1990, pp 19-56.

Buckwalter JA, Roughley PJ, Rosenberg LC. Age-related changes in cartilage proteoglycans: Quantitative electron microscopic studies. Microsc Res Tech 1994; 28:398-408.

Buckwalter JA, Woo SL-Y, Goldberg VM, et al. Soft tissue aging and musculoskeletal function. J Bone Joint Surg 1993; 75A:1533-1548.

Burger H, Van Daele P, Odding E, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. Arthritis Rheum 1996; 39:81-86.

Carroll GJ, Bell MC. Leukemia inhibitory factor stimulates proteoglycan resorption in porcine articular cartilage. Rheumatol 1993; 13:5-8

Chandrasekhar S, Harvey AK, Johnson MG, et al. Osteonectin/SPARC is a product of articular chondrocytes/cartilage and is regulated by cytokines and growth factors. Biochimica et Biophysica Acta 1994; 1221:7-14.

Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: Implications for the role of cytokines in cartilage destruction and repair. Br J Rheumatol 1992; 31:653-661.

Clark JM, Huber JD. The structure of the human subchondral plate. J Bone Joint Surg 1990; 72B:866-873.

Conrozier T, Saxne T, Shan Sei Fan C, et al. Serum concentrations of cartilage oligomeric protein and bone sialoprotein in hip osteoarthritis: A one year prospective study. Ann Rheum Dis 1998 57:527-532.

Cooper C, Egger P, Coggon D, et al. Generalized osteoarthritis in women: pattern of joint involvement and approaches to definition for epidemiological studies. J Rheumatol 1996; 23:1938-1942.

Coventry M. Upper tibial osteotomy for gonarthrosis. The evolution of the operation in the last 18 years and long-term results. Orthop Clin North Am 1979; 10:191-210.

Crofford LJ, Lipsky PR, Brooks P, et al. Basic biology and clinical application of cyclooxgynase-2. Arthritis Rheum 2000; 43:4-13.

Cryer B, Feldman M. Cyclooxygenase-1 amd cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs Am J Med 1998; 302:1179-1185.

Davis MA. Epidemiology of osteoarthritis. Clin Geriat Med 1988; 4:241-255.

Dayer JM, Fenner H. The role of cytokines and their inhibitors in arthritis. Baillieres Clin Rheumatol 1992; 6:485-516

Denko CW, Boja B. Growth factors in assymptomatic osteoarthritis: Insulin, insulin-like growth factor-I, growth hormone. Immunopharmacology 1993; 2:71-76.

Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis: Insulin, IGF-I, growth hormone. J Rheumatol 1990; 17:1217-1221.

Dequeker J. The relationship between osteoporosis and osteoarthritis. Clin Rheum Dis 1985; 11:271-296.

Dequeker J, Boonen S, Aerssens J, et al. Inverse relationship osteoarthritis-osteoporosis: what is the evidence? What are the consequences? Br J Rheumatol 1996; 35:813-820.

Dequeker J, Mokassa L, Aerssens J, et al. Bone density and local growth factors in generalized osteoarthritis. Microsc Res Tech 1997; 37:358-371.

Dieppe P. Osteoarthritis and molecular markers. A rheumatologist's perspective. In: Heinegard D, Lomander S, Saxne T. (Eds); Molecular markers for joint and skeletal diseases. Lund, Sweden; Acta Ortho Scand 1995; pp 1-5.

Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity of oral glucosamine sulfate in osteoarthrosis: a placebo controlled double-blind invesitigation. Clin Ther 1980; 3:260-272.

Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology of disease. FASEB J 1998; 12:1063-1073.

Dye S, Chew M. The use of scintigraphy to detect increased osseous metabolic activity about the knee. J Bone Joint Surg Am 1993; 75:1388-1406.

Eastgate JA, Symons JA, Wood NC. Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis Lancet 1988, 2:706-709.

Ehrich EW, Dallob A, De Lepeleire I, et al. Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. Clin Pharmacol Ther 1999 65:336-347.

Engkvist O, Johansson SH. Perichondrial arthroplasty: a clinical study in twenty-six patients. Scand J Plast Reconstr Surg 1980; 14:71-87.

Felson, DT. Epidemiology of hip and knee osteoarthritis. Epidemiologic Reviews 1988; 10:1-28.

Felson DT, Naimark A, Anderson J, et al. The prevalence of knee osteoarthritis in the elderly. Arthritis Rheum 1987; 30:914-918.

Felson DT, Nevitt MC. The effects of estrogen on osteoarthritis. Curr Opin Rheumtol 1998; 10:269-272.

Felson DT, Zhang Y, Hannan MT, et al. The incidence and natural history of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthritis Rheum 1995; 38:1500-1505.

Finkelstein A, Gross A, Davis A. Varus osteotomy of the distal part of the femur: a survivorship analysis. J Bone Joint Surg Am 1996; 78:1348-1352.

Fischer AE, Carpenter TA, Tyler JA, et al. Visualisation of mass transport of small organic molecules and metal ions through articular cartilage by magnetic resonance imaging. Magn Reson Imaging 1995; 13:819-826.

Fisher NM, Pendergast DR. Effect of a muscle exercise program on exercise capacity in subjects with osteoarthritis. Arch Phys Med Rehabil. 1994; 75:792-797.

Fisher NM, Pendergast DR. Reduced muscle function in patients with osteoarthritis. Scand J Rehabil Med 1997; 29:213-221.

Georges C, Vigneron H, Ayral X, et al. Serum biologic markers as predictors of disease progression in osteoarthritis of the knee. Arthritis Rheum 1997; 40:590-591.

Goldring MB, Sandell LJ, Stephenson ML, Krane SM. Immune interferon suppresses levels of procollagen mRNA and type II collagen synthesis in cultured human articular and costal chondrocytes. J Biol Chem 1986; 261:9049-9056.

Gouze J-N, Bordiji K, Gulberti S, et al. Interleukin-1□down-regulated the expression of glucuronyltransferase I, a key enzyme priming glycosaminoglycans biosynthesis. Arthritis Rheum 2001; 44:351-360.

Grynpas MD, Alpert B, Katz I, et al. Subchondral bone in osteoarthritis. Calcif Tissue Res 1991; 49:20-26.

Guerne PA, Blanco F, Kaelm A, et al. Growth factor responsiveness of human articular chondrocytes in aging and development. Arthritis Rheum 1995; 38:960-968.

Guilak F, Sah R, Setton LA. Physical regulation of cartilage metabolism. In: Mow VC, Hays WC. (Eds); Basic Orthopaedic Biomechanics, 2nd ed. Philadelphia, PA Lippincott-Raven, 1997, pp 179-207.

Gustafsson JA. Therapeutic potential of selective estrogen receptor modulators. Curr Opin Chem Biol 1998; 2:508-511.

Hagiwara H, Schroter-Kermani C, Merker HJ. Localization of collagen type VI in articular cartilage of young and adult mice. Cell Tissue Res 1993; 272:155-160.

Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem 1993; 268:25803-25810.

Hannan MT, Anderon JJ, Zhang Y, et al. Bone mineral density and knee osteoarthritis in elderly men and women: The Framingham Study. Arthritis Rheum 1993; 36:1671-1680.

Hannan MT, Felson DT, Anderson JJ, et al. Estrogen use and radiographic osteoarthritis of the knee in women. The Framingham Osteoarthritis Study. Arthritis Rheum 1990; 525-532.

Hardingham TE, Fosang AJ, Dudhai J. Aggrecan, the chondroitin/sulfate/keratan sulfate proteoglycan from cartilage. In: Kuettner KE, Schleyerbach R, Peyron JG, et al (Eds); Articular Cartilage and Osteoarthritis. New York, NY, Raven Press, 1992, pp 45-63.

Hart DJ, Cronin C, Daniels M, et al. The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee. The Chingford Study. Arthritis Rheum 2002; 46:92-99.

Hart DJ, Mootosamy I, Doyle DV, et al. The relationship between osteoarthritis and osteoporosis in the general population. The Chingford Study. Ann Rheum Dis 1994; 53:158-162.

Hart DJ, Nandra D, Spector TD. Assessing joint space in progression of early osteoarthritis: a comparison of magnified reticule and atlas grades. Arthritis Rheum 1995; 38:S239.

Hass J. Functional arthroplasty. J Bone Joint Surg 1944; 26:297-306.

Hauselmann HJ. Nutripharmaceuticals for osteoarthritis. Best Prac Res Clin Rheumatol 2001; 15:595-607.

Hawkey CJ. COX-2 inhibitors. Lancet 1999; 353:307-314.

Healey JH, Vigorita VJ, Lane JM. The coexistence and characteristics of osteoarthritis and osteoporosis. J Bone and Joint Surg 1985; 67A:586-592.

Hedborn E, Antonsson P, Hjerpe A, et al. Cartilage matrix proteins. An acidic oligomeric matrix protein (COMP) detected only in the cartilage. J Biol Chem 1992; 267:6132-6136.

Heinegard D, Inerot S, Ollsson SE, et al. Cartilage proteoglycans in degenerative joint disease. J Rheumatol 1987; 14(suppl):110-112.

Heinegard D, Saxne T. Molecular markers of processes in cartilage in joint disease. Br J Rheumatol 1991; 30(suppl):21-24.

Hernigou P, Medevielle D, Debeyre J, Goutallier D. Proximal tibial osteotomy for osteoarthritis with varus deformity. A ten- to thirteen-year follow-up study. J Bone Joint Surg Am 1987; 69:332-353.

Hochberg MC, Altman RD, Brandt KD, et al. Guidelines of the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. Arthritis Rheum 1995; 38:1541-1546.

Holt I, Cooper RG, Denton J, et al. Cytokine inter-relationships and their association with disease activity in arthritis. Br J Rheum 1992; 31:725-733.

Homandberg GA, Hui F, Wen C, et al. Fibronectin-fragment-induced cartilage chondrolysis is associated with release of catabolic cytokines. Biochem J 1997; 321:751-757.

Homminga GN, Bulstra SK, Bouwmeester PM, Linden AJVD. Perichondrial grafting for cartilage lesions of the knee. J Bone Joint Surg Br 1990; 72:1003-1007.

Houpt JB, McMillan R, Wein C, et al. Effect of glucosamine hydrochloride in the treatment of osteoarthritis of the knee. J Rheumatol 1999; 26:2423-2430.

Hu B, Trinh K, Figueira WF, Price PA. Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chintinase protein family J Biol Chem 1996; 271:19415-19420.

Hurley MV. The role of muscle weakness in the pathogenesis of osteoarthritis. Rheum Dis Clin North Am 1999; 25:283-298.

Ismaiel S, Atkins RM, Pearse MF, et al. Susceptibility of normal and arthritic human articular cartilage to degradative stimuli. Br J Rheumatol 1992; 31:369-373.

Itay S, Abramovici A, Nevo Z. Use of cultured embryonal chick epiphyseal chondrocytes as grafts for defects in chick articular cartilage. Clin Orthop 1987; 220:284-303.

Itay S, Abramovici A, Ysipovitch Z, Nevo Z. Correction of defects in articular cartilage by implants of cultures of embryonic chondrocytes. Trans Orthrop Res Soc 1988; 13:112.

Iwase T, Hasegawa Y, Kawamoto K, et al. Twenty years' follow-up of intertrochanteric osteotomy for treatment of the dysplastic hip. Clin Orthop 1996; 331:245-255.

Jimenez SA. The effects of glucosamine on human chondrocyte gene expression. The Ninth Eular Symposium, Madrid, Spain;1996; pp 8-10.

Johansen JS, Jensen HS, Price PA. A new biochemical marker for joint injury: analysis of YKL-40 in serum and synovial fluid. Br J Rheumtol 1993; 32:949-955.

Johansen JS, Hvolris J, Hansen M, et al. Serum YKL-40 levels in healthy children and adults: comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. Br J Rheumatol 1996; 35:553-559.

Johansen JS, Christoffersen K, Moller S, et al. Serum YKL-40 is increased in patients with hepatic fibrosis. J Hepatol 2000; 32:911-920.

Johansen JS, Olee T, Price PA, et al. Regulation of YKL-40 production by human articular chondrocytes. Arthritis Rheum 2001; 44:826-837.

Jones G, Nguyen T, Sambrook PN, et al. Osteoarthritis, bone density, postural stability, and osteoporotic fractures: a population based study. J Rheumatol 1995; 22:921-925.

Kamibayashi L, Wyss UP, Cooke TD, Zee B. Trabecular microstructure in the medial condyle of the proximal tibia of patients with knee osteoarthritis. Bone 1995; 17:27-35.

Kellgren JH, Lawrence JS. Radiological assessment of osteoarthritis. Ann Rheum Dis 1957; 16:494-501.

Kellgren JH, Lawrence JS. Atlas of Standard Radiographs, Department of Rheumatology and Medical Illustrations, University of Manchester, Oxford, Blackwell, 1963.

Kirkpatrick RB, Emery JG, Connor JR, et al. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. Exp Cell Res 1997; 237:46-54.

Koch AE, Kunkel SL, Chensue SW, et al. Expression of interleukin-1 and interleukin-1 receptor anatagonist by human rheumatoid synovial tissue macrophages. Clin Immunol Immunopathol 1992; 65:23-29.

Kraus VB. Pathogenesis and treatment of osteoarthritis. Med Clin N Am 1997; 81:85-107.

Kuettner KE, Aydellote MB, Thonar E. Articular cartilage matrix and structure: a minireview. J Rheumatol 1991; 18(suppl):46-48.

Kuettner KE, Goldberg VM. Intr In: Kuettner KE, Goldberg VM, eds. Osteoarthritis Disorders. Rosemont, IL, American Academy of Orthopaedic Surgeons, 1995, pp 11-15.

Kuiper GGJM, Enmark E, Pelto-Huikko M, et al. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996; 93:5925-5930

Kuiper GGJM, Shughrue PJ, Merchenthaler I, Gustaffsson JA. The estrogen receptor b-subtype: a novel mediator of estrogen action in neuroendocrine systems. Front Neuroendocrinol 1998; 19:253-286.

Lai WM, Mow VC, Roth V. Effects of nonlinear strain dependent permeability and rate of compression on the stress behavior of articular cartilage. J Biomech Eng 1981; 103:61-66.

Laine L, Harper S, Simon T, et al. A randomized trial comparing the effect of rofecoxib, a cyclooxygenase-2 specific inhibitor, with that of ibuprofen on gastroduodenal mucosa of patients with osteoarthritis. Gastroenterology 1999; 117:776-783.

Lane NE. Exercise: a cause of osteoarthritis. J Rheumtol Suppl 1995; 43:3-6.

Lane NE, Nevitt MC. Osteoarthritis, bone mass, and fractures: How are they related? Arthritis Rheum 2002; 46:1-4.

Lawrence JS, Bremmer JM, Bier F. Osteo-arthrosis, prevalence in the population and relationship between symptoms and x-ray changes. Ann Rheum Dis 1996; 25:1-23.

Lawrence RC, Helmick CG, Arnett FC, et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 1998; 41:778-799.

Leffler CT, Philippi AF, Leffler SG, et al. Glucosamine, chondroitin and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double blind placebo-controlled pilot study. Mil Med 1999; 164:85-91.

Leivseth G, Tortstensson J, Reikeras O. Effect of passive muscle stretching in osteoarthritis of the hip. Clin Sci 1989; 76:113-117.

Levick JR, McDonald JN, Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. Ann Rheum Dis 1995; 54:417-423.

Lipsky PE, Abramson SB, Crofford L, et al. The classification of cyclooxygenase inhibitors. J Rheumatol 1998; 25:2298-2303.

Loose LD, de Oliveria R, Sipe JD, et al. A possible systematic component of osteoarthritis: elevated concentrations (by ELISA) of C-reactive protein in serum of OA patients and modulation by tenidap. Arthritis Rheum 1993; 36(suppl):S166.

Lozada C, Altman R. Chondroprotection in osteoarthritis. Bull Rheum Dis 1997; 46:5-7.

Mankin HJ. The water of articular cartilage. In: Simon WH (Ed); The Human Joint in Health and Disease. Philadelphia, PA, University of Pennsylvania Press, 1978, pp 37-42.

Mankin HJ, Dorfman HD, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. Correlation of morphology with metabolic data. J Bone Joint Surg 1971; 53A:523.

Mankin HJ, Jennings LC, Treadwell BV, et al. Growth factors and articular cartilage. J Rheumatol 1990; 17:1217-1221.

Mankin HJ, Mow VC, Buckwalter JA, et al. Formation and function of articular cartilage. In: Sheldon R, Simon MD. (Eds); Orthopaedic Basic Science, Rosemont, IL, American Academy of Orthopaedic Surgeons, 1994, pp 1-44.

Marcelino J, McDevitt CA. Attachment of articular cartilage chondrocytes to the tissue form of type VI collagen. Biochim Biophys Acta 1995; 1249:180-188.

March LM. Osteoarthritis. MJA 1997; 166:98-103.

Maroudas A. Physiochemical properties of articular cartilage. In: Freeman MAR, (Ed); Adult Articular Cartilage. New York, Grune & Stratton, 1973, pp 131-170.

Maroudas A, Schneiderman R. "Free" and "exchangeable" or "trapped" and "non-exchangeable" water in cartilage. J Orthop Res 1987; 5:133-138.

Martel-Pelletier J, Cloutier JM, Pelletier JP. Cytokines, interleukin-1 and the tumour necrosis factor in human osteoarthritic tissue. Trans Orthop Res Soc 1990; 15:111.

Martin JA, Buckwalter JA. Articular cartilage aging and degeneration. Sports Med Arthrosc Rev 1996; 4:263-275.

Martin JA, Buckwalter JA.The role of chondrocyte-matrix interactions in maintaining and repairing articular cartilage. Biorheology 2000; 37:129-40.

Matyas JR, Sandell LJ, Adams ME. Gene expression of type II collagens in chondro-osteophytes in experimental osteoarthritis. Osteoarthritis Cartilage 1997; 5:99-105.

Maurer K. Basic data on arthritis: knee, hip, and sacrioliac joints, in adults aged 25-74 years: United States, 1971-1975. Hyattville, MD, National Center for Health Statistics, 1979. Vital and Health Statistics No. 213.

Mazieres B, Combe B, Phan Van A, et al. Chondroitin sulfate in osteoarthritis of the knee: a prospective double blind, placebo-controlled multi-center clincial study. J Rheumatol 2001; 28:171-181.

McAlindon TE, La Valley MP, Gulin JP, et al. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and metaanalysis. JAMA 2000; 283:1469-1475.

Middleton JFS, Tyler JA. Upregulation of IGF-I gene expression in the lesions of osteoarthritic human cartilage. Ann Rheum Dis 1992; 51:440-447.

Milgram JW. Morphologic alterations in the subchondral bone in advanced degenerative arthritis. Clin Orthop 1983; 173:293-312.

Miller VE, Rogers K, Muirden KD. Detection of tumour necrosis factor alpha and interleukin-1 in the rheumatoid osteoarthritic cartilage-pannus junction by immunohistochemical methods. Rheumatol Int 1993, 13:77-82.

Mills MB, Murphy SB, Poss R. Osteotomies about the hip for the prevention and treatment of osteoarthritis. J Bone Joint Surg Am 1995; 77:626-647.

Minor M. Exercise in the treatment of osteoarthritis. Rheum Dis Clin North Am 1999; 25:397-415.

Minor M, Hewett JE, Webel RR, et al. Efficiency of physical conditioning exercise in patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1989; 32:1397-1405.

Mitchell NS, Curess RL. Classification of degenerative arthritis. Can Med Assoc 1977; 117:763-765.

Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg Am 1976; 58:230-233.

Mitrovic DR, Riera H. Synovial, articular cartilage and bone changes in rapidly destructive arthropathy (osteoarthritis) of the hip. Rheumatol Int 1992; 12:17-22.

Moreale P, Manopulo R, Galati M, et al. Comparison of the anti-inflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J Rheumatol 1996; 23:1385-1391.

Moskowitz RW, Boja B, Denko CW. The role of growth factors in degenerative joint disorders. J Rheumatol 1991; 18:147-148.

Mow VC, Rosenwasser MP. Articular cartilage: Biomechanics. In: Woo SLY, Buckwalter JA (Eds), Injury and Repair of the Muscloskeletal Soft Tissue. Park Ridge, IL, American Academy of Orthopaedic Surgeons, 1988, pp 427-463.

Nathan C, Sporn M. Cytokines in context. J Cell Biol 1991, 113:981-986.

Nevitt MC, Arden NK, Lane NE, et al. Incidence of radiographic changes of hip OA in elderly white women. Arthritis Rheum 1998; 41:S181.

Nevitt MC, Cummings S, Lane NE et al. Association of estrogen replacement therapy with the risk of osteoarthritis of the hip in the elderly white women. Arch Intern Med 1996; 156:2073-2080.

Nevitt MC, Lane NE, Scott JC, et al. Study of Osteoporotic Fractures Research Group. Radiographic osteoarthritis of the hip and bone mineral density. Arthritis Rheum 1995; 38:907-916.

Nietfeld JJ, Wilbrink B, Helle M. Interleukin-1 induced interleukin-6 is required for the inhibition of proteoglycan synthesis by interleukin-1 in human articular cartilage. Arthritis Rheum 1990, 33:1695-1701.

Noack W, Fischer M, Forster KK, et al. Glucosamine sulfate in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2:51-59.

Noguchi T, Oka M, Fujino M, et al. Repair of osteochondral defects with grafts of cultured chondrocytes. Comparison of allografts and isografts. Clin Orthop 1994; 302:251-258.

Noyes F, Barber S, Simon R. High tibial osteotomy and ligament reconstruction in varus angulated, anterior cruciate ligament-deficient knees. Am J Sports Med 1993; 21:2-12.

Nyirkos P, Golds EE. Human synovial cells secrete a 39 kDa protein similar to a bovine mammary protein expressed during the non-lactating period. Biochem J 1990; 268:265-268.

O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion. J Bone Joint Surg Am 1988; 70:595-606.

O'Driscoll SW, Salter RB. The repair of major osteochondral defects in joint surfaces by neochondrogenesis and autogenous osteoperiosteal grafts stimulated by continous passive motion. An experimental investigation in rabbit. Clin Orthop 1986; 208:131-140.

Oegema TR, Carpenter RJ, Hofmeister F, Thompson RC. The interaction of the zone of calcified cartilage and the subchondral bone in osteoarthritis. Microsc Res Tech 1997; 37:324-332.

Oliveria SA, Felson DT, Reed JI, et al. Incidence of symptomatic hand, hip, and knee osteoarthritis among patients in a health maintenance organization. Arthritis Rheum 1995; 38:1134-1141.

Osborne KD, Trippel SB, Mankin HJ. Growth factor stimulation of adult articular cartilage. J Orthop Res 1989; 7:35-42.

Paech K, Webb P, Kuiper GGJM, et al. Differential ligand activation of estrogen receptor ERα and ERβ at AP1 sites. Science 1997 277:1508-1510.

Patrignani P, Panara MR, Greco A, et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. J Pharmacol Exp Ther 1994; 271:1705-1712.

Pelletier JP. The influence of tissue cross-talking on OA progression: role of nonsteroidal antiinflammatory drugs. Osteoarthritis Cartilage 1999; 7:374-376.

Pelletier JP, DiBattista JA, Roughley P, et al. Cytokines and inflammation in cartilage degradation. Rheum Dis Clin N Am 1993; 19:545-568.

Pelletier JP, Roughley PJ, DiBattista JA. Are cytokines involved in osteoarthritic pathophysiology. Semin Arthritis Rheum 1991; 20:12-25.

Perlau R, Wilson MG, Poss R. Isolated proximal fermoral osteotomy for treatment of residua of congenital dysplasia or idiopathic osteroarthrosis of the hip. Five to ten years results. J Bone Joint Surg Am 1996; 78:1462-1467.

Petersson IF, Boegard B, Dahlstrom J, et al. Bone scan and serum markers of bone and cartilage in patients with knee pain and osteoarthritis. Osteoarthritis Cart 1998; 6:33-39.

Petersson IF, Boegard B, Svensson D, et al. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. Br J Rheumatol 1998; 37:46-50.

Phillips AC, Simon LS. NSAIDs and the elderly: toxicity and the economic implications. Drugs Ageing 1997; 10:119-130.

Pincus T, Swearingen C, Wolfe R. Toward a Multi-dimensional Health Assessment Questionnaire (MDHAQ): assessment of advanced of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. Arthritis Rheum 1999; 42:2220-2230.

Poole AR. Cartilage in health and disease. In: Koopman WJ. (Eds); Arthritis and Allied Conditions. A Textbook of Rheumatology. 13th ed. Baltimore, MD, Williams & Wilkins, 1997, pp 255-308.

Poole AR, Alini M, Hollander AP. Cellular biology of cartilage degradation. In: Henderson B, Edwards JCW, Pettipher ER (Eds); Mechanisms and Models in Rheumatoid Arthritis. London, Academic Press, 1995, pp 163-204.

Pottenger LA, Phillips FM, Draganich LF. The effect of marginal osteophytes on reduction of varus-valgus instability in osteoarthritic knees. Arthritis Rheum 1990; 33:853-858.

Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. Curr Med Res Opin 1980; 7:110-114.

Qiu GX, Gao SN, Giacovelli G, et al. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. Arzneimittelforschung 1998; 48:469-474.

Radin EL, Paul IL, Rose RM. Role of mechanical factors in the pathogenesis of OA. Lancet 1972; 519-522.

Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. Clin Orthop 1986; 213:34-40.

Reginster JY, Deroisy R, Rovati LC, et al. Long-term effects of glucosamine-sulphate on osteoarthritis progression: a randomized , placebo-controlled clinical trial. Lancet 2001; 357:251-256.

Rickard DJ, Gowen M. Cytokines in arthritis. Arthritis and Rheumatism Council, 1993.

Rosenberg LC. Structure and function of dermatan sulfate proteoglycans in articular cartilage. In: Kuettner KE, Schleyerbach R, Peyron JG, et al. (Eds); Articular Cartilage and Osteoarthritis. New York, NY, Raven Press, 1992, pp 45-63.

Rosenberg LC, Buckwalter JA. Cartilage proteoglycans. In: Kuettner KE, Schleyerbach R, Peyron JG, et al. (Eds); Articular Cartilage and Osteoarthritis. New York, NY, Raven Press, 1992, pp 45-63.

Rosenberg ZS, Shankman S, Steiner GC, et al. Rapid destructive osteoarthritis: clinical, radiographic, and pathologic features. Radiology 1992; 182:213-216.

Rosner IA, Goldberg VM, Moskowitz RW. Estrogens and osteoarthritis. Clin Orthop 1986; 213:77-83.

Rosner IA, Malemud CJ, Goldberg VM, Papay RS, Getzy L, Moskowitz RW. Pathologic and metabolic responses of experimental osteoarthritis to estradiol and an estradiol antagonist. Clin Ortho Rel Res 1982; 171:280-286.

Rosner IA, Manni A, Malemud CJ, Boja B, Moskowitz RW. Estradiol receptors in articular chondrocytes. Biochemical and Biophysical Research Communications 1982; 106:1378-1382.

Roughley PJ, Lee ER. Cartilage proteoglycans: Structure and potential functions. Microsc Res Tech 1994; 28:385-397.

Rovati LC, Giacovelli G, Annefeld M, et al. A large randomised-placebo, controlled, double-blind study of glucosamine sulfate vs prioxicam and vs their association, on the kinetics of the symptomatic effect in knee osteoarthritis. Second Int Congr Osteoarthritis Res Soc 1994; 56.

Samanta A, Jones A, Regan M, et al. Is osteoarthritis in women affected by hormonal changes or smoking? Br J Rheumatol 1993; 68:366-370.

Sandy JD, Garnett D, Thompson V, et al. Chondrocyte-mediated catabolism of aggrecan: aggrecanase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine. Biochem J 1998; 335:59-66.

Saxne T. Differential release of molecular markers in joint disease. In: Heinegard D, Lomander S, Saxne T. (Eds), Molecular markers for joint and skeletal diseases. Lund, Sweden. Acta Orthop Scand 1995; pp 80-83.

Saxne T, Heinegard D. Matrix proteins: Potentials as body fluid markers of changes in the metabolism of cartilage and bone in arthritis. J Rheumatol 1995; 22(Suppl):71-74.

Schilke JM, Johnson GD, Housh TJ, et al. Effect of muscle strength training on functional status of patients with osteoarthritis of the knee joint. Nurs Res 1996; 45:68-72.

Setton LA, Elliott DM, Mow VC. Altered mechanics of cartilage with osteoarthritis: human osteoarthritis and an experimental model of joint destruction. Osteoarthritis Cartilage 1999; 7:2-14.

Sharif M, Saxne T, Sheptone L, et al. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. Br J Rheumatol 1995; 34:306-310.

Shibata H, Spencer TE, Onate SA, et al. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. Recent Prog Horm Res 1997; 52:141-165.

Simkin PA, Bassett JE. Cartilage matrix molecules in serum and synovial fluid. Curr Opin Rheumatol 1995; 7:346-351.

Simon LS, Lanza FL, Lipsky PE, et al. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor: efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, a studies of gastrointestinal and platelet effects. Arthritis Rheum 1998; 41:1591-1602.

Simon LS, Weaver AL, Graham DY, et al. The antiinflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial. JAMA 1999; 282:1921-1928.

Sipe JD. Acute-phase proteins in osteoarthritis. Semin Arthritis Rheum 1995; 25:75-86.

Smith RJ, Clin JE, Sam LM, et al. Biologic effects of an interleukin-1 receptor antagonist protein on interleukin-1 stimulated cartilage erosion and chondrocyte responsiveness. Arthritis Rheum 1991; 34:78-83.

Sokoloff L. Osteoarthritis as a remodeling process. J Rheumatol 1987; 14(suppl):7-10.

Spector T, Campion GC. Generalized osteoarthritis is a hormonally mediated disease. Ann Rheum Dis 1989; 48:256-261.

Spector TD, Hart DJ, Byrne J, et al. Definition of osteoarthritis of the knee for epidemiologic studies. Ann Rheum Dis 1993; 52:790-794.

Spector TD, Hart DJ, Nandra D, et al. Low-level increase in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 1997; 40:723-727.

Spector T, Nandra D, Hart DJ, et al. Is hormone replacement therapy protective for hand and knee osteoarthritis in women? The Chingford Study. Ann Rheum Dis 1997; 56:432-434.

Stockwell RA. The cell density of human articular and costal cartilage. J Anat 1967; 101:753-763.

Stockwell RA. Chondrocytes. J Clin Pathol 1978; 12(Suppl):7-13.

Stockwell RA. Cartilage failure in osteoarthritis: Relevance of normal structure and function: A review. Clin Anat 1993, 199:161-191.

Suomi R, Lindauer S. Effectiveness of Arthritis Foundation Aquatic Program on strength and range of motion in women with arthritis. J Aging Phys Activity 1997; 5:341-351.

Thomas RH, Resnick D, Alazraki, et al. Compartmental evaluation of osteoarthritis of the knee; a comparative study of available diagnostic modalities. Radiology 1975; 116:585-594.

Towheed TE, Hochberg MC. A systematic review of randomized controlled trials of pharmacological therapy in osteoarthritis of the knee. Semin Arthritis Rheum 1997; 27:755-770.

Treadwell BV, Mankin HJ. The synthetic processes of articular cartilage. Clinc Orthop 1986; 213:50-61.

Trippel SB. Growth factor actions on articular cartilage. J Rheum Suppl 1995; 43:129-132.

Trousdale RT, Ekkernkamp A, Ganz R, Wallrichs SL. Periacetabular and intertrochanteric osteotomy for the treatment of osteoarthrosis in dysplastic hips. J Bone Joint Surg Am 1995; 77:73-85.

Tsai CL, Liu TK, Chen TJ. Estrogen and osteoarthritis: A study of synovial estradiol and estradiol receptor binding in human osteoarthritic knees. Biochemical and Biophysical Communications 1992a; 183:1287-1291.

Tsai CL, Lui TK. Upregulation of estrogen receptors in rabbit osteoarthritic cartilage. Life Sci 1992b; 50:1727-1735.

Tyler JA. Insulin-like growth factor-1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cyokines. Biochem J 1989; 260:543-548.

Uebelhart D, Thonar EJ, Zhang J, et al. Protective effect of exogenous chondroitin 4,6-sulfate in the acute degeneration of articular cartilage in the rabbit. Osteoarthritis Cart 1998; 6(suppl):6-13.

Ushiyama T, Ueyama H, Inoue K, et al. Expression of genes for estrogen receptors α and β in human articular cartilage. Osteoarthritis Cartilage 1999; 7;560-566.

Van Saase JLCM, Van Romunde LKJ, Cats A, Vandenbroucke JP, Valkenburg HA. Epidemiology of osteoarthritis: Zoetermeer survey: comparison of radiographic osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheum Dis 1989; 48:271-280.

Vaz AL. Double-blind clinical evaluation of the relative efficacy of glucosamine sulphate in the management of osteoarthritis of the knee in out-patients. Curr Med Res Opin 1982; 8:145-149.

Vilim V, Vytasek R, Olegarova M, et al. Serum cartilage oligomeric matrix protein reflects the presence of clinically diagnosed synovitis in patients with knee osteoarthritis. Osteoarthritis Cart 2001; 9:612-618.

Vingard E, Alfredsson L, Malchau H. Lifestyle factors and hip arthrosis. Acta Orthop Scand 1997; 68:216-220.

Wahl SM, Allen JB, Wong HL. Antagonistic and agonistic effects of transforming growth factor-beta and IL-1 in rheumatoid synovium. J Immunol 1990; 145:2514-2519.

Wakitani S, Kimura T, Hirooka A, et al. Repair of rabbit articular surface with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg Br 1989; 71:74-80.

Warnock MG, Sharif M, Elson CJ. IL-1β at physiological concentration stimulates degradation of human articular cartilage. Bone and Mineral 1994; 25:S36.

Westcott CI, Atkins RM, Dieppe PA. Tumour necrosis factor-alpha receptor expression on chondrocytes isolated from chondrocytes isolated from human articular cartilage. J Rheumatol 1994; 21:1710-1715.

Westcott CI, Ismaiel S, Atkins RM, et al. Human articular cartilage degradation and chondrocyte expression of TNF receptors. Trans Orthop Res Soc 1993; 8:739.

Wilson MG, Michet CJ, Ilstrup DM, Melton LJ. Idiopathic symptomatic osteoarthritis of the hip and knee: a population-based incidence study. Mayo Clin Proc 1990; 65:1214-1221.

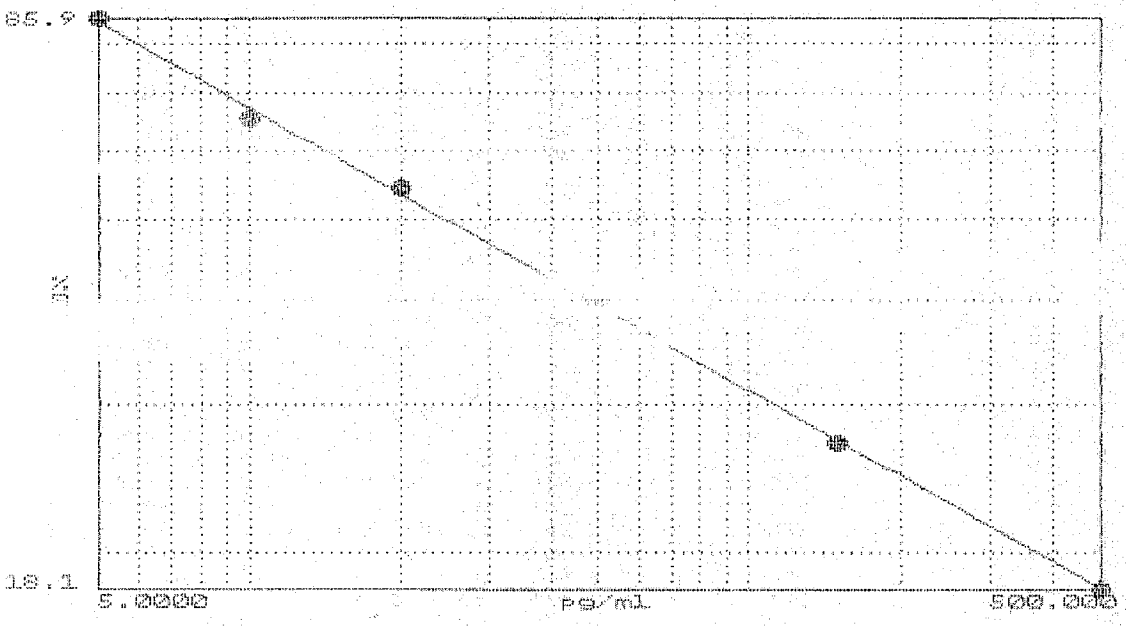
Zhang Y, Hannan MT, Chaisson CE, et al. Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: The Framingham Study. J Rheumatol 2000; 27:1032-1037.

Zhang Y, McAlindon TE, Hannan MT, et al. Estrogen replacement therapy and worsening of radiographic knee osteoarthritis: The Framingham Study. Arthritis Rheum 1998; 41:1867-1873.

APPENDIX A

Protocol #:22 dpc-estradiol User :

Assay Type: RIA %REF. BOUND



STD #	CPM	DEFINED DOSE	%B/T	CALC.DOSE	% DIFF
2	13020	5.00000	85.86	4.87923	-2.42
3	11765	10.00000	77.31	10.94412	9.44
4	10654	20.00000	69.75	18.89515	-5.52
5	8361	50.00000	54.14	48.19784	-3.60
6	5407	150.00000	34.03	153.41620	2.28
7	3065	500.00000	18.08	502.63194	0.53

Curve Fit	: LOGIT	Transform X	: LOG	Transform Y	: LOGIT
Corr.Coef.	: 0.9995			Slope	: -1.6465
Det.Limit	: 5.0000			Para.Coef.	:
%NSB/Total	: 1.1857			%Ref/Total	: 42.681
ED20	: 422.43	ED50	: 60.787	ED80	: 8.7472
%Sigma Std.	: 3.8238				
Intercept	: 2.93712				

APPENDIX B

INSTITUTIONAL REVIEW BOARD APPROVAL FORM

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 03-24-98 **IRB #: HE-98-076**

Proposal Title: THE ROLE OF ESTROGEN AND ESTROGEN RECEPTORS IN KNEE OSTEOARTHRITIS

Principal Investigator(s): Bahram H. Arjmandi, Mark E. Munson

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.
APPROVAL STATUS PERIOD VALID FOR DATA COLLECTION FOR A ONE CALENDAR YEAR PERIOD AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Disapproval are as follows:

Signature: _____ Date: May 4, 1998
Chair of Institutional Review Board

APPENDIX C

Oklahoma State University
Consent for Participation in a Research Study Entitled
The role of estrogen and estrogen receptors in knee osteoarthritis

I, _____ state that I am_____ years of age and agree to participate in research study being conducted by Bahram H. Arjmandi, PhD and Mark E. Munson, M.D. I understand that while the study will be under the supervision of these individuals, the other professionals who work with them may assist or act on their behalves.

Purpose of the Study

The purpose of this study is to examine the relationship between the occurrence of knee osteoarthritis and estrogen levels (a female sex hormone which is also present in males as well but in much lower concentrations) in synovial fluid and the presence of estrogen receptors (estrogen exerts its effect on a tissue through these molecules) in the cartilage. Furthermore, we will investigate the role of other molecules that may function importantly in maintaining the normal physiology and integrity of the articular. In this proposed research, knee cartilage and synovial fluid from both men and women of comparable age with and without knee OA will be obtained.

Procedure
I understand that the following procedures will be performed:

1. I will have about 1 to 4 ml synovial fluid aspirated from knee joint by Dr. Munson during the surgery.
2. The cartilage tissue specimen that is removed during the procedure will be collected by trained personnel and stored appropriately for the experiments proposed by the investigators

Potential Risks
Removal of synovial fluid from my knee may cause slight discomfort.

Potential Benefits

I will receive information on my cartilage status.

Alternatives to Participation

Although my participation in this study would help the healthcare providers to better understand the causes of the disorder, however, the outcome of my surgical treatment is not altered by my lack of participation in this study.

Research/Treatment Cost
I understand that I will not have to pay for the cost of the laboratory work directly associated with this study.

Compensation of Injury

I understand that the research procedures are independent of my surgical procedures. My cartilage tissue and synovial fluid will be examined after the surgery. I understand that the surgeon will not remove additional cartilage for this research.

Confidentiality of Data

I hereby agree to allow my name and medical records to be made available to Drs. Bahram H. Arjmandi, and Mark E Munson involved in the study for the purpose of evaluating the results of this study. I consent to the publication of any data which may result from this investigation, providing that neither my name nor any other identifying information is used in conjunction with such publication. Precautions to maintain confidentiality of my medical records will be taken.

Right to Withdraw

I have been advised by Dr.Munson that I am not obligated to participation in this study.

Right of Refusal

I have been advised that Drs. Arjmandi and Munson will answer any questions that I may have regarding this research study and that I am free to withdraw my consent and discontinue participation at any time without penalty.

I also understand that the investigators have the right to withdraw me from the study at any time.

I acknowledge that _____ has explained to me the risks involved, the need for the research, and has offered to answer any questions which I may have concerning the procedures to be followed. I freely and voluntarily consent to participate in this study. I understand that I may keep a copy of this consent form for my own information.

Signatures

_____	_____
Volunteer (sign and date)	(Print name)
_____	_____
Investigator (sign and date)	(Print name)
_____	_____
Witness of Volunteer's Signature (sign and date)	(Print name)

VITA 2

Shanil Shaban Juma

Candidate for the Degree of Doctor of Philosophy

Thesis: THE ROLE OF ESTROGEN AND ESTROGEN RECEPTORS IN KNEE OSTEOARTHRITIS

Major Field: Human Environmental Sciences

Area of Specialization: Human Nutrition

EDUCATION

1998-2002: Doctorate of Philosophy, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, Stillwater, OK
1994-97:Master's of Science in Human Nutrition, Department of Human Nutrition and Dietetics; College of Associated Health Professions, University of Illinois at Chicago, IL
1989-93:Bachelor's of Science in Health Sciences/Biology, School of Health Sciences, Purdue University, West Lafayette, IN

WORK EXPERIENCE

1998-2002:*Graduate Research Associate*, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, Stillwater, OK
1995-97:*Graduate Research Associate*, Department of Human Nutrition and Dietetics, College of Associated Health Professions, University of Illinois at Chicago, IL
1991-92:*Assistant Technician*, STAT Laboratory, Department of Pathology, Northwestern Memorial Hospital, Chicago, IL (2 Summers)
1990-93:*Research Assistant,* Department of Biochemistry, Purdue University, West Lafayette, IN

AWARDS AND HONORS

2002:*Graduate Research Excellence Award*; Oklahoma State University, Stillwater, OK
2002:*Office of Dietary Supplements Scholar Award*, National Institute of Health
1997:*Winner of Abstract Presentation*, Allied Health Research Forum, College of Associated Health Profession, University of Illinois at Chicago
1996:*Finalist*, 1996-97 Paul D. Doolen Graduate Scholars for the Study of Aging Award, University of Illinois at Urbana-Champaign
1989-93:*Scholarship Recipient*, William J. Cook Foundation Scholarship, Chicago, IL
1989:*Scholarship Recipient*, Union League of Chicago, Chicago, IL

PROFESSIONAL MEMBERSHIP AND AFFILIATIONS

International Bone and Mineral Society
American Society of Bone and Mineral Research
Federation of American Societies of Experimental Biology
Endocrine Society
Arthritis National Research Foundation
American Federation for Aging Research
Associate Member of Lamda Tau Society (Purdue University Chapter)

COMMITTEES

2000:*Graduate Representative*, Student Recognition Committee; College of Human Environmental Sciences (GSHES); Oklahoma State University, Stillwater, OK
2000:*Graduate Representative Judge*, Annual Graduate Research Symposium; Graduate College, Oklahoma State University, Stillwater, OK
1998-2000:*Secretary* (August 1998-May 1999); *Vice-President* (June 1999-May 2000), Graduate Student Council in Human Environmental Sciences (GSHES); Oklahoma State University, Stillwater, OK
1996-97:*Member*, Graduate Student Committee in Human Nutrition and Dietetics; University of Illinois at Chicago, Chicago, IL

TEACHING EXPERIENCE

2000-02:*Guest Lecturer, Nutrition and Aging (NSCI 5393)* Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK
2000-01:*Teaching Assistant, Introductory Nutrition (NSCI 2114)* Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK
1999:*Guest Lecturer, New Findings in Nutrition (NSCI 5230)* Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK
1996:*Guest Lecturer, Science of Food (HND 300)* Department of Human Nutrition and Dietetics, College of Associated Health Professions, University of Illinois at Chicago, IL
1996:*Guest Lecturer, Clinical Nutrition II-Gastroenterology (HND 420)* Department of Human Nutrition and Dietetics, College of Associated Health Professions, University of Illinois at Chicago, IL
1995-97:*Teaching Assistant, Science of Food (HND 300)*, Department of Human Nutrition and Dietetics, College of Associated Health Professions, University of Illinois at Chicago, IL