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Perspective

Review of Nonprimate, Large Animal Models for Osteoporosis Research

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ABSTRACT: Large animal models are required for preclinical prevention and intervention studies related to osteoporosis research. The challenging aspect of this requirement is that no single animal model exactly mimics the progression of this human-specific chronic condition. There are pros and cons associated with the skeletal, hormonal, and metabolic conditions of each species that influence their relevance and applicability to human physiology. Of all larger mammalian species, nonhuman primates (NHPs) are preeminent in terms of replicating important aspects of human physiology. However, NHPs are very expensive, putting them out of reach of the vast majority of researchers. Practical, cost-effective alternatives to NHPs are sought after among ungulate (porcine, caprine, and ovine) and canine species that are the focus of this review. The overriding caveat to using large lower-order species is to take the time in advance to understand and appreciate the limitations and strengths of each animal model. Under these circumstances, experiments can be strategically designed to optimize the potential of an animal to develop the cardinal features of postmenopausal bone loss and/or yield information of relevance to treatment.
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Key words: postmenopause, estrus, estradiol, animal models, osteoporosis, micro-livestock

INTRODUCTION

Osteoporosis is a chronic disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to skeletal fragility. The search for safer, more progressively effective therapeutic treatments to prevent and treat osteoporosis continues. Preclinical studies in animal models that approximate relevant characteristics of human disease processes are essential for research purposes. Guidelines established by the Food and Drug Administration (FDA) indicate that therapeutic treatments designed to attenuate or prevent postmenopausal osteoporosis should, in the first instance, be tested in an ovariectomized rodent model such as the rat because it is comparatively well characterized in terms of bone loss.[(1)] A second larger, longer-lived animal species with intracortical bone remodeling potential is recommended for subsequent examination of treatment effects on bone quality; however, the choice of the larger animal has been largely left to the discretion of investigators.

Although there is no single animal model that precisely replicates all the characteristics of human osteoporosis, Old World monkeys rank very highly as an appropriate animal model of osteopenia because of an evolutionary ancestry that has engendered numerous reproductive and physiologic similarities to humans. A major disincentive associated with the use of nonhuman primates (NHPs) relates to the high cost, both monetarily and time-wise, of breeding and maintaining suitably aged primates or importing mature wild-caught counterparts. Alternative large domestic animal models are therefore required for prevention and intervention studies related to osteoporosis research. Whereas some alternatives have been partially characterized, further studies seem warranted to advance the use of other candidate animal models or to explore potential variations of existing models (e.g., mini/microlivestock). Thorough consideration of associated limitations and benefits of any prospective animal model is advisable before launching into an experiment that involves a substantial investment in time and funds. This review is designed to give an overview of the practical efficacy of using select large, animal species, specifically dogs, sheep, goats, and swine, to meet the requisite needs for an animal model of bone loss; one that is predominantly associated with estrogen deficiency and has some practical relevance to human postmenopausal osteoporosis. A glossary defining terminology relevant to this review appears in Table 1.

ANIMAL MODEL REQUIREMENTS

Severely diminished circulating estradiol concentrations in postmenopausal women are a major factor contributing

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Table 1. Glossary of Terms

	Definition
Animal terms	
Bitch	A female canine animal, especially a dog
Canid	Any animal of the dog family Canidae
Caprine	Of or pertaining to goats
Ewe	A female sheep, especially when full grown
Farrow	To produce a litter of pigs
Gilt	A young female swine, especially one that has not produced a litter
Mare	A fully mature female horse or other equine animal
Ovine	Of, relating to, or characteristic of sheep
Porcine	Of or pertaining to swine
Swine	Stout-bodied, thick-skinned, short-legged omnivorous animal with a moveable snout (e.g., pigs, hogs, and boars)
Ungulate	Mammal with hooves (e.g., goats, sheep and pigs)
Agricultural terms	
Cultivated fodder	Plants grown and raised especially for use as fodder
Fodder	Roughly cut up feed provided to livestock and domestic animals (it may contain hay, agricultural by products, and leftovers like plants or stalks of cereals)
Leguminous	A large family of trees, shrubs, vines, and herbs bearing bean pods
Pastoral grazing	Livestock feeding on naturally-grown grass and other vegetation
Silage	Fodder harvested whereas green and kept succulent by partial fermentation in a silo

to the accelerated rate of bone loss. In contrast to women that have menstrual cycles, most other mammalian placental females, with the exception of Old World monkeys and great apes, generally experience lifelong estrous cycles or only become acyclic at an advanced age in their lifespan that narrowly precedes death.[2] Ovulatory cycles governed by a period of estrus vary in length according to reproductive differences between species, they do not involve an overt shedding of endometrial tissue if conception fails to take place during the period of natural sexual receptivity (i.e., endometrial resorption occurs rather than menstruation), and spontaneous menopause does not occur. Nevertheless, cessation of estrous cycling can be brought about by surgical castration, or bilateral oophorectomy, to induce a postmenopausal-like state in animals that, in many respects, approximately mimics what occurs endocrinologically, and in some circumstances skeletally, in postmenopausal women.

The FDA has recommended ovariectomized animals as the preferred animal model for bone loss research.[1] The "engineered" commonality in mode of onset of bone loss (i.e., ovarian estrogen depletion) provides a reasonable basis on which to gauge the potential clinical outcome of a drug or treatment for osteoporosis. The 1998 World Health Organization guidelines pertaining to preclinical studies for anti-osteoporotic drug development also stipulate that the effects of drugs must be demonstrated in appropriate animal models of osteoporosis.[3] A larger animal's efficacy as a model for postmenopausal osteoporosis in experiments depends on criteria that broadly include the following:

1. Appropriateness as a model of estrogen deficiency (i.e., significant bone loss and a similar, if not identical, tissue level mechanism for bone loss induced by estrogen depletion).
2. Specific biological and physiological characteristics (e.g., osteonal bone remodeling).
3. Cost and availability.
4. Housing/spatial requirements.
5. Manageability during an experiment.
6. Reproducible results.
7. Minimal ethical/societal implications.
8. Predictive of skeletal effects of potential osteoporosis therapies in adult humans (e.g., increased BMD).

If ovariectomy of an animal does not result in significant bone loss within a reasonable period of time, its use as a model for osteoporosis is limited because therapeutic effects of a treatment are likely unable to be adequately assessed. That said, there may be legitimate justification for prior or concurrent perturbation of an animal destined for ovariectomy to maximize the magnitude of bone loss after estrogen insufficiency. Such strategies may involve dietary manipulation by calcium (Ca) restriction or induction of metabolic acidosis.[4]

MODEL PERTURBATION

A chronic calcium (Ca) deficiency adversely impacts the health of the skeleton over time. Data from the 1999–2000 National Health and Nutrition Examination survey indicates that average Ca intakes for women 51–71 yr of age are only 44–56% of the recommended adequate intake. Most laboratory-bred animals and livestock are routinely fed nutrient-adequate rations throughout their entire life before their use in an experiment. Chow formulations often comprise abundantly high concentrations of Ca. In contrast, a high percentage of women, for whatever reasons, do not consistently make appropriate food/nutrient choices and subsist on suboptimal nutrition. Once these women reach middle age, they may exhibit some latent nutrient deficiency effects. A chronic Ca deficiency resulting in bone loss before menopause, together with the amount of bone resorbed in response to estrogen insufficiency, may in part explain why estrogen diminution alone is not enough to stimulate pathological bone loss in all postmenopausal women. Lowering Ca concentrations in the dietary intake of animals leading up to a bone loss experiment to more closely reflect the nutritional state in which many women approach menopause or endure the postmenopausal period may represent a reasonable strategy. However, Ca-deficient diets necessitate the inclusion of a group of intact animals that are also fed a low Ca diet to enable the skeletal effects of estrogen insufficiency and lower dietary Ca to be distinguished.

TABLE 2. APPROXIMATE CIRCULATING ESTRADIOL (17B-E_2) CONCENTRATIONS FOR VARIOUS SPECIES OF MAMMALS

			During reproductive cycling		*Post reproductive period*	
Animal (references)	*Reproductive cycle characteristics*	*Approximate average time in estrus*	*Approximate peak $[E_2]$* (pg/ml)*	*Approximate basal $[E_2]$* (pg/ml)*	*Approximate $[E_2]$* (pg/ml)*	*Time after castration or menopause (M)*
Rat[128–131]	mean: 4.5-day polyestrus	12-h	35 ± 12	10–15	9 ± 5	7 days
					6.6 ± 1.2	4 wk
Dog[24–26,48,132,133]	~7-mo nonseasonal monoestrus	7–9 days	60–70	<20	22.6 ± 6.6	1 mo
					22.0 ± 3.7	3 mo
					8.9 ± 1.6	8.5 mo
					6.5	1 yr
					1	48 wk
Sheep[20–23,134–138]	mean: 17-day temperate breeds are seasonally polyestrus	12–24 h	8–10	2–3	1.2 ± 0.18	1 wk
			20.8		<5	4 wk
					ND	4–6 wk
Goats[108,139–141]	mean: 21-day temperate breeds are seasonally polyestrous	40 h	20–30	5–10	(–42%)	1 mo
					(–52%)	6 mo
Domestic pigs[129,142,143]	mean: 21-day polyestrus	48–60-h	60–70	10–30	—	—
Minipigs[117,125,144,145]	mean: 19.5-day polyestrus	2.9 days	40	10–16	5.5 ± 1.6	16-wk
					<10	3–6-mo
Women (premenopause)[146,147]	mean: 28-day menstrual cycles	NA	~300–600	~100	Younger women <30 yr	
			~150–200	~50	Older women >30 yr	
Women (postmenopause)[148]	NA	NA			14 ± 1	2-yr post-M

Circulating estradiol concentrations can vary with the breed and age of animals, the season, and the assay performed. Therefore, this table gives a brief overview of some of the values that have been cited in research publications.

*Concentration of estradiol.

ND, not detected; NA, not applicable; —, no references found.

IMPORTANCE OF ESTROGEN

In the quest for animal models that mimic key aspects of significant postmenopausal bone loss, it is of interest to consider the extent to which ablation of the ovaries more or less simulates what takes place in women that have transitioned to menopause, particularly in terms of reductions in circulating estradiol concentrations. The natural reproductive endocrinological patterns of nonprimate mammals are not exactly like those of humans. Circulating estrogen concentrations of most healthy women follow an established regular cyclical pattern approximately every 28 days throughout the reproductive years. The substantial diminution in circulating estrogen concentrations in women in the early years after the transition to menopause accelerates bone turnover rate and is a predictable impetus for bone loss.

Estrous cycles vary in length and frequency among different species and involve different basal and peak endogenous estradiol exposures (Table 2). Generally, small short-lived species undergo estrous cycles more frequently, whereas larger long-lived animals have less recurrent regular estrous cycles. Estrous cycling also tends to be more frequent in species that produce larger litters, raising yet another interspecies incongruity that may deserve some consideration when selecting animal models.

In comparison with primates, some laboratory animals can be considered prolific breeders with an endocrine/reproductive capacity that can cope with multiple births per litter numerous times annually. Lactation demands, although they are for comparatively shorter periods in rodents compared with humans, will naturally be endured numerous times and at closer intervals throughout their reproductive life. Rodents are therefore adapted to mobilize comparatively larger amounts of calcium from bone for weaning requirements in a shorter period of time than humans[5] and possess a profound anabolic capacity that facilitates the replacement of bone mass that may have been resorbed for lactation purposes relatively rapidly.[6] The metabolic and endocrinological demands of multiparity may add an inherent robustness to the bone-forming potential of mammals that produce large litters and could represent one component of bone fitness[7] that is not matched by humans.

Whereas there exist variations among breeds within a species, in general, domestic swine farrow large litters. Wild pigs with uncertain food supplies and miniature/micropigs with a smaller uterine capacity on average have greatly reduced litter sizes, or approximately one half the number of offspring per litter compared with large pigs[8] (Table 3). The litter size of beagles can be highly variable, whereas goats and sheep are less prolific.[9,10] Humans and numerous NHPs show characteristics of low reproduction including long gestation periods and in most cases single births.[11] A characteristic trend toward less offspring more closely relates to human reproductive patterns.

When evaluating the use of a large animal model, it is still unclear whether it is preferable to place emphasis on the

TABLE 3. COMPARISON OF SELECT LARGE ANIMAL MODELS FOR OSTEOPOROSIS RESEARCH

	Interspecies comparisons				
Criteria for selection	*Human (♀)*	*Dogs**	*Minipigs*	*Goats*	*Sheep*
Availability		Easy	Easy	Easy	Easy
Per diem cost (U.S. ~$)[†]		≥14	≥11	≥10–12	≥10–12
Cost per skeletally mature laboratory animal (U.S. $)		Hundreds 550	Thousands 3,000–4,000	Couple hundred 250	Couple hundred 250
Floor space requirements per animal based on body weight[‡] (GH) = group-housed		1 dog <15 kg b.w. 0.72 m^2	2–5 pigs(GH) 50–100 kg/b.w. 0.90–1.80 m^2	2–5 goats(GH) <25–>50 kg/b.w. 0.77–1.53 m^2	2–5 sheep(GH) <25–>50 kg/b.w. 0.77–1.53 m^2
Ethical/societal implications		Sensitive	Not sensitive	Not sensitive	Not sensitive
Manageability/handling		Very easy	Less easy(116)	Easy	Easy
Digestive physiology (by nature)	Monogastric, omnivore	Monogastric, carnivore omnivore	Herbivore, ruminant	Herbivore, ruminant	
Offspring (average)	1	6–7	5	2–3	1–2
Average adult b.w. range (kg)	60–72(149)	8–13	65–82	35–40	45–50
Sexual maturity (age in months)	144–156(150)	6–12(151)	6–10(151)§	6(152)	7–9(93)
Skeletal maturity (approximate age in years)	18–25(153)	1.3(154)	>2.5§	2–3	3(93)
Peak bone mass (yr)	~30(155)	6(55)	6(156)	NA	≥5(93)
Life span (~yr)	~70–80	10–12(157)	10–15(158)	10–15(157)	10–15(157)
Remodeling cycle (mo)	~8(16) 40-wk(159)	3(15,160)	3–6(14)	—	—
Osteonal remodeling (age)	Yes (in utero)(32)	Yes(32)(<1-yr)	Yes (≥6-mo)(120)	Yes(32)	Yes(1-yr)(120)
Seasonal changes in BMD	Possible(161,162)	No	No	Likely	Yes(163)
E_2 decrease post-OX (see references in Table 2)	Yes	Variable: yes, no(24)	Yes	Yes	Yes
PostOX bone loss (on a low-moderate Ca intake)	Yes(164)	Yes(43)	Yes(125)	Yes(106–108)	Yes(165)
Sustained bone loss post-OX (>2 remodeling cycles)	Yes	Variable: no,(42) yes(43)	Yes(125)	—	Variable: yes,(84,166) no(22)
BMD (g/cm^3; ±SD)					
Cancellous—vertebra	0.12–0.14(82,167)	0.30–0.39(58)	0.39–0.43(171)	0.39–0.42(107)	0.48–0.60(58,82,168)
Cancellous—distal femur	0.43–0.46(168)	0.44 ± 0.16(168)	—	0.56–0.66(168)	—
Cortical—femur midshaft	1.74 ± 0.06(169)¶	0.37 ± 0.02(170)¶	~1.50(125)¶	1.61 ± 0.09(172) ♀+♂	1.46–1.50(173)
Similarity of bone attributes to humans(82)					
Macrostructure		Moderately similar	Moderately similar	Most similar	Most similar
Microstructure		Moderately similar	Moderately similar	Least similar	Least similar
Bone composition		Most similar	Most similar	Moderately similar	Moderately similar
Bone remodeling		Moderately similar	Most similar	Moderately similar	Moderately similar

* Based on beagle dog breed.

† Per diem based on an approximation of the current average rates published by various institutions.

‡ Based on National Research Council (NCR) recommendations.

§ G Bouchard, Sinclair Research Center, personal communication, 2007.

¶ BMD g/cm^2; ± SD (when vBMD data not available).

OX, castration; —, no references available.

difference in peak estradiol levels for each species during a cycle, the frequency of estrus cycles,[12] or the relative duration of exposure to elevated estradiol concentrations, or whether the magnitude of change in circulating estradiol concentrations during cycling versus noncyclical status for each species is a better indicator of bone loss. Alternatively, a comparison both within and between species, based on a computation of the area under the curve of estradiol concentrations that have been plotted at regular intervals over a period of time equivalent to an estrous/menstrual cycle, both during and after the cessation of reproductive cycling, and/or normalizing for cycle frequency to approximate total estradiol exposure over an identical period of time may represent a plausible approach. Turner[12] has previously speculated that sensitivity to an estrogen deficiency in an ovariectomized animal model may be increased in relation to the frequency of estrous cycles. Consistent with the comparatively shorter estrous cycles and gestation periods, the bone remodeling cycle is generally shorter in smaller animals (e.g., mice and rats[13] < rabbits[14] < dogs[15] < humans[16]) than in larger species of mammals. Overall, some sort of systematic contrast of estradiol status may be warranted to establish whether there is reasonable similarity in terms of cause and effect when it comes to estradiol diminution and bone loss in nonhuman mammals.

Comparisons between animal models can become difficult to fathom considering there are multiple modes of estrous cyclicity between species and numerous external stimuli that cause variations in cycle length and/or frequency within the same species. Some animals are induced ovulators that spontaneously respond to copulation (e.g., cats,[17] bears,[18] ferrets, rabbits[19]). Others are seasonal breeders, dichotomized based on how they synchronize estrous cyclicity with day length. Long-day breeders (e.g., Siberian hamsters and some mares) are not reproductively active during months of the year with short daylight hours and go into heat as the hours of daylight increase. Conversely, a number of ruminant species in the northern hemisphere are short-day breeders: estrous cycles become more numerous as days get shorter. Estradiol (E_2) concentrations in animals with this seasonal aspect to their nature are elevated in the winter and fall months because of estrous cycling and are depressed during the spring and summer seasons when ovarian estrogen secretion is practically negligible and periods of reproductive quiescence prevail. Averaged over the course of an entire year, ovine exposure to estradiol is minimal compared with human or rodent exposures; in fact, in ovariectomized sheep, it only requires 0.4 versus 10 μg E_2/kg in ovariectomized rodents to stimulate estrus-like responses.[20] After ovariectomy, the reduction in circulating estradiol in sheep is not remarkably different from basal concentrations during anestrus[21] or the ovulatory cycle. Extragonadal estradiol, synthesized by peripheral aromatization in adipose tissue, is thought to contribute to low circulating estrogen concentrations in ovariectomized sheep.[22] It is not uncommon for the low estradiol concentrations in ovariectomized sheep to exceed the sensitivity of commercially available assays.[21,23] Goats respond to changing light conditions similarly to sheep. Estrous cycle estradiol peaks are about a magnitude higher in caprine versus ovine species. Conversely, swine are nonseasonal polyestrous animals.

The canine estrous cycle is unusual in that bitches typically only go into heat once or twice a year. Although it can vary widely among dogs, heat cycles include a long anestrus interval of up to 150 days, during which circulating estradiol concentrations remain comparatively low because of ovarian quiescence. During most canine bone loss experiments in which peripheral estradiol concentrations were measured after ovary removal, significant reductions in estradiol have been reported based on comparisons to sham dogs,[24–26] although in one experiment a significant drop in estradiol concentrations took up to 10 wk to become apparent.[27]

Ovariohysterectomy (OHX) or spaying entails physical removal of the ovaries, oviducts, uterine horns, and the uterus. It is a more invasive procedure than OVX and is sometimes the preferred surgical intervention to induce dogs to become osteopenic. OHX can potentially predispose dogs to more long-term urogenital complications.[28,29] Ovarian remnant syndrome has been shown to occur in up to 43% of dogs that have been ovariohysterectomized, although this may be similar to its occurrence in OVX dogs.[28] The deep anatomical location of the right ovary in dogs necessitates necropsy at termination to confirm the complete absence of ovarian tissue in experiments predicated on OVX or OHX (i.e., OX). If OVX rather than OHX has been performed, the uterus should also be examined for signs of significant atrophy. Pseudopregnancy can occur in ovary-intact dogs, and it is not uncommon for OX performed during the luteal phase of the estrous cycle of canids to result in iatrogenic pseudopregnacy. Gestation-like hormonal and physiological changes can persist for weeks or months.[30,31] Monitoring the stages of cyclicity and/or synchronization of estrous cycles in laboratory dogs to enhance endocrinological uniformity and permit strategic timing of castration in relation to estrous cycle stage seems to be a largely unexplored approach in bone research.

GENERAL CONSIDERATIONS

Differences in bone attributes between humans and quadrupeds are fundamental to selecting an appropriate large animal model, using it optimally, and interpreting the data from an informed perspective. For the sake of expediency, the appearance of bone loss very soon after castration is a sought-after outcome for any large animal model of osteopenia. At the outset, this effect is more similar to the human situation when the loss predominantly pertains to cancellous bone. FDA guidelines indicate a preference for assessment of long bones and vertebrae in an animal model of postmenopausal bone loss. Accelerated endocortical resorption in long bones and preferential resorption of transverse trabeculae of cancellous bone epitomizes bone loss during early menopause. The presence of osteonal remodeling is another desirable characteristic in a large animal model, because it confers a characteristic of bone that is relevant to humans and is an improvement on the rodent model with its inherent lack of intracortical remodeling.

In adult humans, practically all normal cortical bone is characterized by secondary osteons made up of layers of remodeled bone (lamellae) that encircle Haversian canals. Conversely, the appendicular cortical bone of other large, rapidly growing terrestrial mammals contains a high percentage of plexiform, or fibrolamellar, bone.[32] This type of primary bone is more prevalent in juvenile animals and forms as a result of subperiosteal or subendocortical budding of a network of small 100- to 150-μm mineralized blocks of bone and is laid down rapidly in a brick-like pattern in laminar layers in a plane parallel to the bone surface. It imparts transverse anisotropic mechanical properties that contribute strength and stiffness beyond that which can be achieved by osteonal bone.[33–35] Accretion of plexiform bone represents an efficient adaptive mechanism that compensates for increased strain magnitudes and frequencies in response to the rapidly increasing weight and sheer size of a growing animal. In contrast, human lamellar growth takes place over a comparatively longer interval of time, and Haversian remodeling is the primary means by which fatigue damage of bone can be arrested and repaired.[36] Large animals eventually remodel some plexiform bone to Haversian bone as they age; however, their need to remodel is typically less than that of humans and is often more or less specific to certain quadrants in long bones. The average size of human Haversian systems is usually larger than most other mammalian species.

Mechanical efficiencies are different in humans and quadrupeds. Humans are at greater risk for injurious falls from higher heights relative to their tall stature, and falls often occur on hard man-made surfaces. Sheep, goats, pigs, and dogs have a lower center of gravity, their body weight is distributed over a larger area, and any fall, if it occurs, is usually onto natural surfaces that are more shock absorbent. Spine orientation and vertebral morphologies also vary with stature.[37] Nevertheless, substantial axial compression forces are common to both bipeds and quadrupeds, although they are generated by divergent mechanics. In quadrupeds, the longitudinal orientation of the spine is supported by large musculature along the animal's back, which can generate four to eight times as much load on the spine as from gravity in the cranial–caudal axis (CH Turner, personal communication, 2006).[38] Human vertebral bodies are larger in diameter than those of dogs, pigs, goats, and sheep, and comparatively enlarged cranial endplates and intervertebral disks increase the surface area[39] to facilitate the redistribution of dynamic loads. Vertebral bodies of mature canine, ovine, and porcine species have more height in proportional terms than they do in humans,[37] and the forces generated by the supporting muscle bulk expose animals to higher axial compression stresses leading to vertebral volumetric BMDs that greatly exceed those of humans.[37,40,41] One of the major challenges when using large animals and inducing osteopenia by ovariectomy is to achieve a severity of bone loss at the spine that is significant and reflects what is generally observed in osteoporotic humans.

The effect of ovariectomy on bone loss and/or bone metabolism is sometimes assessed in the iliac crest of larger animals, particularly dogs[27,42–48] and sheep.[22,49,50] One of the advantages of larger animal models compared with smaller size rodents is the ability to biopsy the iliac crest and the potential for bilateral biopsies at different time points that can yield longitudinal data. This approach provides information that can be compared with humans as histomorphometric studies of treatments for metabolic bone disease are basically restricted to iliac crest biopsies.[51] In ovariectomized animals, iliac crest biopsies can potentially provide a means by which to quantitatively track dynamic changes in bone after in vivo fluorochrome labeling of bone surfaces. However, compared with the femoral neck, the long bones of the lower limbs, and vertebrae, the iliac crest is not a quintessential load-bearing anatomical structure, and considerable variation exists in histomorphometric estimates across different sites.[52] As such, sampling accuracy becomes an issue, and it is debatable whether a single iliac crest bone biopsy captures the true extent of an underlying metabolic bone disease.[53] The efficacy of a castrated animal model for the purpose of osteoporosis-related research may be more directly assessed at major load-bearing sites in termination studies. Aside from remodeling dynamics, ovariectomy-induced changes at the iliac crest may not necessarily reflect the density and microarchitectural changes at usual load bearing sites where pathologic fractures caused by osteoporosis predominate.

DOGS

Bone metabolism in both dogs and humans change from a mode of predominantly modeling during growth to remodeling at maturity.[54] Mature dog bone is very similar in composition to that of human bone.[40] The haversian and cancellous remodeling system in dogs is also analogous to that of humans,[55] although remodeling cycles are ~25% shorter in dogs,[15,56] and the cancellous turnover rate is two to three times that in humans.[57] Osteon population densities in the ribs and long bones of canines match those of humans, although haversian system diameters are relatively smaller.[32] Despite some similarities between human and dog bone, a number of researchers remain uncertain of the potential of ovariectomy as an effective means by which to induce significant bone loss in dogs.[12,42,49,56,58] Thus far, results among laboratories have either differed or have not been entirely encouraging.

The beagle is routinely used in scientific experiments, because of well-documented physiological responses.[55] The fallibility of the castrated dog model for bone loss research may have evolved more as a result of inconsistent study designs rather than an inherent unreliability of the dog itself. A systematic comparison of available OVX and OHX dog studies[24–27,42–48,59–63] showed that the age range of animals has often been wide, the numbers of dogs in experimental groups are sometimes small, use of histomorphometric endpoints (e.g., BV/TV) for small group sizes requires very large differences given the high SD (~25%) for this method of assessment,[57] the site examined may not be representative of bone loss at other sites, dietary calcium is often overabundant or indeterminable, complete extrication of ovarian tissue is not routinely confirmed at necroscopy, and the timing of ovariectomy may not have been well planned in relation to estrous cycle

stage. Castration during times of peak progesterone concentrations can potentially result in heterogeneous hormonal profiles among dogs in shorter-term experiments. Considering the relative infrequency of estrous cycles in canines compared with ungulate or rodent species, experimental manipulation of the OX canine model may require a more standardized approach toward achieving homeostasis. To date, the castrated canine model may not have always been exploited to its full potential, or at least this possibility should be methodically studied before it is summarily discounted. Alternatively, some investigators may be inclined to consider the use of dogs impractical based on the premise that the experimental circumstances required for dogs to possibly yield more consistent bone loss results are too onerous.

Commercial dog foods did not become widely available until the late 1950s and early 1960s. Dry dog chow is often the diet chosen by researchers for their experimental animals. It is formed by extruding pellets formulated with a mixture of rendered meat-and-bone-meal, poultry by-product meal,[64] and grains and flours derived from soybeans and other grains.[65] Dogs do not naturally consume large amounts of bone or cooked and processed bone meal that is exceedingly calcium-rich; nor do they instinctively opt to habitually eat cereal products or grains containing phytoestrogens. The canine digestive tract is relatively short and not as well suited to the fermentation and digestion of plant foods as natural omnivores. Nevertheless, both bone meal and/or soybean meal are common ingredients in commercial dog chows.[65] Normal bone maintenance in dogs has been shown time and again in diets containing 0.5% Ca.[66] Bone meal and other various by-products can provide an overabundance of Ca, and soybean meal provides unnecessarily high concentrations of phytoestrogens[65] that can influence bone metabolism.[67] One only has to peruse the literature related to OVX and OHX dogs that report dietary mineral contents to realize Ca concentrations are routinely excessive (>1%[26,27,44–48,59,63]) in those studies not deliberately restricting or controlling Ca intakes. Martin et al.[26] reported using dog food containing as much as 2.1% Ca (purportedly of a relative equivalency that represents 30–60 times the recommended Ca intake for women at the time) with as little as 1.4% phosphorus for ovariectomized dogs (a Ca:P ratio of 1.5:1.0) when the ideal Ca:P ratio reported by Morgan[68] is 0.50:0.65. Only transient cortical bone loss was observed in response to surgical castration in the study of Martin et al., and perhaps this is not surprising considering the extremely high Ca levels.

Similar to the gain in body weight that follows menopause in many women, dogs also show body weight increases[26,42,48] and changes in fat distribution after the physiological withdrawal of estrogen.[69] Castrated beagles exhibit correlations between bone remodeling variables and percentage marrow adipose tissue volume (%ATV), as well as estrone and estradiol concentrations and %ATV ($r = -0.74$ and -0.65, respectively), reflecting bone metabolic changes common to patients diagnosed as osteoporotic.[69] Uterine tissue has also been shown to be sensitive to estrogen depletion in the canine model (20–30% atrophy).[43] In response to castration, acute bone loss and/or significant changes in bone remodeling, as measured by dynamic and static histomorphometry parameters, have been documented for beagles,[24,26,27,43–48,63] although a number of early changes were considered transient[24,43,48] or were reported for a period of less than two remodeling cycles.[44–47,63] Beyond a 6-mo period, other studies have shown a lack of response for dynamic bone parameters[25,42] and/or a lack of sizeable responses for biochemical parameters.[42] Kimmel[57] has postulated that beagles may have a smaller estrogen-dependent compartment of bone in their skeleton compared with women.

Postmenopausal women are known to sustain spontaneous rib fractures in response to low trauma; rib fractures are known to predict incident limb fractures in humans.[70] Ribs are subject to constant cyclic loading attributable to respiration, and this site has been studied in spayed canines because of its naturally high rate of cortical bone turnover[71] (i.e., ~18%/yr versus <1%/yr in the diaphyses of long bones, although these percentages are subject to the animal's age[72]). Continuous mechanical stimulation of ribs also means that changes in bone remodeling activity are less influenced by levels of physical activity versus inactivity as might be expected with long bones.[73] Anderson and Danylchuk[73] suggested ribs are numerous, and therefore, if required for the purpose of monitoring longitudinal changes in haversian bone remodeling, a rib can be biopsied bilaterally in dogs without undue problems. Wilson et al.[24] have shown that dog ribs appear to be acutely responsive to OVX, with changes in bone formation indices observed between 1 and 4 mo for 7- to 9-yr-old animals. This was despite similar circulating estrogen concentrations for sham and OVX dogs 6 wk after surgery. Sequential midrib biopsies showed that these early changes disappeared by 8.5 mo. Significant differences in cortical bone histologic measurements between different sites (i.e., proximal, distal, and midshaft portions) on the same rib have been documented in canines by Anderson.[74] A different 12-mo experiment showed that OVX increased the resorptive surface on the endocortical envelope of dog ribs ($p < 0.005$), a change that was reversed by the administration of estrogen.[62] In dogs, the ribs may offer a good site to measure intracortical haversian bone remodeling activity if experimental conditions are carefully considered.

Ovariectomy on its own did not result in a significant reduction of BMC or mechanical strength in the lumbar vertebrae of beagle dogs 36 wk after surgery.[59] However, limiting dietary Ca content from 1.4% to 0.1% in addition to ovariectomy in dogs that were ~30 mo old at death resulted in a 31% reduction in BMC for cortical and cancellous bone as assessed by QCT and significantly diminished mechanical strength (40% decrease in maximum load; ~50% decrease in energy absorption based on compression testing of cancellous bone cores taken from vertebral bodies). However, these data are based on small sample sizes ($n \leq 4$ dogs/group). Shen et al.[42] examined the effect of OHX on the bone mass of eight 4-yr-old breeder beagles 6 mo after surgery. The dogs were fed a standard canine chow with an unspecified amount of calcium for the duration of the study. The BMDs (mg/cm^2) of the OHX dogs' lumbar spine (L_2–L_4) did not differ significantly from sham

controls. Conversely, Martin et al.[26] observed a 15% increase in porosity of the fifth lumbar vertebrae of 3- to 7-yr-old ovariectomized dogs by histomorphometric analysis (48 wk after ovariectomy), despite the very high percentage of Ca (2.1%) in the diet of these animals. However, bone composition (i.e., % water, organic, and ash) of the thoracic (T_{13} vertebral bodies) and lumbar (L_3 cancellous cores) spine were not significantly affected by ovariectomy, but biomechanical parameters of work-to-failure and ultimate stress were significantly decreased in OVX dogs compared with sham controls. Dual-photon absorptiometry was used by Guesens et al.[75] to detect significant decreases in the BMD of the second lumbar vertebrae of ovariectomized Ca-restricted (0.06% Ca) beagles versus ovariectomized beagles on Ca-adequate diets 7 mo after castration (i.e., mean ± SD. BMD g/cm^2 for total vertebra 0.340 ± 0.026 versus 0.397 ± 0.021; central region of the vertebrae 0.307 ± 0.036 versus 0.390 ± 0.032). As for postmenopausal women, the calcium content of a canine diet may play an important role in the absence of estrogen.

The added perturbation to bone metabolism brought about by low Ca intakes is considered to be attributable to nutrient-induced secondary hyperparathyroidism in ovariectomized dogs. Dogs fed extremely low Ca levels can develop severe alveolar bone loss and parathyroid changes associated with hyperfunction.[66,76] Cook et al.[61] have shown that dietary restriction of Ca (0.12%) alone alters i-PTH levels in experimental male beagles and promotes the loss of bone. The earliest signs of pathological bone loss induced by Ca deficiency in dogs manifest in the jaw bones and second in skull bones, followed by the ribs, vertebrae, and the long bones.[66] Canine sensitivity to higher calcium intakes was shown in a study of ovariectomized beagles by Shih et al.[77] Twenty-six weeks after castration, dogs administered 250 mg of supplemental Ca had higher bone mineral at the midshaft of the femur than OVX controls not fed supplemental calcium ($p < 0.01$). In this instance, the difference remained significant when BMC was normalized for femur length and femur width.

Iliac crest biopsies yield corticocancellous samples that are valuable in predicting changes in remodeling dynamics. Malluche et al.[47] observed a significant reduction (20.3%) in the iliac crest cancellous bone mass in dogs 4 mo after OHX. Mean values for trabecular wall thickness and density were decreased, whereas trabecular separation was increased, as occurs in patients with osteoporosis. Histomorphometric measures showed the mineral apposition rate (MAR) and bone formation rates (BFR) at the tissue and cellular level were markedly slowed in response to OHX. Despite the increase in osteoblast number and bone-forming cells, this finding was attributed to a decrease in osteoblastic function that had ramifications at the tissue level. Dannucci et al.[27] observed increased osteocalcin levels in dogs after OHX (weeks 8–22), indicating an increased rate of bone turnover similar to what transpire in postmenopausal women with high remodeling osteoporosis. Decremental changes for bone histomorphometric values (BV/TV and Tb.Th) of the iliac crest and for serum biochemical parameters have also been shown by Fukuda and Ilda[54] in 2-yr-old male beagles examined before and 12 mo after orchidectomy. Changes in cancellous bone attributable to a decline in sex hormones may extend to both sexes in canines. Based on percentage change from baseline (±SE), the histomorphometry parameters MAR and BFR/TV are decreased in beagles by 12.3 ± 4.2% and 3.8 ± 4.2%, respectively, in response to oophorectomy; these changes were measured on thin sections taken from iliac crest biopsies in different studies (i.e., a total of 12 baseline versus OVX group comparisons[26,42,44,47,48,78,79]).

There are few data showing osteopenic changes in the hind- or forelimb bones of dogs. Mechanical parameters of the femoral neck of beagles has been evaluated by Nagai and Shen[59] after calcium restriction in conjunction with OVX. Maximum load and energy absorption of the femoral neck were significantly lower than in sham dogs that were not calcium restricted. Martin et al.[26] have reported a significant increase in the number of osteonal resorption spaces on the ulna of ovariectomized beagles. In the same study, cortical bone areas of the radius, ulna, and humerus were not different between sham and OVX animals, but mechanical test data for the femoral neck and shaft regions showed a trend for lower strength, although differences were not consistently significant. Cortical bone microstructure of the canine hip is considered similar to that of humans as is the cancellous bone distribution along the medullary canal of their femur.[80]

SHEEP

Generally sheep have an agreeable disposition, are compliant, and can be trained to perform routine tasks (e.g., treadmill walking or running).[81] Thus far, sheep have proven invaluable in orthopedic research,[82] and their use in the study of alveolar and mandibular bone loss has been effective.[83,84] They are a less expensive animal model than pigs or dogs, and their size is conducive to the insertion of prosthetic implants comparable to those implanted in humans.[85] The effect of osteopenia on the biological response of ovine bone to implants (e.g., osseointegration) and various prosthetic devices can be reliably assessed in studies that allow enough time for a nontransient osteopenic state to develop.[86] The age of the animals, the timing of the study, and the duration of the experiment have to be considered in sheep versus other species. Use of a few widely accepted standardized sheep breeds would make interlaboratory comparisons easier, and disclosure of reproductive (i.e., the parturitional and lactational) and dietary histories could further aid our understanding of the efficacy of this animal model.

The compact bone of sheep is predominantly plexiform until the animals reach 3 or 4 yr old.[82] Thereafter, most of the bone that is laid down is primary osteonal bone that is not remodeled to secondary bone until the animals reach a reasonably old age (7–9 yr).[81,82] Aging results in secondary haversian remodeling that becomes more extensive at specific locations such as the posterior aspect of long bones and in ribs. Haversian canal distribution has been shown to be less dense in ovine bone than in human bone[32] and has been described as nonuniformly distributed.[82] Aged sheep exhibit an 8–10% loss of cortical bone and <1% loss of

cancellous bone at the distal tibia 6 mo after ovariectomy based on pQCT measurements.[87] Reports of acute changes in bone remodeling and bone mass in older sheep (as early as 3–6-mo, respectively) in response to ovariectomy[22,88,89] are encouraging but are countered by data indicating potential rebound effects over time[22,90] and spinal idiosyncrasies that need to be taken into account before embarking on a study of bone loss in an ovine model.

Chavassieux et al.[89] showed that ovariectomy in retired breeder ewes (mean age, 8 ± 1 yr) on normal calcium intakes (4.5 g/d) for 6 mo resulted in significant effects on cortical bone parameters 6 mo after ovariectomy (i.e., increased cortical erosion and cortical porosity) compared with sham animals. Ovariectomy also significantly decreased BMD of the femur but not the lumbar vertebrae by 6 mo. In the distal radius of ovariectomized sheep, Sigrist et al.[22] observed a rapid initial phase of cancellous bone loss that averaged 12.7% between 0 and 4 mo after ovariectomy. Thereafter, a slight rebound effect occurred in terms of the percentage change from baseline in BMD of OVX animals. This change corresponded to a slight decline from baseline BMD in control animals, such that significant differences between controls and OVX sheep disappeared from 4 to 17 mo. This rebound effect was attributed to the likelihood of extragonadal estrogen synthesis that may have caused BMD to be regained. Three and 6 mo after ovariectomy, the distal radius of 7- to 9-yr-old ex-breeder ewes had no significant bone loss.[88] After 6 and 12 mo, OVX alone was not determined to be an effective means by which to significantly reduce the proximal tibia BMD of skeletally mature Merino sheep.[90] 3D histomorphometric analysis of ovine tibial bone biopsies yielded data showing only slight reductions in trabecular parameters between OVX and sham animals; however, OVX sheep administered glucocorticoids did present with significant cancellous bone loss.

Reports of significant rapid bone loss and architectural changes in the vertebrae of sheep after ovariectomy are relatively scarce. Compared with intact young and aged sheep, progressive bone rarefication has been measured in the vertebrae (L_5) of OVX sheep 24 mo after surgery by Borsari et al.[86] Rocca et al.[85] also observed differences in the areal BMD of the fifth lumbar vertebrae from OVX versus sham sheep (0.695 ± 0.070 versus 0.893 ± 0.035 g/cm^2, respectively) 24 mo after castration when the animals were between 5 and 6 yr of age. Turner et al.[88] reported some significant BMD (g/cm^2) differences in the lumbar vertebrae of OVX versus sham sheep 3 (L_4) and 6 mo (L_4, L_6, L_4–L_6/L_5–L_7) after ovariectomy. However, these differences were not necessarily caused by bone loss: the BMD of sham sheep seemed to increase over 6 mo, whereas the BMD of the OVX sheep barely changed from baseline and was only decreased by 0–2%. Results of these in vivo scans were reported as a percentage change from baseline. In contrast, MacLeay et al.[4] was not able to detect areal BMD changes in the lumbar vertebrae (L_4–L_7) of mature (4–7 yr old) ovariectomized sheep 90 days after surgery.[4] A study of similar length (3 mo) was performed by Pogoda et al.[50] Static histomorphometry of the lumbar spine (L_3–L_5) showed no structural differences in trabecular bone between sham and ovariectomized sheep, although cellular histomorphometry in vertebrae showed that osteoclast number/bone perimeter (mm^{-1}) and osteoclast surface/bone surface (%) were significantly increased in response to ovariectomy. Twenty-four months after OVX surgery in 6-yr-old ewes, significant microarchitectural changes in vertebral cancellous bone were detected by measurement of static and dynamic histomorphometric parameters (i.e., decreased BV/TV ~30%, Tb.Th ~13%, and Ac.f ~58% and increased Tb.Sp ~46%) compared with sham animals.[91] Mechanical parameters tested by vertical compression were also significantly compromised in these 6-yr-old OVX sheep. Collectively, these results suggest that significant bone loss in the vertebrae of older ovariectomized sheep may require longer-term studies (>12 mo).

The lumbar spine of sheep is slightly kyphotic rather than lordotic,[92] the number of vertebrae can be variable,[4,88,92] and cancellous BMD can be extraordinarily high (400–600 mg/cm^3).[41] Ovine vertebral bodies are also remarkably tall in the cervical region,[92] and it is the thoracic and lumbar vertebrae of sheep that are most grossly similar to human vertebral dimensions.[37,93] Projectional in vivo DXA measurements of vertebrae can be easily distorted in sheep because of positioning issues associated with the amount of subcutaneous fat and fleece.[22] Moreover, biomechanical parameters have been shown not to correlate with BMD assessed by DXA in ewes treated with bone loss–inducing glucocorticoids; this perceived inconsistency was attributed to the anatomically large vertebral posterior arches in sheep that are predominantly comprised of cortical bone.[94]

As herbivores, the gastric systems of most ruminants are characterized by a complex four-compartment stomach to facilitate digestion of vast amounts of plant cellulose. Consequently, the mode of action of some nutrients, pharmaceuticals, or other bioactive materials delivered orally may be altered in polygastric animals before absorption in their true glandular stomach.[95] An example of this phenomenon is forestomach microbial fermentation of phytoestrogens from various fodder crops being converted into more potent estrogen-like molecules, which can cause serious health problems in sheep.[96] Creative measures to regulate and protect the potency/viability of an orally administered therapeutic substance in ruminants may need to be used.[12,97–100] Ruminants become a more appropriate model when therapeutic agents are administered by injection rather than by the oral route.

In experiments where ruminants are left to graze in pastures, the potential phytoestrogen content of leguminous fodder consumed should be considered as a factor that can influence the metabolism of estrogen-responsive tissues and organs such as bone. Leguminous silages can be rich in phytoestrogenic compounds that act as selective estrogen receptor modulators (SERMs). Ewes have been shown to be sensitive to the effects of both naturally occurring[101] and pharmaceutical SERMs.[89]

Although estimations can be variable, a high percentage of the body weight of sheep is associated with rumen fluid/contents (~13%) as well as fleece and pelt weight (~12%),[102] making drug dosages and clearance rates difficult to calculate. Establishing a comparable clinically rel-

evant dose of a pharmaceutical agent in sheep or goats based on a comparable mg/kg/day basis for humans will require more work in the case of ruminants. As Smith and Minium[103] have indicated previously, good experimental animal models typically simplify complex systems rather than complicate them.

GOATS

Goats are generally classified as gentle, inquisitive, placid, and easy to handle, as well as clean and hardy in nature. Like sheep, they are polygastric ruminants and face most of the same issues related to their gastrointestinal physiology. Most goats are maintained in a farm environment for a large part of their life and their dietary history (pastoral grazing versus cultivated fodder versus silage) should routinely be disclosed when data are reported. The rumen in goats and sheep represents a significant site of Ca absorption,[104] and goats in particular require time to adjust to dietary changes and are predisposed to serious illnesses and urinary calculi if the calcium:phosphorus ratio is $\leq$1.5:1.0.[105] Considering goats are a seasonally polyestrous species, the seasons spanned and geographical location inhabited during the experimental period should also be stated for contextual interpretation. Goats continue to gain acceptance as an established large model for orthopedic research and surgical training,[105] although their use as an osteopenic animal model is currently infrequent. Most data available on the effect of ovariectomy on caprine bones emanates from China and is for Chinese mountain goats for which the extrapolation of findings to alternate breeds common in other nations is practically untested.

The limited data that do exist for goats indicate ovariectomy can induce significant bone loss and low Ca intake augments osteopenia. He et al.[106] measured BMD of the femora and tibias of ovariectomized goats 6, 12, and 18 mo after surgery and observed the progression of osteopenia at all time points; however, at 12 versus 18 mo, the decrease in BMD was not statistically different at these sites. The cortices of the long bones of immature goats are similar in microstructure to those of sheep, with copious plexiform tissue that is remodeled to haversian bone with advancing age. The distribution of haversian systems in goats has been described as heterogeneous; dense haversian tissue has been observed near the endocortical surface, with primary and sporadic haversian systems in the central cortical region, and combinations of plexiform and scattered haversian bone at the periosteal surface.[32]

Very little has been published on ovariectomy-induced osteopenia in the goat spine. He et al.[106] observed significant decremental changes in the microstructure of the first lumbar vertebrae of OVX goats. The analysis was based on μCT analysis of trabecular bone 6 and 18 mo after ovariectomy, and comparisons were made with intact and sham-operated goats. Leung et al.[107] fed a low-calcium diet (0.5% Ca) for 6 mo to ovariectomized 3.4-yr-old mountain goats, and the median reduction in pQCT volumetric BMD for L_2 and L_7 lumbar vertebrae of OVX animals compared with sham-operated animals was 32.76% ($p = 0.01$) and 30% ($p = 0.011$), respectively. Median percentage change data tends to suggest statistical nonnormality and variability in measurements between animals. Leung et al. have also reported sizable median bone BMD (g/cm^3) decreases of 32.84% and 23.91% for the calcaneus and humeral head of OVX goats compared with sham controls. The volumetric BMD (pQCT) of the iliac crest bone of skeletally mature goats 6 mo after ovariectomy has been shown to be reduced by 16.26% and is accompanied by a deterioration of trabecular architecture as measured by μCT.[108] Other data indicate significant decreases in BMD and decreases in structural and material mechanical properties 180 days after OVX; however, full details are not accessible because of the lack of translation.[109]

Mini- and microbreeds of goats represent essentially unexplored animal models for ovariectomy-induced bone loss. It is unknown whether their characteristic early maturity and small size are associated with less plexiform bone at a younger age. The long life span ($\geq$20 yr) of these low maintenance compact breeds, their high reproductive rate, and adaptability to environmental conditions seem to make them an attractive prospect for future study in circumstances not unduly complicated by their digestive physiology.

PIGS

Because of the well-characterized and striking anatomical similarities that exist between pigs and humans, in addition to their general good health as a species, swine are used in biomedical research as relevant models for numerous human conditions and diseases[110] and have emerged as the preferred donor for xenotransplantation in humans. The increased availability of genetically defined/modified pigs further improves their usefulness as a versatile large animal model.[111] An overwhelming body of research attests to the notion that bone metabolism is significantly affected by diet and nutrition in humans and animals. The food sources from which pigs derive their energy and the gross gastrointestinal physiological function of swine are more similar to humans[112] than most other species. Humans and swine are both monogastric and true omnivores, factors of relevance considering physiology and food intake can influence drug metabolism and renal elimination in mammals.[102] Interspecies differences in renal function take on importance with respect to pharmacokinetic profiles of nonmetabolizable drugs such as bisphosphonates[113] used in the treatment of various bone metabolism disorders. Swine represent an animal model considered to approximate human renal physiology.[114]

One major liability associated with management of domestic pigs is their massive size. Domestic pigs grow rapidly from their birth weight of 1.2 kg and can attain body weights of 150 to $\geq$200 kg at maturity, hampering their usefulness in chronic studies. Their massiveness can even pose an occupational safety hazard for personnel handling them.[112] Swine can also exhibit an intermittently stubborn and/or aggressive disposition and tend to be noisy.[115] Substituting domestic pigs with miniature or micropigs can circumvent many issues related to their size disadvantage. However, occasional human-directed aggression is a docu-

mented problem in pet miniature pigs,[116] as is obesity if food intake is not regulated.[117] The most commonly used breeds of miniature pigs in laboratory research are Yucatan miniature and micropigs, Hanford, Sinclair, and Gottingen pigs.[118]

By nature, pigs are socially inclined and quickly establish dominance hierarchies within groups. They are intelligent, emotional animals and, when handled and housed correctly, aggression problems are less likely encountered.[112–119] The ease of handling reduced size livestock has definite advantages in terms of space requirements and reduced housing and food costs. Benefits extend to include reduced bone scan times, smaller size samples for analysis, and the smaller quantity of drugs required to elicit therapeutic effects. However, adequate quantities of blood and tissue are available from larger animals.

The femoral compact bone of juvenile swine is predominantly plexiform in nature, only a small percentage of which is remodeled over time and converted to osteonal bone. Pigs remodel bone earlier during their growth than do sheep or calves.[120] Large-sized, skeletally mature breeds of swine continue to exhibit a high proportion of plexiform bone in femoral bones with dense haversian bone being localized in the posterior quadrant.[32] There is some overlap in haversian canal diameters and secondary osteon dimensions among humans, goats, sheep, and pigs,[32] with the osteonal bone of swine appearing most similar to humans for these parameters.[121] Marinez-Gonzales et al.[14] report a close similarity between the bone remodeling rates of pigs and humans. However, based on a steriologic study, Kragstup and Agerbaek[122] estimated the cortical bone of 14-mo-old domestic gilts was remodeled at a rate of ~10%/mo, whereas only ~3%/yr of cortical bone is turned over in humans.

The growth pattern of minipigs is quite different compared with large-sized pigs bred for their meat.[123] It takes minipigs ~2.4 times as long to grow to 40% of their mature weight as it does for intensely or restrictively fed fattening pigs.[123] A relatively slow linear growth pattern early in life may decrease the amount of plexiform bone formed in miniature pigs and increase the amount of osteonal bone present in mature animals. Currently, data of this nature for skeletally mature small-sized pigs or other miniature or microlivestock do not exist to substantiate or discount that notion.

Of the various animals mentioned, hybrid miniature and microporcine strains probably show more morphological similarities to the human spine than do the other species.[37] They reach skeletal maturity at a body weight comparable to that of humans (70–80 kg), and structurally, the vertebral body dimensions are closest to those of humans. However, there exists a shortage of data on studies performed to assess vertebral bone loss and bone architectural changes in skeletally mature (≥36 mo) ovariectomized mini, micro, or even domestic pigs. Borah et al.[124] has reported that ovariectomy of Sinclair minipigs resulted in no significant bone volume and/or architectural changes in L_4 trabecular vertebral cores as measured by μCT analysis in animals that were operated on at 18 mo and killed by the age of 36 mo; however, during this period of time, pigs are considered to still be growing. In the same study, administration of risedronate after OVX enhanced trabecular architecture and increased bone strength in OVX animals.

Mosekilde et al.[125] conducted a pilot study that examined the long-term effects of lowered Ca levels (0.9% versus 0.75%, and 0.5% Ca in the diet for 12 mo) on the axial and appendicular skeleton of sexually mature Sinclair minipigs 6 mo after ovariectomy versus sham minipigs (0.9% Ca). Mild Ca restriction (0.75% Ca) 6 mo before and 6 mo after ovariectomy was found to be most effective at stimulating vertebral bone loss (–10% areal BMD) and deterioration of vertebral architecture (Tb.N and Tb.Th) and mechanical strength. The remodeling parameter MAR (μm/d) of vertebral cancellous bone of the 0.75% and 0.9% Ca + OVX groups was significantly reduced compared with sham pigs, and rib cortical bone geometry was compromised after 0.5% and 0.75% Ca + OVX. Increased endocortical resorption was observed to decrease BMD at the midshaft of the femur and radius of pigs fed 0.75% and 0.5% dietary Ca, although midfemur cortical bone histomorphometric parameters were not different among groups. Osteoclast activity at the level of the remodeling unit reduced cancellous bone and deteriorated cancellous bone microstructure. However, ~25% of the diet of swine in this study was comprised of food sources rich in endocrine-active phytoestrogens (i.e., soymeal and alfalfa) that could have influenced the amount of bone loss after ovariectomy.

Vertebral trabecular separation in minipigs is less than that observed in pre- and postmenopausal women (200–300 versus 500–700 and 700–1000 μm, respectively), although vertebral trabeculae are thinner in minipigs (~100 μm) than in humans (150–170 μm).[41] On this basis, Gluer[41] speculated there may be an increased propensity for trabecular perforation in the event of increased bone resorptive activity in minipigs. Such an outcome has been shown in young growing minipigs that were ovariectomized and placed on a Ca-restricted diet.[126] Vertebral bone loss resulting from increased resorptive activity converted vertebral trabecular plates into rods, which was purported to increase trabecular connectivity concurrent with the decrease in spine BMD. Osteopenic changes in the vertebrae have thus far been the focus of studies on older swine, and more research is needed to characterize the responses to ovariectomy in the limb bones of this species.

Use of the veterinary drug tetracycline (TC) as a broad-spectrum antibiotic occurs frequently in swine production.[127] This agent is used prophylactically and/or therapeutically at various dosages. TC is one of a number of fluorochromes available for labeling mineralizing bone surfaces for the purpose of histomorphometric analyses. Multiple therapeutic TC doses can cause intense discrete fluorescing bands at bone surfaces and interfere with bone labels administered by investigators intending to perform ex vivo dynamic histomorphometry. Avoiding swine that have been routinely treated with TC is advisable to prevent superfluous bone labels.

CONCLUSION

In an economic climate where financial resources for research are becoming increasingly limited, the choice of ex-

perimental animals must be scientifically and ethically justifiable as well as cost-effective.[104] Skeletally mature animals are obligatory for osteoporosis research to avoid the numerous confounding factors that are introduced with animals that are still growing and accruing bone. If ovariectomy alone, or ovariectomy coupled with dietary/nutrient perturbation, can induce significant bone loss in a species relatively rapidly, the use of the model then becomes contingent on the degree to which the tissue-level mechanisms of bone loss are similar to those of humans and on the potential of the model to make reliable predictions relevant to prevention and therapeutic treatment. Biological and physiological discrepancies will always exist between species; therefore, all foreseeable limitations must be carefully considered so that the rationale for choosing the animal is a judicious one that enables the researcher to best answer the questions being posed. The fewest number of animals may suffice when the chosen species shows bone metabolism and disease characteristics most analogous to those of humans. To effectively formulate or test hypotheses, the physiological characteristics of specific animal models must be reasonably well understood. Conflicting bone loss data for a particular breed of a species across similar studies suggests more variables in experiments need to be systematically controlled before a consensus can be reached on its usefulness as a model of osteopenia. Overall, primates are most physiologically similar to humans. However, other large animal models may closely mimic one or more relevant aspects of human physiology making them a better choice based on cost, time, labor, availability, and ease of management. The predictive validity of a large animal model is paramount in preclinical research as it serves as a prelude to human clinical trials.

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