[Frontiers in Bioscience 3, c17-26, April 16, 1998]

IN SEARCH OF AN ANIMAL MODEL FOR POSTMENOPAUSAL DISEASES

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Received 9/25/97 Accepted 4/10/98

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1. ABSTRACT

The purpose of this review is to discuss the use of the aged ovariectomized ewe as a cost-effective large animal model to study coronary artery disease (CAD), osteoporosis, osteoarthritis (OA), and oral bone loss - conditions seen after menopause. Earlier studies from our laboratory showed a significant decline in the bone mineral density (BMD) of the iliac crest following ovariectomy in sheep, while subsequent studies demonstrated decreased bone loss (measured by dual energy X-ray absorptiometry (DXA)) in the lumbar vertebrae following ovariectomy. We examined the effects of estrogen deficiency and estrogen therapy on the terminal aorta of the aged ovariectomized (OVX) ewes and demonstrated subintimal thickening in the distal aorta of animals that were estrogen deficient when compared to the control groups. A popular model to study OA is the knee joint of sheep following medial or lateral meniscus removal combined with exercise, but there is a need for an estrogen-deficient large animal model of OA to study articular cartilage changes occurring after menopause. We saw an effect of ovariectomy on the biomechanical properties (aggregate modulus and shear modulus) of articular cartilage. Estrogen deficiency had a detrimental effect on the articular cartilage of the knee even though the cartilage of the OVX animals appeared grossly normal. In another study, 13.5 months following ovariectomy, we found an increase in estrogen receptor binding capacity of the articular cartilage suggesting that articular cartilage is a sex-hormone sensitive tissue. There is intense interest in the correlation between systemic osteoporosis and bone loss of the mandible and maxilla. We studied mandibular bone loss in OVX sheep using DXA. The mean BMD of the OVX group versus sham and estradiol-treated animals was lower, indicating that systemic bone loss in OVX ewes may be accompanied by oral bone loss. Coronary artery disease, osteoporosis, osteoarthritis (OA) and oral bone loss all have a major impact on women's heath after menopause and we found that certain characteristics of these conditions can be reproduced in the skeletally mature or aged estrogen-deficient sheep. It is premature to promote the sheep as the only model to study estrogen deficiency and the many differences from small animal omnivores and non-human primates need to be overcome and a search for more economical models must continue. This model, however, may offer the opportunity to study postmenopausal conditions and the safety and efficacy of new therapeutic agents.

2. GENERAL INTRODUCTION TO ANIMAL MODELS

To clarify the etiology and improve the management of certain diseases experienced by women after menopause, it is necessary to establish a suitable animal model to validate safety and efficacy of therapy. Previously, researchers have used non-human primates, dogs, cats, rabbits, guinea pigs, pigs, and minipigs, each of which these animal models possesses certain advantages and disadvantages (1). (table 1) In order to select an appropriate animal model for a given study, the investigator must take into consideration a wide variety of factors: 1) appropriateness as a model for estrogen deficiency , 2) genetic homogeneity of organism, 3) background knowledge of biological properties, 4) cost and availability, 5) ease of experimental manipulation, 6) ecological considerations, and 7) ethical and societal

Table 1. Characteristics of six animal models used for the study of postmenopausal disease

Animal	Cynomologus	Sheep	Mongrel Dog	Beagle	Swine	Rodents
Cost (ea.) US $	2000	165	250	350	350	12
Maintainence Cost/day)	4.75/day	1.50/day	2.20/day	2.31/day	2.31/day	28c/rat/day
Handling/Trained Tech.	Aggressive/Yes	Docile/No	No	No	Agressive/Yes	Some training reqd.
Societal concerns	High	Low	High	High	Low	Low
Estrus Cycle	Polyestrus	Seasonal-Polyest.	Diestrus	Diestrus	Polyestrus	Polyestrus 4-5 days
Physiology	Monogastric	Ruminant/herbivore	Monogastric	Monogastric	Monogastric/Omnivore	Monogastric

implications (1). It is of great importance that the model selected does not add too many new variables to an already complex problem (2); therefore, Ideally, the model chosen should closely mimic human diseases in its induction, progression, and pathology"(2). Obviously, no animal model can meet every criterion. In real life, compromises must be made and the *best* possible model is selected (1). This article will demonstrate that the sheep is a practical and highly suitable large animal model, for human postmenopausal diseases.

The sheep, more specifically the skeletally mature (> 3.5 yrs) or aged (>7 yrs.) ewe, is gaining recognition as a cost-effective large animal model for studying four of the most important postmenopausal diseases: CAD, osteoporosis, OA, and oral bone loss. This animal model can be used for studies directed at clarifying the details of pathogenesis that remain obscure, and it also can meet the criteria needed to test new therapeutic strategies that could help to prevent these diseases (3).

The sheep as an experimental animal model possesses numerous advantages. Although larger than many research animals, sheep are docile, compliant, and therefore easy to handle and house. Because sheep are raised in flocks, they experience little stress when they are housed in groups of two or more. For most people, there is less emotional attachment to sheep as compared to companion animals such as cats and dogs, which eases some of the ethical stigma associated with animal research (1). In an era of spiralling research costs, one clear advantage of using sheep as a large animal model is their relatively low maintenance costs (table 1). Finally, sheep are available in large numbers of "homogeneous populations" of known age, making them ideal for large scale studies (1).

3. A BRIEF OVERVIEW OF CONDITIONS ASSOCIATED WITH CESSATION OF OVARIAN FUNCTION

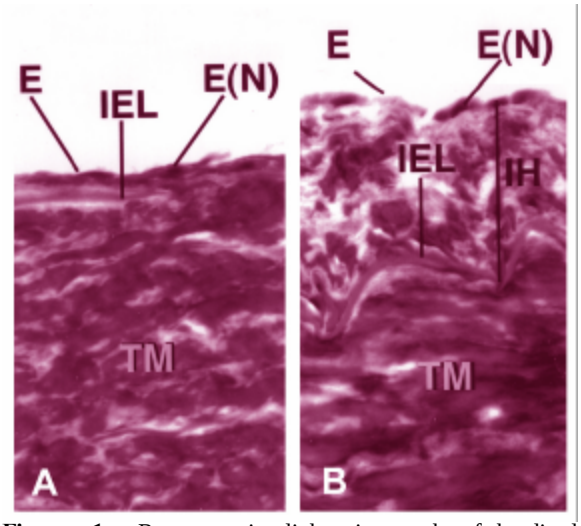
Current trends in population dynamics fuel the interest in conditions associated with menopause. Because of decreases in maternal mortality and deaths from infectious disease, today's average white female will survive to the age of 79 (4) which means that she can expect to live 1/3 of her life in a postmenopausal hypoestrogenic state (5). Further, one of the most alarming statistics related to this demographic shift is that, within approximately the next 40 years, the population of women in the United States over the age of 65 will double, reaching 65 million (6). Accordingly, this population shift will make menopause a highly prevalent state and one which recent epidemiological studies indicate is a risk factor for several subsequent chronic diseases (7). There is a well-documented and ever-increasing body of evidence appearing in the medical literature linking menopause and the subsequent decrease in estrogen production with four serious conditions: CAD, osteoporosis, OA, and oral bone loss. In addition to these conditions, there are a number of well-researched symptoms associated with menopause such as vasomotor abnormalities ("hot flashes"), sleep disturbances, urogenital atrophy, vaginal dryness, insomnia, depression and perhaps fatigue. It is beyond the scope of this article to discuss the pathophysiology and management of these symptoms. We will discuss some of characteristics of certain conditions associated with cessation of ovarian function as seen in ovariectomized sheep. The medical and economic impact of postmenopausal diseases, a great need to search for the appropriate treatments, examining not only the role of estrogen but also estrogen-like agents and other pharmacological alternatives in the treatment and prevention of these diseases.

4. CORONARY ARTERY DISEASE (CAD)

4.1 Animal Models Used to Study CAD

Many key questions regarding CAD and estrogen replacement therapy (ERT) have been answered with the assistance of animal models. In the 1950's, for instance, the Michael Reese Research Institute used the hen as a model (8). They were the first to provide evidence that estrogen inhibited the progression of the disease.

Rodents and rabbits are practical and economical animals for the study of CAD. Most studies utilizing these species involve supraphysiologic lipid-concentrated diets with or without some form of vascular injury. In these models, the vascular injury (produced by intimal abrasion using an inflated balloon) induces a highly reproducible intimal migration and proliferation of vascular smooth muscle cells mimicking the early injury phase of atherosclerosis. However, there are still unanswered questions as to how these models relate to spontaneous human atherosclerosis. Mice (9), rats (10) and rabbits (11) have also been used to examine the effects of estrogen deficiency and arterial lipoprotein metabolism.



Figures. 1. Representative light micrographs of the distal aorta in a sham-operated ewe (a) and an ovariectomized (estrogen-deficient) ewe after 6 months (b). There is marked subintimal thickening in the ovariectomized ewe.. E, endothelium; IEL, internal elastic lamina; E(N), endothelial nuclei; TM, tunica media; IH, intimal hyperplasia. Trichrome stain. Original magnification, X 40.

In the 1970's, Magill and colleagues used the OVX baboon as a model to determine the effects of exogenous estrogen on atherosclerosis (12). This work led to the discovery that baboons, unlike many other primate species and humans, are resistant to dietary-induced atherosclerosis. There were no significant effects of estrogen replacement therapy, and no difference between male and female in the extent of dietary-induced coronary artery disease (11). This research contradicted data that had already been established with human studies (11) and suggested that the baboon was a poor model for some of the conditions seen after menopause.

The cynomolgus macaque (*Macaca fascicularis)* has traditionally been the non-human primate used most extensively in CAD research because of its susceptibility to dietary-induced coronary atherosclerosis (11). Its reproductive physiology is also comparable to that of humans, with a 28-day menstrual cycle, similar circulating hormonal patterns, and naturally occurring menopause (11). However, the acquisition of aged female monkeys is becoming more difficult and more costly (1). In addition, sophisticated housing facilities and technicians specially trained are required to handle the sometimes aggressive primate. One of the greatest disadvantages of using primates as an animal model is the potential for transmission of zoonotic diseases such as the Marburg virus disease, Ebola virus disease, viral hepatitis, *Herpes virus simae,* and tuberculosis (1). Thus, despite the large amount of productive research that has resulted from using the monkey, this model is extremely expensive and potentially dangerous. Additionally, use of this model is also complicated by the general distaste of the public for primate research.

4.2. The Use of Sheep to Study CAD

To date, few studies have used sheep as an animal model for CAD because of researchers' unfamiliarity with ovine physiology, husbandry and veterinary requirements. In our laboratory, we examined the terminal aorta of OVX, sham-operated, and ovariectomized estrogen-supplemented (OVXE) ewes to determine the effects of estrogen deficiency and estrogen therapy. Histological analysis by light microscopy demonstrated that there was diffuse subintimal thickening in the distal aorta of animals that were estrogen deficient when compared to the control groups (figure 1). This marked subintimal thickening was significantly less in the sham-operated group and the estrogen supplemented group. With the use of an actin stain it was confirmed that smooth muscle proliferation was in fact partially responsible for the subintimal hyperplasia in the OVX animals .

In the same study, we also compared serum cholesterol and triglyceride levels at four time points (0, 4 , 8, and 12 months) following OVX or sham surgery and found no effect of treatment on lipid profiles. The results strongly suggested that the myointimal thickening observed in OVX sheep was caused by estrogen deficiency rather than unfavorable changes in lipid profiles. In other words, intimal hyperplasia occurred despite normolipemia, and the hormonal influence on the vasculature was independent of lipid metabolism.

Numerous studies in humans have shown that increases in plasma total cholesterol or low density lipoprotein (LDL) cholesterol can increase the risk of CAD (13). Furthermore, it is believed that the effect of estrogen deficiency on CAD is partially mediated by an increase in high density lipoprotein (HDL) cholesterol. Although vascular pathology occurred without measurable changes in plasma lipids in sheep, these changes strongly suggest a link to alterations in reproductive hormone levels and it is likely that smooth muscle proliferation causing subintimal thickening was a result of OVX.

Future research using the sheep model should focus on whether increasing dietary fat can induce atherogenic changes. The diet of the grazing ruminant animal consists of pasture grasses and legumes which are low in lipid content (5-10 g lipid per 100g dry plant tissue) (14). Much larger intake of dietary lipid may be achieved in ruminant animals such as sheep by feeding diets containing "protected" lipid supplements. Previously, these have been used to increase energy intake or to produce polyunsaturated meat or milk. By spraying a casein/vegetable-oil emulsion with formaldehyde, the product provides an encapsulated form of lipid that is protected against lipolysis and hydrogenation by the rumen microflora but is well digested and absorbed from the lower gastrointestinal tract.

Providing "protected" lipid supplements would add little to the cost of the sheep model. Therefore, one of the most exciting questions regarding the use of the sheep model for CAD is: What would be the effect of a high fat diet (using "protected" lipid supplements) superimposed upon estrogen deficiency?

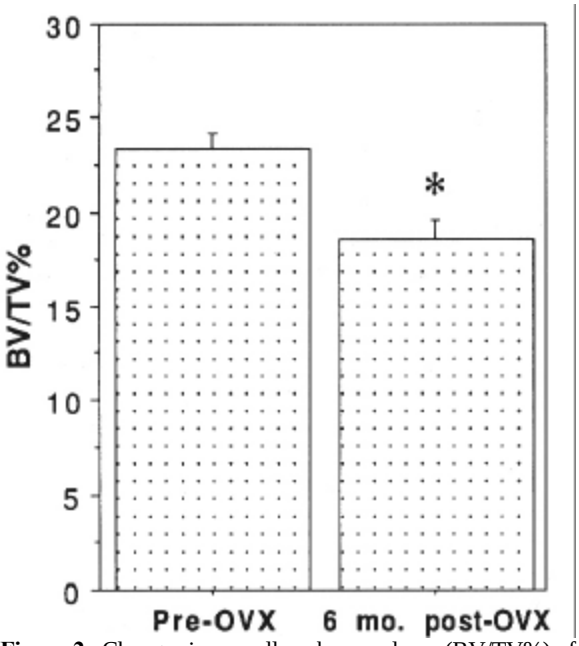


Figure 2. Changes in cancellous bone volume (BV/TV%) of the iliac crest in 14 skeletally mature ewes (mean + S.E) six months after ovariectomy (OVX). * p = 0.0012; Wilcoxon Signed-Rank Test.

Phytoestrogens are nonsteroidal plant compounds, most of which are weakly estrogenic unless ingested in large quantities. They were responsible for infertility in sheep in southwestern Australia in the 1940s when red clover (with large quantities of estrogenic isoflavones) was ingested. Estrogenic isoflavones have been found in commercial feline diets containing soymeal, and there has also been a growing interest in the use of the estrogenic response of dietary soy in postmenopausal women (15). Unless the source of the sheep is from pastures with clover and there is evidence suggesting infertility, researchers using sheep can usually be assured that phytoestrogens are unlikely to be a confounding factor.

5. OSTEOPOROSIS

5.1 Animal Models to Study Osteoporosis

Previously, rats, mice, dogs, swine, and non-human primates have been used to study the pathophysiology of bone loss (table 1). However, none of these animals perfectly mimic human osteoporosis because of obvious differences in reproductive physiology, hormone profiles and biomechanical bone characteristics. Even though most of these animal models do not experience a natural menopause, this state can be induced with some reliability by ovariectomy.

The most commonly used animal model for osteoporosis is the rat, and there is extensive literature studying the ovariectomized rat as a model for histomorphometric changes, biochemical markers, methodology for bone densitometry and evaluation of bone fragility (16-20). Rats are inexpensive, easy to house, and do not carry the societal concerns of the other models; their shorter life span also facilitates studies on the effects of aging on the bone. Because the rodent has been used so extensively in research of all types, much is known about bone turnover and the effect of diet on this process. Cortical thinning and increased fragility are well documented in aging rat and mouse bones, but it is unclear if this results in increased incidence of fractures.

There are however, disadvantages in the use of rodents as a model for osteoporosis. Rodents do not experience a natural menopause, but ovariectomy has been used to produce an artificial menopause. (16-20) Furthermore, although aged rodents have Haversian systems and ovariectomy results in a significant bone loss, they have a limited naturally occurring basic multicellular unit-based remodeling. Rats also have lamellar bone (although most is "fine-fibered"), trabecular remodeling, and no intracortical remodeling. (18-20) Longitudinal bone growth increases transiently after ovariectomy in long bones of rats, but this can be minimized by the use of aged rats (9-12 months old) or of skeletal sites where the longitudinal growth is greatly reduced (e.g. lumbar vertebrae) (18). Another limitation is the absence of impaired osteoblast function during the late stages of estrogen deficiency (18) which may be due to the decrease in bone fatigue experienced by small quadruped animals such as the rodent. Longer term studies which require several biopsies, or large blood samples, also are impossible in such a small animal. For a detailed review of other models of osteoporosis see reference 1 (1).

5.2 Sheep as a Model for Osteoporosis

Sheep are well suited for the study of osteoporosis for the following reasons: (1) bone loss associated with estrogen deficiency has been documented (21-25); (2) the hormone profiles of ewes are temporally and quantitatively similar to those of women (26); (3) the osteoblast product, osteocalcin, has been clearly defined in the sheep model (27-30); (4) the size of the sheep permits prosthetic implantation to evaluate performance in osteoporotic bone (31-33); and (5) the large size of these animals allows for the collection of substantial blood and urine samples, as well as multiple iliac crest biopsies (1,34).

Our group previously conducted studies of osteopenia in sheep following OVX which showed a significant decline in cancellous bone volume (BV/TV%) of the iliac crest following OVX (35) (figure 2). Subsequent studies (24,25) demonstrated bone loss (measured by dual energy X-ray absorptiometry (DXA)) in the lumbar vertebrae following OVX (figures 3). The activity of bone-specific alkaline phosphatase (BSAP), an isoenzyme involved in bone formation and skeletal mineralization, was found to be significantly increased in both the sham group and the group which underwent OVX and received estradiol implants (OVXE) (figure 4). This demonstrated that bone loss in the lumbar vertebrae of sheep following OVX was ameliorated by estrogen replacement therapy (ERT). The increase in activity of BSAP in the OVX group was highly indicative of accelerated bone turnover and was in agreement with the changes seen in aging adult humans, particularly postmenopausal women (36,37). Future studies using OVX sheep should investigate other newer biochemical markers indicative of type I collagen synthesis and degradation that are most likely to reflect bone turnover. Such markers include the

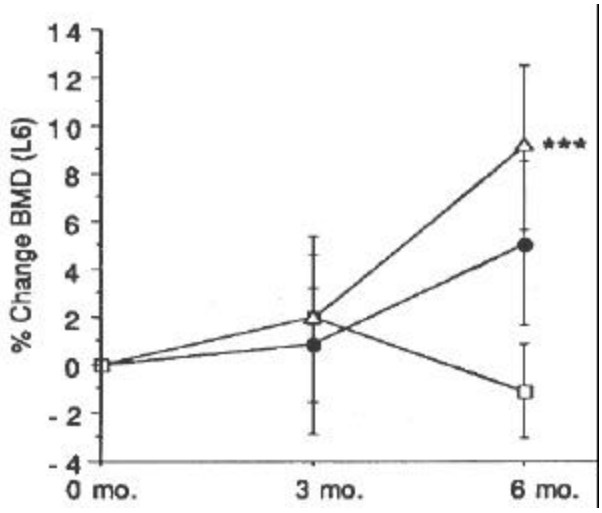


Figure 3. Changes in BMD of the 6th lumbar vertebra at 3 and 6 months expressed as percent change from baseline (Mean ± S.E.) in sham (filled dots), ovariectomized (OVX, open squares) and ovariectomized estrogen-treated (OVXE, open triangles) ewes. Significant difference compared to OVX; * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$. (See Ref # 25 for details).

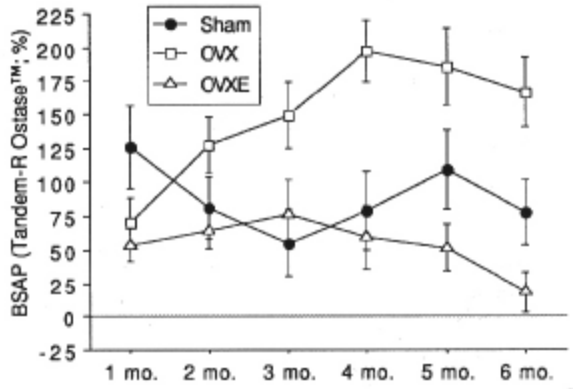


Figure 4. Percentage change (mean ± S.E.) of bone-specific alkaline phosphatase (BSAP). Estrogen treatment did not change BSAP at any time point compared to sham, however OVX significantly increased BSAP at 3, 4, 5 and 6 months compared to Sham and OVXE groups (* $p < 0.05$; based on ANOVA). See Ref # 24 for details).

carboxy-terminal propeptide (PICP) to reflect bone matrix synthesis and the carboxy-terminal telopeptide (ICTP) which reflects bone matrix degradation (37,38). These biochemical markers will be important in monitoring new therapeutic regimens for osteoporosis when using animal models.

The bony changes seen in the proximal femur following menopause have been studied intensively for many years. We documented a statistically different Singh index in the proximal femur in OVX ewes compared to both young and old sheep (35). For this model, precise measurements of excised sheep femora using DXA are possible (39). However, one distinct disadvantage of using a quadruped animal (including dogs) for measuring longitudinal (temporal) changes in BMD using DXA is the technical difficulty in positioning the animal to examine the femoral neck region. In longitudinal studies, it is critical that an identical region of interest is evaluated on a repeatable basis.

Sheep do not experience spontaneous menopause and extremely old animals are usually culled for economic reasons. Therefore finding animals with osteoporotic fractures of the spine and femoral neck is unlikely.

As densitometric techniques such as DXA do not furnish sufficient information about the quality of bone, an important endpoint when using all animal models of osteoporosis is the fragility of bone as a result of OVX. Thus, one of the important steps in the characterization of the aged OVX ewe as a model for osteoporosis will be determination of biomechanical changes of the bone.

Questions yet to be answered with this model of osteoporosis include:
1) At what time of the year should ovariectomy be performed to demonstrate the most rapid bone loss? In other words, should ovariectomy be performed in anestrus prior to when estrus cycles begin or during the breeding season when estrus cycles are occurring regularly?
2) Is skeletal fragility likely to occur after many years following ovariectomy?
3) Are there seasonal and circadian fluctuations in bone density, or fluctuations associated with parity, pregnancy and number of offspring and lactation?
4) What are the most suitable sites for measuring change in bone mass in sheep?
5) What extrinsic factors are likely to confound the bone loss following ovarian hormone deficiency? Such extrinsic factors may include breed, diet (e.g. Ca:P ratio) and exposure to sunlight (e.g. sunny or cloudy environment).
6) Will societal pressures (animal rights groups), lead to difficulties in using this model ?

6. OSTEOARTHRITIS (OA)

Osteoarthritis is defined as "transient and progressive structural changes in joint tissue, principally in articular cartilage, subchondral bone , synovium, and synovial fluid" and is characterized by mild cartilage degeneration with chondromalacia and death of chondrocytes (3). When this disease progresses to extremes, it can result in complete loss of articular surface as well as exposure of subchondral cartilage (2). Consequently, OA has a major impact on the functioning of the elderly and costs of care (40).

6.1 The Relationship Between Estrogen and OA

There is increasing evidence that sex hormones play a role in the development of OA in women. Specifically, the prevalence of OA increases in women following menopause and rises faster with age in women than men. Some women experience rapidly progressing OA in the hand at the time of menopause (40,41). The cross-sectional association of postmenopausal ERT with prevalence of radiographic findings of OA of the hip in a large cohort of elderly white women showed that women who used oral estrogen had a significantly reduced risk of OA in the hip

(42). Furthermore, epidemiological studies have implicated estrogen deficiency as a risk factor for OA (43).

Ex vivo studies of cell and organ cultures have provided insight into the biochemical events within cartilage, bone, and synovial fluid (3). Unfortunately, these studies provide little information about the structural changes in the joint. It is difficult to study OA in humans because of genetic variation, numerous nutritional and biochemical variables, and difficulty in clearly identifying the *early* stages of this disease (3). Furthermore, the elucidation of the *early* events of OA is difficult when using human subjects because patients usually do not seek medical attention until the pathological changes are advanced (end stages of the disease).

There are few reports on the effects of OVX or chronic sex steroid administration on the properties of articular cartilage. Most animal studies have been inconclusive in proving a relationship between OA and estrogens. A high prevalence of OA lesions (subchondral plate thickening, osteophytes , subchondral cysts, articular cartilage fibrillation, and clefts) was observed in the knee joints of relatively young intact monkeys. However, this study failed to detect an effect of chronic sex steroid administration or OVX on severity of OA (44).

There is a need for an *estrogen-deficient* large animal model of OA to study the complex structural changes in tissues that evolve over time, spontaneously or following experimental injury, and to determine how constitutive, environmental or biomechanical risk factors may initiate, promote or otherwise regulate these changes" (3). Such a model would be useful in investigating novel therapeutic and pharmaceutical strategies (eg. new estrogen-progestin products or selective estrogen receptor modulators such as raloxifene) which could then be used to control progression of this disease in the postmenopausal women.

6.2 The Use of Sheep as a Model for OA

A popular site to study OA is the knee joint of sheep following unilateral medial or lateral meniscus removal combined with exercise (45-47). One advantage of the sheep over smaller animals such as rats, mice, guinea pigs and rabbits, is the increased amount of tissue available for biochemical, histological and biomechanical studies. Sheep have also been used to assess the effects of therapeutic agents on joint tissues (48). Because sheep have a longer lifespan than rodents, long term changes induced by OA can be studied with non-invasive methods like magnetic resonance imaging (MRI) (3).

In a recent study, sheep were exercised on hard concrete surfaces while a control group of sheep were exercised on soft wood chips. The stress to the joints from the concrete induced degenerative changes in the articular cartilage and subchondral bone. This study provided some insight into the role of mechanical determinants on chondrocyte metabolism (3).

6.3 The Relationship between Estrogen and OA in Sheep

We investigated the effect of OVX with or without ERT on the intrinsic biomechanical properties of articular cartilage in sheep. Skeletally mature ewes were divided into four groups: Sham treated, OVX, OVX plus an estradiol implant (OVXE), OVX and two estradiol implants (OVX2E). Twelve months after the OVX or sham procedure, sheep were euthanized and the left knee joints were disarticulated and separated from the surrounding soft tissues, leaving the articular tissue intact. Using creep indentation methods (49), biomechanical testing of several properties of the cartilage specimens was performed including aggregate modulus (H_A), Poisson's ratio (v_s), shear modulus (microns), and permeability (k). In the OVX articular cartilage, H_A and shear modulus were significantly lower than in the other groups (50), suggesting that OVX may have a detrimental effect on intrinsic material properties of the articular cartilage of the knee even though the cartilage of the OVX animals appeared grossly normal. Treatment with estradiol implants prevented these deleterious effects; sham, OVXE and OVX2E were not different from each other. Gross signs of OA in the estrogen-deficient sheep model were not observed. However in another study, 13.5 months following OVX an increase in estrogen receptor binding capacity of the articular cartilage in sheep was observed (51). These observations support those of others (52), suggesting that articular cartilage is indeed a sex-hormone sensitive tissue.

7. ORAL BONE LOSS

Oral bone loss affects up to 94% of the elderly population (presently about 30 million); it is likely to increase in the future as the number of elderly increases (53). There is an intense interest to show correlation between systemic osteoporosis (specifically the hip and spine) and bone loss of the mandible and maxilla (54). Some studies have shown that bone density of the mandible is diminished in post-menopausal women suffering from osteoporosis when compared to controls (53). Others have shown that oral bone loss of the maxilla and mandible associated with osteoporosis of the hip and spine, as well as atrophy of the residual ridges after tooth loss, occurs frequently and inhibits prosthetic rehabilitation (53).

The relationship between osteoporosis and oral bone loss is poorly understood. A large animal model exhibiting bone loss of the maxofacial region along with systemic osteoporosis would greatly clarify this relationship. To date, however, there has been little to no development of a model that could be used to study *both* of these diseases. The non-human primate has always been a promising model with obvious anatomical and physiological similarities, along with similar endocrine and sex steroid metabolism (55), but the disadvantages outlined in table 1 have restricted the use of primates.

7.1 The Use of Sheep to Study Oral Bone Loss

Changes observed in mandibular bone at various stages of development in sheep have been studied (56). It was suggested that the sheep may be a model for human eduntulism. We have studied mandibular bone loss in OVX sheep using DXA (57). Skeletally mature ewes (4-5 years old; same breed and housing; $n = 42$) were divided into four groups: sham treated, OVX, OVX plus one estradiol (E2) implant (OVXE) and OVX plus two E2 implants (OVX2E).

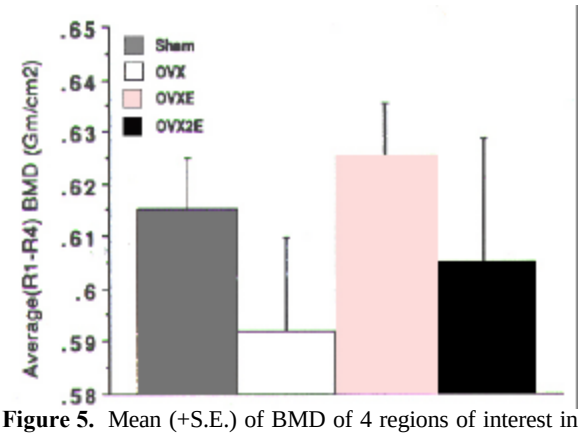


Figure 5. Mean (+S.E.) of BMD of 4 regions of interest in the mandible in Sham, OVX, OVXE and OVX2E groups.

After 12 months, bone mineral density (BMD) of 4 regions of interest of excised mandibles (ROI: R1, R2, R3, and R4) was measured with DXA. Two scans of each bone were obtained and a mean BMD of each ROI was determined. Biopsies of both cortical and trabecular bone were obtained from the angles of randomly selected left mandible, four from OVX and four from sham, for histomorphometry. The BMD at all 4 ROI was lower in the OVX group than in the sham, OVXE or OVX2E groups. The mean BMD of the OVX group versus all others was lower but was not statistically significant ($p = 0.26$). (The means of BMD of all regions combined are shown in figure 5.) Cancellous bone volume was 24% and 36% in the OVX and sham groups respectively. The number of active remodeling sites (cuboidal osteoblasts) was 3.63 and 1.38 in the OVX and sham groups. Only modest changes in mandibular bone density was demonstrated after a year of estrogen deficiency. Although these changes did not reach statistical significance, larger numbers of animals or longer periods of follow-up will be needed to determine if the sheep can be used as a model for oral bone loss.

This study, based on DXA and histomorphometry of the mandible, provided some evidence that systemic bone loss in OVX ewes may be accompanied by oral bone loss. Further, there was evidence that the prophylactic effect of estrogen occurred with oral bone loss as well as with osteoporosis. This supported the epidemiological data from one population of older women in a California retirement community, where estrogen users had retained more natural teeth than the non-users (58). Others have demonstrated that ERT had a positive affect on bone mass in the mandible and lumbar spine (59).

The OVX ewe may be a useful large animal model in the study of mandibular bone loss and its manifestations. Although associations have been made between the extent of reduction of the residual ridges and osteoporosis in people, the sheep model may allow more controlled study of the effects of osteoporosis on bone remodeling following tooth loss and on the rate of success of dental implants and bone substitute materials in osteoporotic sites. The sheep model may also be useful in the study of therapeutic agents (e.g. estrogen analogs) and their protection against oral bone loss. Compared to laboratory animals, the sheep is particularly attractive to those studying the osseous responses to loaded implants and fixtures. The size of the ovine mandible allows implants of a more realistic size and loading to be used with better appreciation of the response to the tissues surrounding the implants.

8. PERSPECTIVE

Coronary artery disease, osteoporosis, osteoarthritis and oral bone loss can occur after menopause and all have a major impact on women's health. It is premature to promote the sheep as a model to study estrogen deficiency; the many differences from small animal omnivores and non-human primates need to be overcome. Specifically, there is a need to validate the aged OVX ewe as a model for CAD. Although dietary fat has a depressive effect on rumen fermentation (60), it would be of interest to feed sheep modified dietary fat, (encapsulated or as calcium soaps, which are utilized to avoid desaturation within the rumen bacteria) to OVX ewes and then search for atherogenic changes.

Female sex steroids have also been shown to affect the composition and structure of many tissues other than the reproductive organs. Recently, estrogen and progesterone receptors were identified in synoviocytes in the synovial lining, in fibroblasts in the anterior cruciate ligament stroma, and in the cells in the blood vessel walls of the ligament (61). Demonstration of sex hormone receptors in these tissues suggests that future studies using animal models could provide further answers to a variety of other orthopaedic conditions associated with menopause.

Clinical and epidemiological studies have suggested a protective (or an onset-delaying) affect of estrogen against Alzheimer's disease (AD) (62-64). Findings imply that estrogen may also protect cognitive functions associated with aging. Further, estrogen replacement therapy may protect against memory decline in nondemented postmeniopausal women and has a beneficial role on cognitive functioning (65). Additional studies of the neurobiological mechanisms for ERT in large animals may help characterize AD well before the diagnosis of the disease.

A series of questions remain unanswered until estrogen-deficient sheep have been studied more intensively: (1) Do the various breeds of sheep differ in their response to estrogen deficiency? (2) What is the effect of parity on bone loss associated with estrogen deficiency? (3) Should OVX be performed during anestrus, prior to regular estrus cycles or when regular estrus cycles are occurring? (4) Is climate (exposure to sunlight) a factor? (5) Can physiological measurements (e.g. core body temperature) for hot flashes be developed in this model?

As the financial climate in biomedical research continues to deteriorate, the search for more economical models must continue. One step is to network or collaborate with other researchers interested in similar models or conditions. In our laboratory for instance, a group of OVX ewes, a control group (sham) and treatment groups can provide specimens for those interested in CAD, osteoporosis, OA, and oral bone loss, as well as other diseases. The skeletally mature OVX ewe therefore offers the opportunity to

study the pathophysiology of the postmenopausal diseases and research on new therapeutic agents.

9. ACKNOWLEDGEMENT

The authors would like to thank Drs. Dan Robinson and Ann Wagner for advice and suggestions in the preparation of the manuscript.

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Key Words: Animal Models, Sheep, Menopause, Coronary Artery Disease, Osteoporosis, Osteoarthritis, Oral Bone Loss, Estrogen

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