mass and especially mineralization of cortical bone (enhanced microporosity induced by stimulation of Haversian remodeling), maintaining the diaphyseal design relatively stable, thus slowing (yet not completely preventing) the development of bone fragility. (3) Bone *mechanostat* remains healthy (yet metabolically disturbed) after MP; hence, the status of this system should be also evaluated for diagnosing osteoporosis, avoiding to establish this diagnosis based only on the severity of the osteopenic condition (DXA-BMD t-scores). (4) Comparison of BMC between predominantly trabecular or cortical sites in the same individual would allow detecting a relative deterioration of the former compared to the latter, as determined in "systemic" (primary or endocrine-metabolic) osteopenias. (5) In practice, a *Z-scorization* of the described natural relationships will provide suitable reference charts for an original, biomechanical diagnosis of osteoporosis, and a differential diagnosis between "disuse" and "systemic" osteopenias (disuse would correspond to a normal bone/muscle proportionality, while a systemic disturbance would tend to reduce both the bone/muscle proportionality and the trabecular/cortical bone mass ratio), with important therapeutic implications (exercise in the first case, drugs or hormones in the second one).

The authors have no conflict of interest.

P-3

Determination of Carrier Status in ADO2: An *In Vitro* Study of Clinically Unaffected Gene Carriers

*K. Chu, R. Snyder, M.J. Econs

Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

E-mail: kachu@iupui.edu

Autosomal dominant osteopetrosis type II (ADO2) is a heritable osteosclerotic disorder that results from heterozygous mutations in the ClCN7 gene. However, of those individuals with a ClCN7 mutation, 1/3 are asymptomatic carriers. Disease severity in the remaining 2/3 is highly variable. The most severely affected manifest multiple fractures, osteomyelitis, visual loss and, occasionally, bone marrow failure. There is no genotype/ phenotype correlation and families frequently have both carriers and severely affected individuals.

To determine whether the carrier status results from the bone microenvironment (i.e., secretion of cytokines, hormonal environment, and condition of the other bone cells) or is intrinsic to the osteoclasts, we performed *in vitro* osteoclast cell culture in affected patients, carriers and normal controls (n=5 in each group). Subjects were matched for age, sex and ethnicity. Human peripheral blood mononuclear cells were isolated by magnetic beads and differentiated into osteoclasts by stimulation with hRANK-L and hM-CSF. Cell fusion, F-actin ring formation, TRAP activity, cell acidic microenvironment analysis were investigated and no significant difference was found between the three groups. However, osteoclasts from the carrier and normal groups generated more extensive and continuous resorption pits compared to the affected groups, which had rare and restricted pits. Moreover, the pits from the carrier and the normal groups formed much earlier and extended much more rapidly than the pits from the affected groups. There were no significant differences between the carrier and normal groups at any time point.

In conclusion, osteoclasts from the carriers, in contrast to those from the affected individuals (who had the same ClCN7 mutations) function normally in cell culture. This finding supports the hypothesis that intrinsic osteoclast factors determine whether an individual with a ClCN7 mutation becomes an affected patient or an asymptomatic carrier. Further understanding of this mechanism is likely to lead to the development of new approaches for the treatment of affected patients.

The authors have no conflict of interest.

P-4

68. Comparison of Osteon Histomorphometry in Human Ribs to Equine Appendicular Bones

G.C. Clark, S.M. Sorenson, K. Taylor, J. Hoopes, J.G. Skedros

University of Utah Department of Orthopaedic Surgery, SLC, UT, USA

E-mail: jskedros@utahboneandjoint.com

Introduction. Data regarding osteonal remodeling in human ribs have been broadly extrapolated to appendicular bones of various mammalian species. However, ribs have notable differences when compared to appendicular long bones; ribs 1) are derived from sclerotomes of the somites, in contrast to limb bones that are derived from lateral plate mesoderm, 2) are phylogenetically primitive, occurring in the fossil record much earlier than limb bones, 3) are metabolically more active and sensitive to hormonal changes such as during lactation, and 4) receive frequent and low-strain loading; e.g., loading occurs even when the animal is recumbent. The present study is the second (the first study examined horse radii) of a series of investigations assessing the appropriateness of using osteonal rib data for broad extrapolations to limb bones. In view of these differences, the

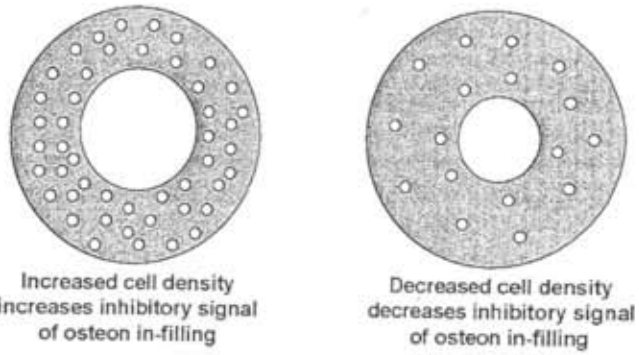

Figure 1. Two osteons showing the relationships of cell density with osteonal in-filling/bone formation.

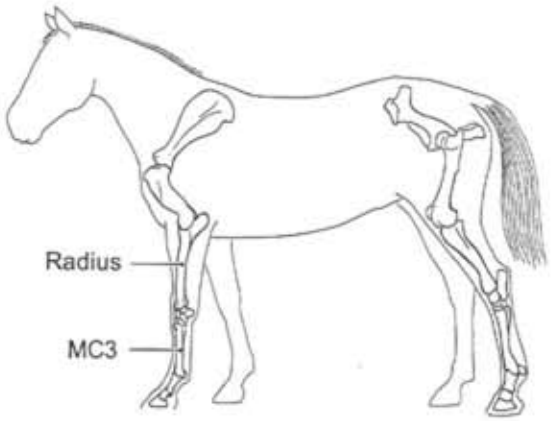

Figure 2. Horse skeleton showing bone types used.

		Human Rib			Equine Radius			Equine MC3		
		On.Ar	Lc.N/ On	Lc.N/ B.Ar	On.Ar	Lc.N/ On	Lc.N/ B.Ar	On.Ar	Lc.N/ On	Lc.N/ B.Ar
Hc.Ar	r	0.64	0.56	–	0.47	0.34	–	0.79	0.01	–
	p	<0.01	<0.01	–	<0.01	<0.01	–	<0.01	0.55	–
Hc.Pm	r	0.67	0.58	–	0.49	0.36	–	0.72	0.10	–
	p	<0.01	<0.01	–	<0.01	<0.01	–	<0.01	<0.01	–
Hc.Pm/ B.Ar	r	0.70	–	0.14	-0.60	–	0.43	-0.10	–	0.48
	p	<0.01	–	<0.01	<0.01	–	<0.01	<0.01	–	<0.01
Lc.N/ On	r	0.93	–	–	0.79	–	–	0.48	–	–
	p	<0.01	–	–	<0.01	–	–	<0.01	–	–
Lc.N/ B.Ar	r	0.14	–	–	-0.32	–	–	-0.23	–	–
	p	<0.05	–	–	<0.01	–	–	<0.01	–	–
Hc.Ar/ On.Ar	r	–	–	0.10	–	–	0.31	–	–	0.37
	p	–	–	<0.05	–	–	<0.01	–	–	<0.01

Results Table

present study further tests the hypothesis presented in Figure 1, which shows two osteons. The hypothesis that increased osteocyte densities correlate with reduced osteon in-filling reflects the idea that these cells form a network that represses osteonal bone formation (Martin, 2000).

Methods. 50X backscattered electron images were obtained from mid-third diaphyses of 10 mature (ages 2-10) equine third metacarpals (MC3s) and radii from mixed breeds (**MC3s:** in eight radial sectors: dorsal, dorsal-lateral, lateral, palmar-lateral, palmar, palmar-medial, medial, and dorsal-medial; **Radii:** cranial, caudal, medial, and lateral cortices). Using the histomorphometry parameters examined by Qiu et al. (2003) in human male ribs, approximately 2,470 (MC3s) and 2,350 (horse radii) osteons were examined for osteonal area (On.Ar), osteonal bone area (B.Ar), central (Haversian) canal area (Hc.Ar) and perimeter (Hc.Pm), number of osteocyte lacunae per osteon (Lc.N/On) and osteonal bone area (Lc.N/B.Ar), and the Haversian canal perimeter to osteonal bone area ratio (Hc.Pm/B.Ar). According to the ASBMR Histomorphometry Nomenclature Committee (Parfitt et al., 1987), the 2-D parameters (B.Ar and Hc.Pm) in osteons can be defined as the 3-D parameters as the BS/BV ratio. Thus Hc.Pm/B.Ar can be regarded as the bone surface to bone volume (BS/BV) ratio (Qiu et al., 2003). The data in this study are compared to human rib data (mature males) reported by Qiu et al. (2003).

Results (see table). Equine MC3 data: Results showed that, similar to human ribs, On.Ar in the equine MC3s had a strong positive correlation with Hc.Ar and Hc.Pm ($r = 0.79, 0.72$; $p < 0.01$, respectively). However, in contrast to human ribs, Lc.N/On showed little, if any, correlation to Hc.Ar and Hc.Pm ($r = 0.01, 0.10$; $p < 0.01$, respectively) in the MC3s.

Equine Radius data: Equine radii showed lower correlation (but still positive and statistically significant) of On.Ar to Hc.Ar and Hc.Pm ($r = 0.47, 0.49$; $p < 0.01$, respectively) than human ribs. Furthermore, compared to human ribs, horse radii showed lower correlation of Lc.N/On to Hc.Ar and Hc.Pm ($r = 0.34, 0.36$; $p < 0.01$, respectively).

Analysis of all data in each bone revealed that BS/BV (Hc.Pm/B.Ar) was highest in the MC3, next highest in horse radii, and lowest in human ribs ($p < 0.001$ for each paired comparison). Additionally, Lc.N/On was significantly lower in the MC3 than in the horse radius and human rib ($p < 0.001$).

Discussion. These results suggest that the putative coordination between the formation of an "optimal" Haversian canal and osteocytes found in human ribs (Qiu et al., 2003) is present but weak in horse radii and virtually non-existent in the MC3s as indicated by the low correlation co-efficients comparing Lc.N/On to Hc.Ar and Hc.Pm. These relationships may not exist in bones where there are large regional variations in strain gradients and/or a highly non-uniform strain distribution, which produce relatively greater regional variations in trans-cortical fluid flow. For example, the MC3 exhibits dramatic trans-cortical strain gradients; the cranio-lateral cortex experiences ~50x less strain than the caudal-medial cortex (Gross et al., 1992). Regions of bones experiencing high strain gradients may also have more efficient nutrient delivery, which may explain why the MC3s have greater Hc.Pm/B.Ar but lower Lc.N/On than horse radii and human ribs. Beyond interspecies differences, there may be other important considerations when attempting to extrapolate human rib data to other mammalian appendicular bones. For example, it is possible that thresholds for metabolic activity of bone cells in ribs may be fundamentally different when compared to cells in appendicular bones. Rib bone is exquisitely sensitive to hormonal changes associated with lactation and appendicular bones are not. Although this is gender specific (i.e., occurs in females) it illustrates how metabolic demands can affect osteonal histomorphology in some regions differently from others. Reasons for these differences are not fully understood but do point out that histomorphometric data considered "optimal" in human ribs might not apply to other mammalian appendicular bones.

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The authors have no conflict of interest.

P-5

Structural Properties of Florida Manatee Ribs

K.B. Clifton[1], J.J. Mecholsky Jr[2], R.L. Reep[1]
Departments of [1]Physiological Sciences and [2]Materials Science and Engineering, University of Florida, Gainesville, FL, USA
E-mail: cliftonk@mail.vetmed.ufl.edu

On average, 25% of all Florida manatees that die each year are killed by boats. Boat strikes account for 85% of deaths attributed to humans. Reducing watercraft-related mortality is a high priority in state and federal manatee recovery efforts, which focus primarily on regulating boating activities. In order to establish safe boat speeds for manatee protection, an estimate of the forces required to fracture their bone is needed. The goal of this project is to estimate the mechanical properties of whole manatee ribs. Impact tests were used to estimate the load required to initiate a fracture, and the amount of energy needed to fracture whole ribs. Manatee ribs from the mid-thoracic region were impacted with an air cannon. Fractographic analysis was applied to the fracture surfaces to calculate the loads that led to failure. Loads ranged from 91-173 MPa. Strain gauges were used on some ribs to validate these calculations. Fractal geometry was used to calculate the fractal dimensional increment (D^*), a quantitative measure of the topography of an irregular fracture surface. D^* is directly related to the fracture energy of a material; the higher D^*, the greater the fracture energy. Results indicated D^* for manatee bone (.08-.22) is at the lower end of the range for ceramics, suggesting a low amount of energy is needed to cause fracture. These impact tests will allow us to determine whether the kinetic energy generated by typical watercraft under normal operation is sufficient to fracture manatee ribs.

The authors have no conflict of interest.

P-6

Re-establishment of Normal Cancellous Bone Turnover Differs Following The Withdrawal of Alendronate and Risedronate Treatment in Ovariectomized Rats

*R.K. Fuchs, D.B. Burr
Indiana University Medical School, Indianapolis, IN, USA
E-mail: rfuchs@iupui.edu

It has been suggested that alendronate (ALN) and risedronate (RIS) have different skeletal pharmacokinetics, with ALN having a longer terminal half-life than RIS. The aim of this study was to investigate the skeletal response to the withdrawal of RIS and ALN using an ovariectomized (ovx) animal model. We hypothesized that normal bone turnover rates would be re-established sooner following the withdrawal of bisphosphonate therapy in ovx rats treated with RIS than in those treated dose-equivalently with ALN. Female Sprague Dawley rats (n=210) were ovariectomized and treated 3x/wk for 8 wks with either saline-vehicle (CON), ALN (2.4 μg/kg), low-dose RIS (1.2 μg/kg: RISlow), or high-dose RIS (2.4 μg/kg: RIShigh) via subcutaneous injection. Doses of ALN and RIS were based on clinical dose levels (on an oral mg/kg basis) and on doses known to inhibit bone loss in ovx rats. From a clinical perspective, 1 remodeling cycle is equal to ~1 month in rats and ~4-6 months in humans. Fluorochrome label (calcein) was administered 10 and 4 days prior to sacrifice for histological measurements. Animals (10/group) were sacrificed at 0, 4, 8, 12 and 16 wks after treatment withdrawal. Indices of bone turnover were significantly suppressed in all drug-treated groups compared to vehicle-treated CON after 8 wks of treatment (all $P<0.05$), and BMD of the distal femur increased significantly in all drug-treated groups after 8 wks of treatment compared to vehicle-treated CON ($P<0.05$). Trabecular bone turnover in the RISlow and RIShigh groups increased steadily following the withdrawal of treatment. In contrast, trabecular bone turnover in the ALN-treated groups did not demonstrate increases in bone turnover at any time point during treatment withdrawal. The ALN-treated groups remained significantly lower than vehicle-treated CON at all time points ($P<0.05$). At 16 wks the RIS treated groups were not significantly different from vehicle-treated CON ($P>0.05$) and there were no significant differences between the