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Perspective Article

Hylonome

Perspectives on using nonhuman primates to understand the etiology and treatment of postmenopausal osteoporosis

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Abstract

The reproductive physiology and skeletal anatomy of nonhuman primates are very similar to those of women and these similarities have prompted studies of the effects of ovariectomy in monkeys on bone metabolism. Following ovariectomy, monkey bone exhibits increases in remodeling activity resulting in bone loss. Since similar bone changes occur after menopause in women, ovariectomized monkeys provide an excellent model of the early skeletal events following menopause and have been employed to study the skeletal actions of drugs designed to treat postmenopausal osteoporosis. This review describes the motivations for examining monkeys, practical aspects of working with monkeys, comparisons of human and monkey bone anatomy, endocrinological aspects of monkey bone metabolism, and the available data obtained in monkeys related to postmenopausal and other forms of osteoporosis.

Keywords: Nonhuman Primates, Monkeys, Osteoporosis, Bone Metabolism, Ovariectomy

Overview and motivations for studying monkeys

Scientists interested in anthropology, behavior, cardiovascular disease, infectious disease, primatology, reproduction and veterinary medicine, among other areas, study nonhuman primates. Each scientific discipline has its own perspectives on the advantages and limitations of using monkeys to understand human biology and disease. As summarized below, the similarities in reproductive endocrinology and skeletal anatomy between humans and nonhuman primates provide a motivation for undertaking bone studies in monkeys. This review summarizes the personal reflections of a bone biologist who has examined cynomolgus monkeys for five years in an effort to better understand the development of postmenopausal osteoporosis and potential therapeutic approaches to its treatment. The reader is assumed to have knowledge of bone metabolism and osteoporosis and is also directed to another recent review on this topic[1].

Nonhuman primates are categorized into two groups, New World and Old World monkeys that separated in evolution about 60 million years ago. Humans diverged from Old World primates (baboons and macaques) about 35 million years ago, with the separation of humans from chimpanzees and bonobo apes occurring about 6 million years ago[2-4]. Most recent investigations of bone metabolism have examined rhesus, cynomolgus or pigtailed macaques, baboons or African green (vervet) monkeys, all of which are Old World monkeys. Rhesus monkeys are native to India and China whereas cynomolgus monkeys (also known as crab-eating macaques) come from Malaysia, Indonesia and the Philippines[5]. The origin of the name cynomolgus is obscure since its Latin root refers to the drinking of milk from dogs. Interestingly, dogs have been observed to act as maternal surrogates to infant rhesus macaques[6].

There are two main reasons to study bone metabolism in monkeys. First, monkeys have a menstrual cycle very similar to that of women. Ovulatory cycles have been extensively characterized in baboons[7] and in rhesus[8,9] and cynomolgus macaques[10-13] and involve a follicular phase, ovulation, a luteal phase and menstruation over four weeks. A natural menopause occurs in baboons[14] and rhesus monkeys[15-18] during their third decade of life. In contrast, dogs have a seasonal estrus, rabbits ovulate upon mating, and rodents have a four to five day estrous cycle that does not involve a true luteal phase unless mating (or pseudopregnancy) occurs. Normally cycling cynomolgus macaques appear to have reduced bone resorption during the late follicular phase of their menstrual cycle, when circulating estrogen levels are highest[19].

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The second reason skeletal metabolism is examined in monkeys involves the presence of cortical bone osteons, first identified by the English physician Clopton Havers in 1691[20]. Monkey cortical bone undergoes osteonal (Haversian) remodeling which does not normally occur in rodents. Osteons are cylindrically oriented structural units resulting from the resorption of old bone and its replacement by new bone. Among the many publications describing osteonal biology, the recent book by Martin, Burr and Sharkey[21] and papers by Cooper et al.[22], Enlow[23], Ingram et al.[24], Marotti[25], Piekarski and Munro[26], Robling and Stout[27], Stout et al.[28], and Villanueva and Frost[29] are particularly recommended. Blood vessels run longitudinally through osteons and supply nutrients to osteocytes embedded within mineralized bone. Unmyelinated nerves are also preset alongside the vasculature[30]. Activation of osteonal remode-ling is believed to occur in response to bone microdamage[31,32] that disrupts the communication among osteocytes and between osteocytes and bone lining cells[33]. With osteonal remodeling there is a continual repair and replacement of old bone with new bone to minimize the accumulation of microdamage. Additional information on cortical bone biology and osteonal remodeling in monkeys can be found in several publications[34-38] and Figure 1 shows a photograph of a midshaft fibula section containing osteons obtained from a cynomolgus monkey. Excellent microradiographs showing variations in mineral density among various osteons in cross-sections of baboon tibia are presented in the review by Meunier and Boivin[39].

In addition to the repair of microdamage, three anatomical aspects of osteonal remodeling emphasize the critical role this process plays in the adaptation of bone metabolism to physical forces resulting from mechanical loading. First, osteonal remodeling in monkey mandibles is influenced by the hardness of the diet consumed and is thus related to the physical forces expended by the jaw during eating[40,41]. Second, as Enlow[23] has pointed out, there are increased numbers of secondary osteons in cortical bone adjacent to sites of tendon insertion. Bones are exposed to physical forces from muscular activity at these sites. Third, Marotti[25] has shown that the locations of active osteonal remodeling in the long bones of dogs (femur, tibia, humerus, radius and ulna) are essentially identical when comparable cross-sections are examined in contralateral limbs. This remodeling activity is not spaced uniformly throughout the cross-sections, but occurs in locations specific to each bone and varies along their longitudinal axes. Since dogs utilize their contralateral limbs equally, the sites of osteonal remodeling presumably reflect the distribution of physical forces during normal activity.

There have been few studies of the effects of menopause (or estrogen deficiency) on cortical bone osteonal remodeling in women. Olah and Schenk[42] examined ribs obtained at autopsy from 49 men and 46 women of various ages. Prior to embedding in plastic, the bones were incubated in basic fuchsin to stain unmineralized osteoid. After sectioning, osteonal remodeling was measured by determining the number of osteons with mineralizing osteoid seams, indicative of new osteons being formed. As shown in Figure 2, ribs from men and women have similar osteon densities that increase slightly with age. The number of remodeling osteons is also similar in men and women, with the exception that 50 to 60-year-old women exhibit a doubling of osteonal remodeling. Since menopause normally occurs around age 50, osteonal remodeling appears to increase transiently in women during the decade following menopause.

Two studies have examined cortical bone remodeling in iliac biopsies obtained from women. In the first study[43] the activation frequency of osteonal remodeling was elevated 3-fold in postmenopausal compared to younger women. In estrogen-deprived young women, Bell and colleagues[44] found

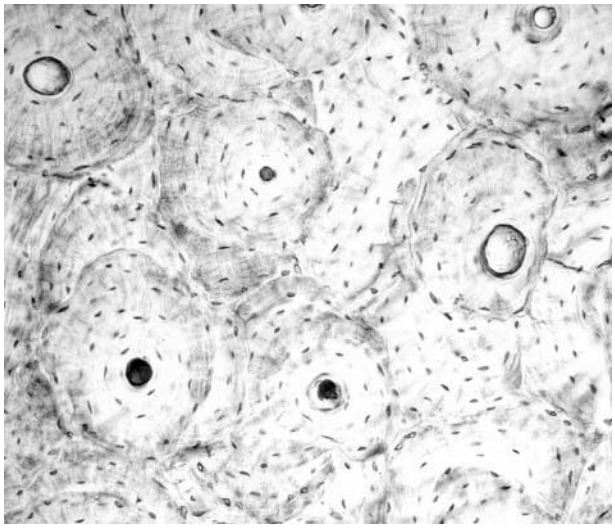

Figure 1. Photograph of a midshaft fibula section obtained from an adult female cynomolgus macaque. Notice the lamellar structure of the interstitial bone and the presence of osteocytes and secondary osteons.

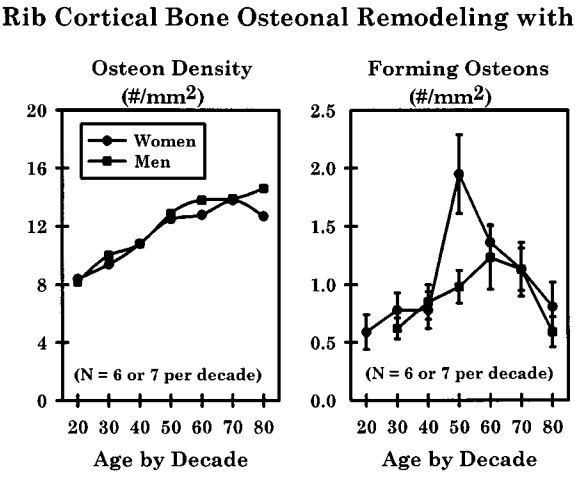

Figure 2. Osteonal remodeling in human rib cortical bone as a function of age and gender. Figure prepared from data presented by Olah and Schenk[42]. Error bars represent SEM.

a doubling of osteonal resorbing surface with a greater than 3-fold increase in double tetracycline labels in paired biopsies obtained before and 6 months after inhibition of estrogen production. In these same iliac biopsy samples, there were alterations in cancellous bone structural parameters, but no change in cancellous bone remodeling[45].

The mechanism by which estrogen deficiency after menopause leads to increased cortical bone osteonal remodeling is not known. One intriguing hypothesis involves osteocyte apoptosis, since estrogen withdrawal leads to programmed cell death in osteocytes[46]. The region of bone without functional osteocytes is detected as damaged and "directed" remodeling occurs to replace the "dead" bone with new bone. Parfitt[47,48] has elegantly reviewed our incomplete understanding of the processes involved in directing osteoclast precursors to the correct remodeling location and osteoblasts to sites recently excavated by osteoclasts.

Although there is no doubt that loss of cancellous bone following menopause contributes to reduced bone strength, cortical bone loss is an important and often underappreciated factor in bone fragility[49,50]. Since each bone has a unique structure and is subjected to unique physical forces [51], generalizations concerning the relative contributions of cancellous and cortical bone to skeletal strength cannot be made. Vertebral bodies have a thin cortical shell (~ 250 μm in humans) with cancellous bone providing the major structural support. In comparison, the femoral neck has a thicker cortex (~ 700 μm in humans), contributing 60 to 70% of the total breaking strength in humans[52,53]. Numerous studies have described the thinning of cortical bone with aging, with the recent NHANES III data[54] perhaps the most complete. These investigators made morphometric measurements on DXA scan images obtained from 2719 men and 2904 women from the ages of 20 through 80 years. Mean cortical thickness of the femur shaft declined from 5.6 to 4.4 mm in men and from 5.0 to 3.5 mm in women. Mean cortical thickness in the femoral neck declined from 2.1 to 1.5 mm in men and from 1.9 to 1.3 mm in women.

Bone mass gains in imported monkeys

A consistent observation is that spine BMD values for adult female monkeys imported from the wild increase several percent during the first several years of captivity[55], and these increases are not observed in monkeys housed in captivity for extended periods prior to examination[55,56]. Since the ages of wild-caught monkeys can never be known, the only method of verifying maturity is radiographic evidence of closed growth plates. Excluding adolescent monkeys with open growth plates from studies does not prevent these gains in spine BMD. From the point of view of bone biology, increases in spine BMD during captivity are fascinating because some unidentified component of "civilization" may have a beneficial effect on bone mass. However, gains in spine BMD in control, sham-ovariectomized monkeys are a complication in studies on osteoporosis because, although the ovariectomized monkeys lose spine BMD relative to controls, they may show only small declines or even gains in spine BMD during the experiment. The factors responsible for increased spine BMD during captivity have not been definitively identified, but several possible explanations are worth discussing.

Since bone densitometry only provides information on the entire bone analyzed, the exact site(s) undergoing mineralization are not known. However, the impression gained while examining cross-sections of long bones obtained from control monkeys after long-term studies has been that endocortical bone formation occurred during the experiment. In our most recent study we administered the bone fluorochrome alizarin to monkeys during their initial month of quarantine upon arriving in captivity. These monkeys were necropsied about one year later and preliminary examination of midshaft fibula cross-sections indicates that endocortical bone formation occurred in many, but not all, of the imported monkeys soon after arrival. Thus, part of the gains in bone mass observed during early captivity in monkeys appears to occur at the endocortical surface.

Radiographs are normally taken of monkey extremities to verify closed growth plates. Nonetheless, histologic examinations of iliac crest biopsies show the occasional continued presence (15/125 in a recent study) of a growth plate at this site. Since the growth plates of the axial skeleton close after those of the appendicular skeleton[57], this finding is not surprising, but indicates that some imported monkeys only reach full skeletal maturity after arrival. Another factor potentially influencing spine BMD is the possibility that some monkeys were removed from the wild soon after lactation. Spine BMD declines during lactation in monkeys and recovery of this bone loss occurs following weaning[58-60]. No method exists to identify a monkey that has recently been lactating. Confirmation that a monkey has been pregnant at least once in her lifetime can be made by examination of the uterus at necropsy[60A], and in a recent study 111/119 monkeys were so characterized (Mark Cline, personal communication). The third developmental aspect involved in captivity-associated gains in bone mass is the observation that healthy women continue to gain spine BMD during their third decade of life[61] and therefore young adult female monkeys should be expected to have similar increments in spine BMD.

In addition to the potential effects of aging and reproduction on bone mass accumulation during captivity, environmental factors must be considered. The most obvious environmental factor different between the wild and captivity is nutrition. Negative effects of a marginal dietary calcium deficiency on bone mass are well known. Recent epidemiological data from NHANES III indicate that variations of dietary protein intake within the range consumed by adults in the USA affect total hip BMD[62] and the loss of both spine and femur BMD over four years[63]. Slight reductions in protein consumption rapidly lead to declines in intestinal calcium absorption[64] and secondary

hyperparathyroidism[65] in women. Unfortunately, the nutritional status of monkeys in the wild is difficult to ascertain and testing the hypothesis that a nutritional deficiency is corrected in captivity would involve purposely feeding a group of monkeys an incomplete diet. Retrospective analyses of past studies suggest that the dietary calcium content clearly has an important influence on the gain in spine BMD observed in newly arrived monkeys, but other factors are also involved. An investigation of the acute effects of marginal dietary protein restriction on serum levels of parathyroid hormone and other parameters involved in bone metabolism is currently underway and the results of this study might provide additional insights into this issue.

Additional evidence that imported monkeys can have a deficit in bone mineralization comes from recent examinations of iliac crest biopsies using backscattered electron microscopy and confocal microscopy (Janet Hock and Alan Boyde, personal communication). Some osteons were poorly mineralized and contained unmineralized cement lines, consistent with a history of mineral disturbances during the formation of that bone. Based on the location of fluorochrome labels given during the study, regions of defective mineralization appeared to be in bone made prior to captivity. As bone undergoes normal remodeling, defectively mineralized regions are gradually replaced with concomitant gains in BMD.

Another environmental factor related to bone metabolism is fluoride. Monkeys consuming fluoridated tap water (1mg/liter F) as their sole source of water may ingest sufficient fluoride to positively influence bone mass. Two recent epidemiological studies[66,67] found a relationship between long-term consumption of fluoridated tap water and higher values of spine and proximal femur BMD. Using standard methods[68], vertebral cancellous bone fluoride content was determined in both colony-raised and imported adult monkeys. As shown in Table 1, bone fluoride content increases with age in colony-raised monkeys and the length of time wild-caught monkeys are kept in captivity. The highest bone fluoride contents measured in monkeys (0.21% of bone ash weight) are essentially identical to levels observed in humans drinking fluoridated water for more than a decade. Although a definitive study comparing bone mass accumulation in imported monkeys consuming fluoridated and nonfluoridated water has not been performed, the epidemiological data suggest that continuous exposure to fluoride at the levels present in tap water increases spine BMD.

Given the multiple potential factors influencing bone mass, gains in spine BMD observed in monkeys during their first several years in captivity are likely to result from multiple causes. Data from sham and ovariectomized control groups in the four most recent studies performed at Wake Forest University are presented in Figure 3. Dietary calcium levels appear to play an important role, but other factors appear to be involved. Inclusion of sham-ovariectomized, control monkeys in studies has shown that ovariectomy consistently leads to osteopenia. Gains in spine BMD by these control monkeys can be minimized by extending the duration between importation and the start of the study or using colony-raised monkeys[56]. Pigtailed, cynomolgus and rhesus macaques raised in colonies attain peak bone mass at the ages of approximately 7, 9, and 10 to 11 years, respectively[69-72]. However, the availability of colony-raised monkeys is clearly limited[73] and maintaining monkeys in captivity in advance of starting a study increases its expense. Another important perspective often overlooked is that monkeys are employed as a model of postmenopausal osteoporosis in women. Spine BMD values of the placebo control groups in most recent clinical trials usually decline by less than 2% during the study. The rapid loss of large amounts of cancellous bone in the proximal tibia of rats following ovariectomy does not reflect the rate or extent of bone changes after menopause in women.

Study Group	Age	N	Bone Fluoride (% Ash Weight)
Colony-raised monkeys	6±1 years	11	0.09±0.01
Colony-raised monkeys	11±1 years	13	0.17±0.01
Colony-raised monkeys	13±1 years	13	0.19±0.01
Colony-raised monkeys	15±1 years	13	0.21±0.01
Imported monkeys ~4 months after arrival	adult	7	0.08±0.01
Imported monkeys 13 months after arrival	adult	10	0.14±0.01

All data are means±SEM. Vertebral bodies were ashed and approximately 12 mg of cancellous bone was dissolved in perchloric acid to which was brought to pH 6.5 with a solution containing citrate to chelate calcium and CDTA to chelate iron and aluminum, which bind fluoride and interfere with its determination. Fluoride was then measured with an ion-specific electrode. The measured potential was linearly related to the logarithm of the fluoride concentration with a slope close to 57 mV per log unit. Analysis of five separate samples from a single bone gave a coefficient of variation of 3.2%.

Table 1. Bone fluoride in colony-raised and imported female monkeys

Practical aspects of studying monkeys

Monkeys are housed either individually or in social groups, depending upon the experience and facilities available at the research center involved. Group housing has the advantage that interaction with peers is an important aspect of psychosocial enrichment[74] and the monkeys generally have sufficient space for adequate physical exercise. However, monkeys housed together form hierarchical social groups with the subordinate individuals subject to continual psychological stress from losing fights and continual harassment. In extreme instances, introducing a new monkey into an established social group can result in the death of the new monkey[75]. In studies involving intact, non-ovariectomized monkeys, the subordinate animals experience ovarian dysfunction and develop more severe atherosclerosis[76,77]. Although individual housing eliminates these potential complications, the cages usually do not allow much room for movement and weight-bearing exercise, with the possible complication that bone loss due to inactivity might be encountered. A reasonable compromise might be pair housing, which allows psychosocial enrichment and a more easily established social hierarchy.

Most investigators have performed surgical ovariectomy to mimic the declines in ovarian estrogen production following menopause. Careful attention to surgical technique is essential, as cynomolgus monkeys continue to cycle normally with as little as 5% of their ovarian tissue present[78]. Anecdotal evidence exists that two (unpublished) studies from separate institutions have been compromised by the failure to remove all ovarian tissue in the majority of monkeys. The success of ovariectomy in individual monkeys can be monitored by serum estradiol levels, which should remain consistently below 10 pg/ml. Inhibition of menstrual cyclicity with the use of GnRH agonists and antagonists is also possible (chemical ovariectomy), with the advantage that the loss of ovarian function is reversible[79-83].

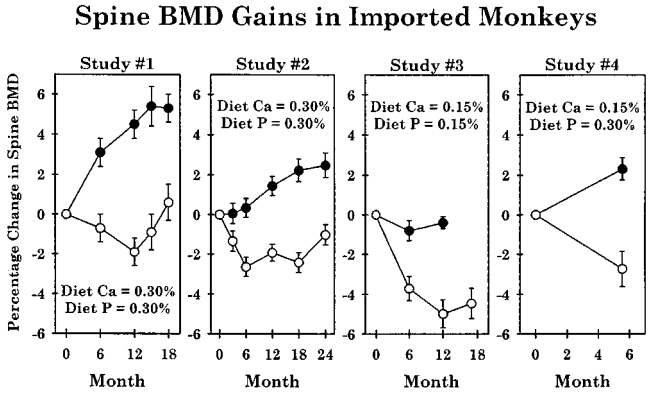

Figure 3. Gains in spine BMD measured by DXA in the four most recent studies performed on imported cynomolgus monkeys at Wake Forest University. For each study, data from the two control groups (Sham monkeys in filled circles and OVX monkeys in open circles) are presented. Durations between importation and initiation of the experiments averaged 3, 20, 11 and 6 months for studies 1, 2, 3, and 4, respectively. Dietary Ca and P contents are indicated on each graph. Studies 1 through 4 were performed to examine the skeletal actions of rhPTH-(1-34), lasofoxifene, levormeloxifene and recombinant salmon calcitonin, respectively. Scans were obtained with a Hologic QDR-1500 Bone Densitometer.

Bone biopsies for chemical and histomorphometic analyses can be taken from monkeys at the start of a study to obtain baseline values or during a study to obtain intermediate data. As is the case in humans, the iliac crest is the easiest and most reliable site to obtain a biopsy with minimal complications and several technical procedures to obtain such biopsies have been published[84-87]. More limited experience indicates that biopsies of the rib[60,88], (personal communication from Cynthia Lees), vertebral body[89] and humerus[60] are possible. Rib biopsies are of particular interest because they provide the opportunity to examine osteonal bone metabolism.

A major concern when working with nonhuman primates involves the possibility of disease transmission between humans and monkeys. Monkeys have little natural resistance to tuberculosis and therefore the spread of this bacterium among monkeys or from people to monkeys can rapidly lead to the death of an entire colony. Routine monitoring of both monkeys and the people coming in contact with them is a standard practice, as is the isolation of any monkey suspected to have contracted tuberculosis. Monkeys can also transmit diseases to humans[90], with the herpes B virus the most serious[91,92]. With proper safety precautions such transmissions are extremely rare, but in a tragic case during 1997 a 22-year-old woman died 42 days after an exposure through her eye[93].

Given the specialized expertise and facilities required to work with nonhuman primates, the number of research centers performing bone studies in monkeys is limited. A list of such facilities is presented in Table 2 to provide a starting point for gathering further information.

Endocrinology of calcium metabolism

Two early studies in rhesus macaques[94,95] examined calcium dynamics and observed results similar to those in humans. Harris and colleagues[95] emphasized their finding that the ratio of urine to fecal ^{45}Ca excretion during the first 5 days after an intravenous injection of ^{45}Ca was similar in humans and monkeys, and much higher than in dogs and rats. Renal calcium excretion is known to be a relatively larger contributor to calcium balance in humans than many other animals (but not rabbits and hamsters). As an example, a person consuming 1000 mg of calcium each day typically has a urinary calcium excretion of 250 mg, or 25% of the calcium ingested. In contrast, a rat consuming 20 grams of a 0.5% calcium diet ingests 100 mg of calcium a day. With a urinary calcium excretion of 5 mg/day, urinary calcium excretion in rats averages 5% of the calcium consumed. In this regard, monkeys provide a better model for human calcium metabolism than dogs and rats.

Minimal information is available on intestinal calcium absorption in monkeys. A balance study performed in ten adolescent pigtailed macaques[96] determined fractional intestinal calcium absorption to be 37% of intake, similar to results observed in human adolescents. Intestinal calcium absorption averaged 40% of intake in adult male cynomolgus macaques[97]. Additional studies on this topic would be informative, particularly the influences of calcitriol and dietary calcium intake.

Light microscopy of the rhesus[98] and ultrastructure of the baboon[99] parathyroid glands have been described. Serum PTH levels, readily measured in baboons[100], rhesus[101] and cynomolgus macaques[102,103] and African green monkeys[104], respond in the appropriate fashion to acute alterations of serum calcium concentrations in cynomolgus macaques[103] and African green monkeys[104]. Serum levels of calcitonin have been determined in baboons[100] and rhesus monkeys[105] and also respond appropriately to serum calcium levels in rhesus monkeys[105]. The method of anesthesia is an important technical consideration as the gaseous anesthetic isoflurane causes a decrease in serum ionized calcium levels with a corresponding stimulation of PTH secretion[103]. Ketamine anesthesia is recommended when obtaining blood for PTH measurements.

Serum levels of calcitriol, the active metabolite and hormonal form of vitamin D, are typically observed to 5 to 10-fold higher in baboons[100] and macaques[106-108] than humans. The binding affinity for calcidiol of the circulating vitamin D binding protein in rhesus monkeys appears to be normal[109]. Knowledge of the binding affinity of the monkey intracellular vitamin D receptor for calcitriol would help in understanding why circulating calcitriol levels are higher than those observed in humans and other animals. Polymorphisms of the circulating vitamin D binding protein have been described in cynomolgus monkeys[110] to help understand phylogeny[111] and genetic variations among native populations[5].

With the exception of osteocalcin in the cynomolgus macaque[112], the amino acid sequences of hormones and proteins involved in bone metabolism have not been determined. Measurement of monkey hormones and bone proteins using reagents designed for use in human samples has been successful to date. Nonetheless, caution is always appropriate and all assays of monkey proteins should be adequately validated before use.

Bone structure and turnover

Adult female cynomolgus and rhesus macaques, weighing approximately 3 and 6 kg, respectively, are considerably smaller than women and the dimensions of the monkey skeleton reduced proportionally. Since bone dimensions and architecture may be important in regulating the rates, extent, and location of bone remodeling, a comparison of measured histomorphometric parameters in bones obtained from monkeys and humans is worthwhile. Such comparisons can be readily made for biopsy samples from the iliac crest, as extensive human data are available. However, minimal information is available about dynamic aspects of bone formation in human vertebrae, distal radius, or femoral neck. For these three bones, only structural bone parameters can be compared.

Of the numerous studies examining human iliac biopsies, the work by Han and colleagues[113,114] will be summarized

Facility	Location	Species	Comments
AEA Technology	Harwell, England	Cynomolgus	Contract Research Organization
Centre International de Toxicologie	Evreux, France	Cynomolgus	Contract Research Organization
ClinTrials BioResearch	Senneville, Quebec	Cynomolgus/Rhesus	Contract Research Organization
Covance Laboratories	Reston, Virginia	Cynomolgus/Rhesus	Contract Research Organization
National Institute of Aging	Poolesville, Maryland	Rhesus	Long-term caloric restriction study
Shin Nippon Biomedical Laboratories	Yoshida, Japan	Cynomolgus	Contract Research Organization
Skele Tech, Inc	Bothell, Washington	Cynomolgus	Contract Research Organization
Southwest Foundation for Biomedical Research	San Antonio, Texas	Baboons	Inheritance/pedigree studies
Tsukuba Primate Center	Ibaraki, Japan	African Green Monkeys	Identified 5 publications in 1994-1996
University of Michigan	Ann Arbor, Michigan	Rhesus	Bone research currently performed
University of Washington	Medical Lake, Washington	Pigtailed Macaque	Regional Primate Research Center
University of Wisconsin	Madison,Wisconsin	Rhesus	Regional Primate Research Center
Wake Forest University	Winston-Salem, North Carolina	Cynomolgus	Bone research performed since 1985

Table 2. Centers with recent experience performing bone studies in monkeys

since static and dynamic measurements were made in both cancellous and cortical bone and the effects of age/menopause are described. Cortical thickness in the ilium of premenopausal women (mean age = 37 years) was 1400 μm and declined in postmenopausal women (mean age = 62 years) to 1230 μm. Cortical porosity increased with age as a consequence of higher osteonal density without changes in osteonal canal diameter. Cancellous bone volume declined with age (26 to 21%) as trabecular thickness remained stable near 140 μm. For the entire population, surface-based bone formation rates were higher in endocortical and intracortical bone (~21 mm^3/mm^2/year) than cancellous bone (~14 mm^3/mm^2/year) as was wall thickness (41 versus 35 μm). Bone formation rate increased with age on intracortical, but not cancellous or endocortical surfaces. Cortical thinning appeared to result from enhanced osteoclast erosion depth with age, presumably due to delayed osteoclast apoptosis. No changes in erosion depth were apparent in cancellous or intracortical bone.

Cancellous bone volumes in iliac crest biopsies from nonhuman primates have been reported to average 25% in baboons[115], 28% in rhesus macaques[116] and 26% in cynomolgus macaques[117]. Cortical thickness is 460 μm in rhesus monkeys and trabecular thickness is approximately 100 μm in both rhesus and cynomolgus macaques. Values for surface-based cancellous bone formation rates ($\mu m^3/\mu m^2$/year) average 40 in baboons[115] and 31 in cynomolgus monkeys[117].

The cortex of human vertebrae has an average thickness in the range of 240 to 290 μm with thinner cortices observed in osteoporotic patients[118]. Cancellous bone volume in the lumbar spine averages 15% in young adults and declines with age[119]. The thickness of horizontal trabeculae decreases from ~180 to ~90 mm with age whereas vertical trabeculae maintain a thickness of ~215 μm[120]. In cynomolgus monkeys, sections of the second lumbar vertebrae prepared in the transverse plane have a trabecular thickness of 100 μm, cancellous bone volume of 25% and cancellous bone formation rates (bone volume referent) averaging 90%/year[121]. Analyses of the fifth lumbar vertebrae in baboons showed a cancellous bone volume of 28%, a trabecular thickness of 160 μm and cancellous bone formation rates (bone volume referent) averaging 12%/year[115]. For both humans[51] and cynomolgus monkeys [117,122], variations exist in trabecular architecture between the center of the vertebral body and the regions nearer the intervertebral discs. Wall thickness of trabecular remodeling units is increased (22 to 26 μm) by ovariectomy in cynomolgus monkeys, suggesting increased osteoclastic erosion depth[117]. In this latter study, the normal activation frequency was determined to be 1.4/year and appeared to increase to 2.2/year following ovariectomy[117].

Compared to the iliac crest, the human distal radius has a thinner cortex, lower cancellous bone volume, thinner trabeculae and a reduced trabecular wall thickness[123]. Values of cancellous bone volume (15% versus 18%) and trabecular thickness (80 μm versus 95 μm) are similar in cynomolgus monkeys[121] and humans[123]. Cancellous bone formation rates (bone volume referent) average 60%/year in cynomolgus monkeys[121].

The structure and architecture of the human femoral neck and its muscle attachments are different from that of all other primates, including apes[124,125]. This uniqueness is primarily related to the evolutionary change from arboreal to terrestrial environments, which involved the development of bipedalism. Compared to nonhuman primates, the human femoral neck is less "rounded" in shape[126], with a cortical bone shell that is thin relative to the cross-sectional area. The cortex is thickest in the inferior (medial) aspect in both humans (~6 mm)[127] and cynomolgus monkeys (~1.5 mm)[126]. This region is subjected to the greatest compressive forces during standing[124]. Cortical width in the superior region decreases to ~300 μm in humans[127] and ~200 μm in cynomolgus monkeys[126]. Values of femoral neck cancellous bone volume in humans have been reported to average from 15 to 23%[51,127,128], in contrast to 45% in cynomolgus monkeys [121]. Trabecular thickness averages 279 μm in humans[127] and 160 μm in cynomolgus macaques[121], with a cancellous bone formation rate (bone volume referent) of 50%/year in monkeys[121].

Evaluation of biopsies of the proximal humerus of young adult female pigtailed macaques (with growth plates present in the humerus) gave values of 29% for cancellous bone volume and 106 mm for trabecular thickness[60]. Using the measured data for mineral apposition rate (1.17 μm/day) and mineralizing surface (11.8%), the calculated surface-referent bone formation rate is ~50 $\mu m^3/\mu m^2$/year.

No simple conclusion can be drawn from these various comparisons of bone architecture between humans and nonhuman primates. Although the general patterns of bone structure are similar, there are differences, particularly in the femoral neck. Extending these comparisons to include other species, such as sheep, pigs, dogs, rabbits, rats and mice, would be informative. As developed in the next two sections of this review, considerations of the changes in skeletal metabolism in response to estrogen withdrawal and to treatment with agents known to influence bone in humans are also important considerations in evaluating the appropriateness of any species as a model for postmenopausal osteoporosis.

Development of the monkey model of postmenopausal osteoporosis

Examination of ovariectomized monkeys for possible bone loss began at Wake Forest University in the mid-1980s as an outgrowth of a cardiovascular research program. The techniques initially employed to study bone metabolism were simple by today's standards[129-132] but became more sophisticated as technical advances were made in the bone field. Experimental techniques employed during past

decades include determinations of BMD by DEXA and peripheral QCT[133], histological processing of plastic embedded sections of undecalcified bone[134], static and dynamic histomorphometic measurements of bone structure and activity[117], bone immunohistochemistry[135,136], bone biomechanical measurements[137], serum hormone analyses[103], and determination of various urine and serum markers of skeletal metabolism[117,138,139]. Most importantly, other laboratories[56,80,140,141] have independently confirmed these results, establishing ovariectomized rhesus and cynomolgus macaques as excellent models for postmenopausal osteoporosis. Ovariectomized baboons also exhibit the skeletal changes observed following menopause in women[100,115].

Ovariectomized monkeys consistently exhibit bone loss, as measured by spine BMD, when compared to sham-ovariectomized controls. As indicated by measurements of serum and urine markers of bone formation and resorption, ovariectomy-induced bone loss is associated with an elevation of bone remodeling. Markers of bone turnover successfully employed include serum activities of tartrate-resistant acid phosphatase and both total and bone-specific alkaline phosphatase, serum levels of osteocalcin and assays measuring the urinary excretion of various degradation products of bone collagen fragments. Recent experience indicates that bone resorption can be reliably estimated by analyzing serum levels of the C-terminal crosslinked peptide of Type I collagen (CTX), thereby avoiding the need to collect urine.

Limited experience indicates that ovariectomy does not influence serum PTH levels, but results in lower serum calcitriol levels[108]. However, the "intact" PTH assay employed detects the PTH-(7-84) fragment in addition to PTH-(1-84) and the results may be misleading, especially since a recent study using a more accurate "whole" PTH-(1-84) assay showed that postmenopausal women in the PEPI trial responded to hormone replacement therapy with an increase in "whole" PTH-(1-84), but not "intact" PTH-(1-84) levels[142]. Although not proven, an intriguing hypothesis to explain various observations in postmenopausal women involves the following series of events: increased net calcium release from bone, lower serum PTH levels, decreased circulating levels of calcitriol and ultimately, reduced intestinal calcium absorption. This potential scenario may be easier to test in ovariectomized monkeys than women because monkeys are fed defined diets with constant levels of calcium and phosphorus, thereby eliminating the variations in serum levels of PTH and calcitriol that result from the variable dietary intakes of calcium and phosphorus.

The most consistent bone histomorphometric finding in ovariectomized monkeys is an increase in osteonal bone remodeling in the midshafts of the femur, radius and humerus. This observation agrees perfectly with the previously discussed cortical bone data obtained in women[42-44]. Cancellous bone remodeling in the ilium and vertebrae of baboons increases following ovariectomy[115]. However, ovariectomy in cynomolgus macaques has not induced consistent elevations of cancellous bone remodeling measured histomorphometrically. This observation might simply reflect the high variability inherent in histomorphometric measurements of bone turnover. As mentioned above[45], analyses of paired-biopsies obtained before and after 6 months of estrogen withdrawal in young women failed to show changes in cancellous bone remodeling. A recent study by Eriksen and colleagues[143] of paired biopsies obtained two years apart showed that hormone replacement therapy (HRT) blocked the menopause-associated increase in osteoclast erosion depth but had no effect on cancellous bone formation parameters. HRT was effective in reducing cancellous bone remodeling by ~25% (as indicated by volume- and surface-referent bone formation rates) in paired biopsies obtained 6 months apart from postmenopausal women[144]. Given the difficulty in demonstrating expected estrogen-dependent changes in cancellous bone remodeling by histomorphometry in women, a failure to consistently observe such a phenomenon in monkeys may not be surprising.

Most importantly, bone breaking strength in the spine and femoral neck is decreased by ovariectomy[137]. However, since bone mechanical parameters have a reasonably high variability, studies must include sufficient numbers of monkeys to detect statistically significant effects. Inhibition or reversal of this ovariectomy-induced skeletal fragility at two clinically relevant sites by potential drugs provides some confidence that such drugs will be efficacious in preventing osteoporotic fractures in postmenopausal women.

A key aspect in using ovariectomized monkeys to model postmenopausal osteoporosis is that the skeletal changes induced by ovariectomy are reversed by treatment with estrogens. Conjugated equine estrogens (CEE - Premarin) can be provided in the diet[145] or given by daily oral doses and 17ß-estradiol has also been successfully administered orally[146] and via silastic implants[117]. Although the progestin medroxyprogesterone acetate had minimal effect when given alone, it does not interfere with the beneficial skeletal actions of estrogens when the two agents are administered together[117,147-149].

Our understanding of the mechanistic aspects of estrogen action on the skeleton remains incomplete[150]. Recent evidence suggests estrogens influence bone metabolism through actions on TNF-α[151] and osteoprotegerin[152,153]. Nonetheless, serum levels of IL-1, IL-6, IL-6R and TNF-α are not influenced by ovariectomy in rhesus macaques[154,155]. Because the details of estrogen actions on bone remain to be identified, the mechanism(s) by which estrogen and its analogues inhibit bone remodeling and loss following estrogen deficiency in monkeys (and other species) is not clear.

Evaluating drugs in ovariectomized monkeys

As the validity of the ovariectomized monkey model of postmenopausal osteoporosis became established, several

drugs thought to be capable of preventing and/or treating postmenopausal osteoporosis were examined (Table 3). These studies will be summarized briefly with detailed protocols and results found in the original citations. In every instance, conclusions from these monkey trials agree completely with available knowledge of the actions of the drugs in women. Agents presently under examination for which no published data are available include lasofoxifene and salmon calcitonin.

Nandrolone decanoate, an anabolic steroid, acted like estrogens by decreasing bone turnover and loss[156-158]. Although this agent clearly had anabolic actions on muscle mass, there was no evidence of an anabolic skeletal effect. Tamoxifen had a minimal effect on spine BMD, but suppressed bone turnover measured histomorphometrically[147-149]. The SERMs raloxifene[159] and levormeloxifene[146] blocked increases in serum and urine markers of bone turnover induced by ovariectomy, but did not completely inhibit loss of BMD. In this regard, data obtained in monkeys mimics findings in postmenopausal women in whom raloxifene is less active than estrogen[144,160]. In contrast, studies in ovariectomized rats have found raloxifene and other SERMs to be fully active estrogen agonists on bone.

The bisphosphonates tiludronate[161] and alendronate[39,100, 115] have been examined in intact and ovariectomized baboons, zoledronate has been studied following ovariectomy in rhesus macaques[56] whereas ovariectomized cynomolgus macaques have been employed to test clodronate[162], ibandronate[163,164] and MCC-565, an estrogen-bisphosphonate conjugate[165]. Each bisphosphonate blocked all indices of bone turnover and produced increases in bone mass. Most importantly, clodronate administration[162] increased the strength of the femur and spine, ibandronate treatment[164] increased spine strength and alendronate treatment increased the strength of 5.1 mm diameter cores of cancellous bone obtained from the midbody of LV4 [115.] Neither tiludronate nor alendronate had any negative effects on bone strength.

Parathyroid hormone and its analogues have been thoroughly studied in cynomolgus and rhesus monkeys due to concerns that this anabolic agent might compromise the strength of cortical bone[166], Initial studies, with a 6-month treatment duration, examined PTH-(1-34)[167] and SDZ PTS 893[168], an analogue of PTH-(1-38), in ovariectomized cynomolgus monkeys and PTH-(1-84) in intact rhesus monkeys[169-171]. More comprehensive subsequent studies have examined PTH-(1-84) given for 16 months to ovariectomized rhesus monkeys[172,173] and PTH-(1-34) given for 18 months to ovariectomized cynomolgus monkeys[108,174-178]. The two longer-term studies have recently been completed and at the time of preparation of this review, many of the results have appeared only in abstracts. As is the case in human clinical studies, PTH has a strong anabolic action on bone in monkeys, increasing BMD and bone strength in both

Agent Examined	Reference	Species	Brief Description
Alendronate	39,100,115	Baboon	Antiresorptive Bisphosphonate
CEE (*Premarin)*	numerous	Cynomolgus	Full Estrogen Activity on Bone
Clodronate	162	Cynomolgus	Antiresorptive Bisphosphonate
Estradiol	117,146	Cynomolgus	Given as Control Treatment
Growth Hormone	102	Rhesus	Increased Spine BMD
Ibandronate	163,163	Cynomolgus	Antiresorptive Bisphosphonate
Levormeloxifene	146	Cynomolgus	SERM, Partial Estrogen Agonist
MCC-565	165	Cynomolgus	Estrogen-Bisphosphonate Conjugate
Nandrolone	156-158	Cynomolgus	Estrogen-Like Actions on Bone
PTH-(1-34)	167	Cynomolgus	6-Month Anabolic Study
PTH-(1-34)	108,174-178	Cynomolgus	18-Month Anabolic Study
PTH-(1-84)	172,173	Rhesus	16-Month Anabolic Study
Raloxifene	159	Cynomolgus	SERM, Partial Estrogen Agonist
SB267268	83	Cynomolgus	Vitronectin Receptor Antagonist
SDZ PTS 893	168	Cynomolgus	Analogue of PTH-(1-38)
Soy Protein in Diet	179	Cynomolgus	No Activity on Cortical Bone
Tamoxifen	147-149	Cynomolgus	Partial Estrogen Agonist
Zoledronate	56	Rhesus	Antiresorptive Bisphosphonate

Table 3. Summary of ovariectomized monkey studies evaluating drugs for postmenopausal osteoporosis

the spine and femoral neck. PTH administration does stimulate osteonal remodeling in cortical bone, but cortical bone strength is not compromised because most of the activated remodeling activity occurs in the endocortical region of the cortex. Basic biomechanical principles dictate that the strength of hollow cylinders such as the shafts of long bones results primarily from periosteal rather than endosteal bone.

Growth hormone has been administered to chemically-ovariectomized cynomolgus monkeys for 10 months[102] and to intact adult female rhesus monkeys for 7 weeks[179]. In the first study, treatment partially prevented the loss of spine BMD whereas in the second study, histomorphometrically-determined cancellous bone formation rates were increased in femoral necks and proximal tibias. Feeding monkeys soy protein containing phytoestrogens is ineffective in preventing both the elevation in osteonal bone remodeling observed after ovariectomy[179] and loss of spine BMD (Tom Register, personal communication). Preliminary data suggest that a vitronectin receptor antagonist[83] inhibits bone resorption in ovariectomized monkeys.

Osteoporosis resulting from aging, immobilization and glucocorticoid excess

Although postmenopausal osteoporosis is the most common metabolic bone disease, aging, immobilization, androgen deficiency in males, and chronic glucocorticoid excess also promote osteoporosis. Moreover, considerable attention has been given to the important roles of both genetics and bone mass accumulation during growth and early adulthood on the development of osteoporosis. No studies have been performed in monkeys examining osteoporosis in males or the deleterious actions of glucocorticoid excess on bone mass. A recent short-term study in adult female cynomolgus monkeys showed that glucocorticoid administration has similar actions on serum

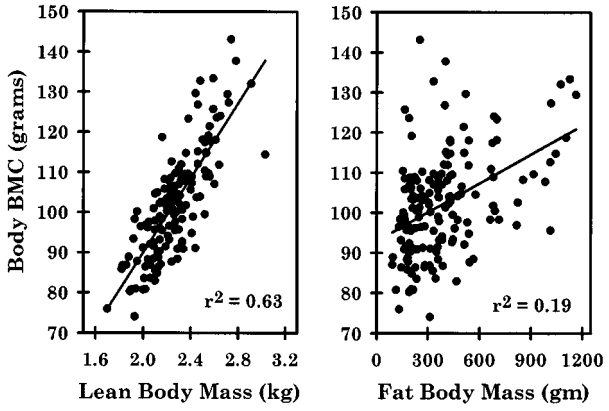

Figure 4. Body bone mineral content (BMC) as a function of lean body mass and fat body mass. Data were obtained at baseline from 158 adult female cynomolgus monkeys in the levormeloxifene study[146]. The correlation coefficients are presented. Scans were obtained with a Hologic QDR-1500 Bone Densitometer.

parameters in humans and monkeys (Brommage and Allison, unpublished data). Briefly, dexamethasone given every other day for a total of six doses resulted in complete suppression of endogenous cortisol secretion, transient hyperglycemia, mild hypercalcemia, elevated serum levels of both insulin and IGF-I, and reductions in serum levels of osteocalcin. Bone resorption, as indicated by serum levels of CTX, increased slightly. Longer-term studies are clearly required to understand the skeletal actions of glucocorticoid excess in monkeys. Since glucocorticoids are thought to promote osteocyte apoptosis[180], the existence of osteonal bone remodeling in monkeys makes them an attractive species to examine the effects of glucocorticoid excess on cortical bone.

The loss of bone with age in monkeys has been characterized[181-188], but little effort has been made to elaborate mechanisms leading to these declines. The impression gathered is that available aged monkeys were examined without prospective studies exploring methods to ameliorate the effects of age on the skeleton. However, two research centers have started long-term studies in rhesus monkeys designed to investigate the beneficial effects on health, including bone metabolism, of moderate caloric restriction[189,190], with initial results showing delays in skeletal development[191,192].

In addition to the tiludronate[161] and alendronate[39,100,115] studies described above, other groups have examined bone metabolism in baboons. Feeding growing baboons a low calcium diet produces osteomalacia and secondary hyperparathyroidism[193]. Baboons lose bone with age[194,195] and following ovariectomy[196] and detailed histomorphometric analyses of iliac crest biopsies have been performed[197]. Pilot studies involving the implantation of strain gauges within the spinal column to provide real-time telemetry of strains generated during normal activities have been successful[198]. The Southwest Foundation for Biomedical Research in Texas has approximately 2000 baboons with known pedigrees being used for analyses of the genetics of osteoporosis[199,200] with initial results reported[201,202]. The development of a genetic linkage map of the baboon genome[203] should speed up progress in this field.

Monkeys have been employed for studies on immobilization osteoporosis[204-209] with analyses of rates and locations of the bone loss described, and data on recovery. One study found that exercise reduces bone turnover in iliac crest biopsies[116]. As shown in Figure 4, whole body bone mineral content in monkeys correlates much better with lean body mass than with fat mass, a finding consistent with the known influence of muscle activity on bone mass.

New World monkeys

The previous discussion has focused solely on Old World primates because of the numerous bone studies in which they have been examined and their close evolutionary relationship to humans. New World primates have not been

extensively studied in part because of a generalized resistance to the actions of glucocorticoids[210-212] and other steroid hormones, including vitamin D[213-216]. This hormone resistance is associated with high circulating levels of calcitriol[217,218] and other steroid hormones. The explanation for this hormone resistance involves the overexpression of binding proteins that shuttle hormones between the plasma membrane and intracellular organelles[219,220]. Because of resistance to vitamin D, New World monkeys have high requirements for this vitamin and can easily become deficient unless supplemented with higher than usual amounts of vitamin D. Another unusual finding in New World monkeys is their poor ability to use vitamin D_2 [221-223] and thus they are protected from vitamin D_2 toxicity[213].

Summary

Ovariectomized baboons and both rhesus and cynomolgus macaques are good models of the initial skeletal events following estrogen deficiency in women. Following ovariectomy, the monkey skeleton undergoes an elevation in remodeling with a net loss of bone. Bone loss can be readily demonstrated by in vivo densitometric techniques and elevations in remodeling detected using serum and urine markers of bone formation and resorption. These changes are similar to events following menopause in women and both monkeys and women respond in a similar fashion to drugs designed to prevent and/or treat osteoporosis. As is the case in human clinical trials, bone biopsies can be obtained in monkey studies for histomorphometric analyses of skeletal dynamics. At the conclusion of monkey studies, all relevant bones can be obtained for detailed chemical, biomechanical and histomorphometric analyses.

Although valuable information is obtained in studies of rats and mice, the extent and rapidity of the changes observed in metaphyseal bone following ovariectomy in rodents does not mimic postmenopausal osteoporosis. Moreover, in contrast to humans and monkeys, the rodent skeleton does not undergo osteonal remodeling. Osteonal biology can be examined in dogs and rabbits, but these species do not mimic the reproductive physiology of women and therefore their bones do not respond to ovariectomy in the same fashion as women and monkeys. Nonhuman primates are believed to the best species for understanding the effects of estrogens on osteonal remodeling in women.

Update from the author:

In agreement with a previous human study examining raloxifene, the SERM levormeloxifene is also less effective than estrogen therapy in preventing postmenopausal declines in spine BMD[224]. This result agrees with data obtained in monkeys[146,225]. Three papers[226-228] by the NIA group examining the effects of chronic caloric restriction have recently appeared.

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