mineral particles. Previous studies have shown that bone mineral crystallinity can be inferred from the width of the phosphate vibrational Raman band at 960 cm^{-1}. Also, the peak location of this band shifts towards higher frequencies in bone matrix under compression and lower frequencies under tension. The objective of this study was to apply Raman imaging to investigate the composition of bone matrix in and around osteocyte lacunae and changes to the matrix microstructural composition around and within lacunae in response to mechanical strain. Human femoral cortical bone was milled into a 3x3x30 mm beam specimen and polished using silicon carbide papers followed by refinement with progressive diamond slurries (from 6 micron down to 0.25 micron). The specimen was loaded using three-point bending in a custom microscopy load frame that operates within the Raman imaging microscope (Renishaw 2000). Three direct Raman images at 952, 960, and 968 cm^{-1} bands were recorded for lacunae located at the tension side of the specimen. As the specimen was loaded to approximately 2000 microstrain on the tension side of the beam, the Raman phosphate vibrational band (960 cm^{-1}) shifted to higher frequencies on one side of the lacuna and towards lower frequencies on the opposite side, indicating that the lacuna is subjected to a complex strain field. Furthermore, the 960 cm^{-1} peak bandwidth was higher for the matrix material within the lacunae as compared to bone matrix material surrounding the lacunae. This change in crystallinity became more pronounced as the bone specimen was deformed. This information indicates that the bone mineral within the osteocyte lacuna is less crystalline compared to the surrounding bone matrix and that the degree of crystallinity decreases as the lacunae undergoes increasing deformation.

Disclosures: ***D. Nicolella****, None.*

SA285

The Chemokine, MCP-3, Is Produced by Osteocytes and Protects against Glucocorticoid-Induced Apoptosis. Y. Kitase[1], S. Ko[2], J. Gluhak-Heinrich[3], S. E. Harris[3], L. F. Bonewald[1]. [1]Oral Biology, UMKC, Kansas City, MO, USA, [2]Kangnung National University, College of Dentistry, Kangnung, Republic of Korea, [3]Periodontics and Cellular and Structural Biology, U of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

The monocyte chemoattractant proteins, MCPs, were so named for their capacity to induce chemotaxis of monocytes during inflammation. This chemotactic property was thought to be their principal function. We found that MLO-Y4 osteocyte-like cells produce MCP-3 (20ng/ml/10^4 cells or 500X compared to 2T3 osteoblasts) and MCP1 (40X). This osteocyte model also expresses the receptor for this chemokine, CCR2. *In vitro*, MCP3 is regulated by fluid flow shear stress at 16 dynes/cm^2 increasing 3-4 fold and *in vivo*, using *in situ* hybridization, MCP-3 was found to increase in osteocytes near the surface of alveolar bone and to dramatically increase in periodontal ligament cells in response to tooth movement. Conditioned media from MLO-Y4 cells (10%) stimulates osteoclast precursor (Raw264.7 or MOCP-5) chemotaxis, however, chemotaxis could not be abrogated by neutralizing antibody to MCP3 (10ug/ml). Recombinant MCP-3 (1~100ng/ml) only modestly stimulated Raw264.7 chemotaxis compared to MLO-Y4 conditioned media and had no effect on MOCP-5 chemotaxis suggesting another function for MCP-3. Recently, several studies have shown protective effects by members of this family. MCP-1 has been shown to be produced by osteoblasts and indirectly increases osteoblast number (Posner et al, Bone, 1997) and protects neurons and astrocytes from apoptosis (Eugenin et al, J Neurochem, 2003). RANTES, a related chemokine, promotes osteoblast survival (Yano et al, Endocrinol. 2005). We have found that MCP-3 (100 ng/ml) will block apoptosis of MLO-Y4 cells due to dexamethasone (10^{-8} M) and etoposide (50uM) using both the nuclear fragmentation and the trypan blue exclusion assay. These studies suggest that MCP-3 is produced by osteocytes in response to mechanical strain and that a major function may be not only to recruit osteoclast precursors, but to protect osteocytes against cell death. This study also has important implications for the treatment of glucocorticoid induced osteoporosis.

Disclosures: ***Y. Kitase****, None.*

SA286

See Friday Plenary number F286.

SA287

A Proposed Function for E11 in Osteoblast to Osteocyte Differentiation and Mineralization. C. Barragan-Adjemian, D. Guo, J. Rosser*, L. Bonewald. Oral Biology, University Missouri Kansas City, Kansas City, MO, USA.

E11 is the earliest osteocyte-specific antigen expressed as the osteoblast becomes embedded in osteoid and changes shape to a dendritic morphology. This membrane protein is also found in other cell types that express cytoplasmic extensions and form a barrier with fluid such as kidney podocytes or with air as in type II alveolar lung cells. As osteocytes are in contact with bone fluid and express extensive dendritic networks, it was hypothesized that E11 is responsible for one or both of these functions. To determine the function of E11 in bone cells, MLO-A5 cells were used as a model of late osteoblasts/early osteocytes and MLO-Y4 cells as a model of early osteocytes. MLO-A5 cells express low levels of E11 that increase with time in culture coinciding with the generation of dendritic processes at 3-4 days, maximizes by 9 days when sheets of mineral form and begins to decrease after 12 days of culture. This *in vitro* expression recapitulates *in vivo* expression in bone where E11 appears as the late osteoblast embeds in osteoid, is highest in the osteoid-osteocyte and is reduced in expression in the mature osteocyte surrounded by mineral. The MLO-Y4 cells express very high levels of E11 (100 fold) compared to confluent MLO-A5 cells. To inhibit E11 expression in order to determine function, such a dendrite formation, siRNA was designed. Sequences used include 66-87, 165-185 and 397-417. It was found that a combination of all three siRNAs at 25nM would inhibit E11 expression 80% in MLO-A5 cells and 84% in MLO-Y4 cells. Unfortunately, several commercial transfection vehicles [Transit-ko (Mirus), Lipofectamine 2000 (Invitrogen), Silent (Bio-Rad)] were found to dramatically change cell morphology making assessment of changes in dendricity in MLO-Y4 cells impossible. Vehicle effects were also found on mineralization by MLO-A5 cells. The vehicle Lipofectamine plus (Invitrogen) was found to have no effects on either parameter. Preliminary experiments on MLO-A5 cells at five days of culture after two 24 hr exposures to siRNA suggest that siRNA inhibits mineralization. These cells have been shown to produce 20-50nm mineralized spherical structures as they generate dendritic processes, responsible for initiation of mineralization in these cultures. Experiments are underway to determine if blocking dendrite formation will block the generation of these mineralizing structures.

Disclosures: ***C. Barragan-Adjemian****, None.*

SA288

Proteomic Comparison of Osteoblasts and Osteocytes Reveal Unique Protein Expression Patterns. D. Guo[1], J. Guthrie*[2], J. Zhao*[1], L. Barragan*[1], S. Harris[3], L. Bonewald[1]. [1]UMKC, Kansas City, MO, USA, [2]Midwest Res. Inst., Kansas City, MO, USA, [3]UTHSCSA, San Antonio, TX, USA.

Genome-scale molecular profiling is used to examine global patterns of transcription in cell systems. However, mRNA does not always correlate with corresponding protein abundance, nor with post-translational modifications. Our hypothesis for these studies was that osteocyte proteomic expression will reflect osteocyte function. Total cell lysates of MC3T3 and 2T3 osteoblast cells and MLO-Y4 osteocyte-like cells were applied to 2D gels, analyzed by PDQuest, and differentially expressed spots excised for protein identification using mass spectrometry. Comparison of the protein expression profiles between the two osteoblast-like cells, showed only a 1-2% difference while comparison between MC3T3 and MLO-Y4 cells showed a 25% difference in expressed proteins. 80% of 69 spots were identified; 20 only detectable in MLO-Y4, 27 at least two fold greater in MLO-Y4 compared to MC3T3, 15 only expressed in MC3T3 and 7 two fold greater in MC3T3 compared to MLO-Y4. Direction of change in protein expression generally correlated with gene expression except for vimentin and lamin A where different forms were expressed between MLO-Y4 and MC3T3. Tubulin α6 was only present in osteoblasts while tubulin α3 was only present in MLO-Y4 cells. Collagenα 1 and 2 were only observed in the osteoblasts. Three proteins with greater expression in MLO-Y4 osteocytes compared to osteoblasts, were chosen for *in vivo* validation because of a potential role in osteocyte function. These include; Cap G (macrophage capping protein P24452), destrin (Q9ROP5), and Hypoxia up-regulated factor ORP150 (NP_067370), expressed 14, 5, and 62X greater respectively in MLO-Y4 cells compared to the osteoblast cells and 3 and 5X higher in the gene arrays (ORP150 was not on the array). Cap G and destrin are involved in cytoskeletal rearrangement and therefore may play a role in dendrite formation. CapG is a member of the gelsolin family responsible for capping the barbed ends of actin filaments and destrin, also known as actin depolymerizing factor, is essential in regulating actin filament turnover by severing and enhancing depolymerization of actin filaments. ORP150 is important in neuronal survival in glutamate toxicity and oxygen deprivation and therefore could play a role in osteocyte survival. Immunostaining of murine bone showed that destrin is highly expressed in the embedding osteocyte compared to osteoblasts or mature osteocytes. Staining for the other two proteins are in progress. Comparison of differences in the proteome of osteocytes with osteoblasts should provide considerable insight into the various molecules and pathways responsible for osteocyte function.

Disclosures: ***D. Guo****, None.*

SA289

See Friday Plenary number F289.

SA290

67. Surface Area/Volume Relationships of Secondary Osteons in Equine Radii: Implications for Regional Variations in Convective Nutrient Delivery. S. M. Sorenson*, G. Clark*, J. Hoopes*, W. E. Anderson*, K. Taylor*, J. G. Skedros*. Univ. Utah Dept. Orthop. Surg., SLC, UT, USA.

In human ribs, positive correlations have been reported between osteon area (On.Ar) and Haversian canal area (Hc.Ar), perimeter (Hc.Pm), and osteocyte lacunae number/osteon (Lc.N/On). Hc.Ar and Hc.Pm also increase with increasing Lc.N/On [Qiu et al. 2003 Anat. Rec]. These data imply a coordination between the Haversian canal and osteocytes that may be dependent on nutrient delivery. However, it is unclear if these associations broadly apply in other mammals. Additionally, these relationships may not exist in bones where there are large regional variations in strain gradients and trans-cortical fluid flow. For example, these relationships may differ in bones that receive a non-uniform strain distribution [e.g., tension (T), compression (C), and shear (S)]. We examined 2,350 secondary osteons to evaluate relationships between Hc.Ar, Hc.Pm, On.Ar, and Lc.N/On in a bone that habitually experiences non-uniform strains (hence large regional variations in strain gradients and fluid flow). 50X backscattered electron images were obtained from 10 equine radii at mid-diaphysis in cranial "T", caudal "C", and medial/lateral "neutral axis" S" cortices. Sections were also examined in circularly polarized light to determine if osteon morphotypes differ in these regions. Results showed that the T, C, and S cortices exhibited similar correlations despite having variations in strain gradients: moderate-to-high positive correlations between On.Ar and Hc.Ar, Hc.Pm, and Lc.N/On; low positive correlations between Lc.N/On and Hc.Ar and Hc.Pm (Table). The largest surface-to-volume ratios (Hc.Pm/B.Ar) occurred in neutral axis (NA) regions where strain gradients are highest ($p<0.001$ vs. T & C). Additonally, the NA regions had significantly lower Lc.N/On than

the other regions* ($p<0.05$). These results may reflect more efficient nutrient transport across NA regions. However, a potentially confounding factor in studies that deem lacuno-canalicular geometries as similar among *all* secondary osteons is that osteons with alternating lamellar organization are prevalent in the "C" and "S" cortices while the "T" cortex consists mostly of parallel-fibered osteons. Lacuno-canalicular geometries, hence conduits for convective fluid flow, might differ between these two osteon morphotypes.

	Correlation Coefficients (r values)	
	On.Ar Cranial "T"; Caudal "C"; M/L "S"	**Lc.N/On** Cranial "T"; Caudal "C"; M/L "S"
Hc.Ar	0.46; 0.55; 0.49	0.36; 0.37; 0.37
Hc.Pm	0.49; 0.53; 0.52	0.36; 0.36; 0.40
Lc.N/On	0.79; 0.78; 0.80	ALL P values are ≤ 0.0001

T = Tension; C = Compression; S = Shear [Medial (M) and Lateral (L) Cortices]

Disclosures: ***S.M. Sorenson****, None.*

SA291

Gene Expression Signatures of the Mouse MLO-Y4 Osteocyte Cell Model: Pathway and Gene Set Enrichment Analysis. W. Yang[1], M. A. Harris[1], D. Guo[2], L. F. Bonewald[2], S. E. Harris[1]. [1]Dept. of Periodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Dept. of Oral biology, University of Missouri at Kansas City, Kansas City, MO, USA.

The MLO-Y4 mouse osteocyte cell model is widely used to study osteocyte biology *in vitro*. Few markers for osteocytes are recognized and the gene expression patterns and signaling pathways of osteocytes are not well defined. Identifying gene expression sets and signaling pathways should provide information concerning osteocyte function. Therefore, we investigated gene expression patterns of MLO-Y4 cells at low and high density for comparison to 2T3 osteoblast cells at subconfluent and confluent stages of osteoblast differentiation. After hybridization using Clontech Mouse 5K Microarray, gene expression intensity was quantified and statistically analyzed using triplicate experiments, classified using standard clustering algorithms and functionally organized using DAVID-EASE, a program that explore biological themes of microarray results. Northern analysis was performed to validate expression of key genes. From a data set of 181 genes highly overexpressed in MLO-Y4 osteocytes compared to osteoblasts, we built pathway maps to model the pathways that reflect the MLO-Y4 osteocyte gene expression signature. This signature was then compared to a variety of public gene sets characteristic of bone cells such as osteoblasts, osteoclasts, and a variety of other cell types such as macrophages using Gene Set Enrichment Analysis (GSEA). From the pathway analysis, MLO-Y4 osteocyte cells have patterns of expression that reflect activation of acute and defense response pathways, the TGFβ pathway, the interferon activated pathway, and other closely related immune response pathways. As the cells are selected for their dendritic morphology, they have a set of genes that are involved in formation of cell processes or dendrites such as E11, SPP1, CD44, LAMA5. Several genes that inhibit apoptosis are also in the 181 MLO-Y4 gene set, such as Myc, Bag3. From the GSEA analysis, the MLO-Y4 181 gene set has high enrichment scores with other osteoblast differentiation models such as Col1α1 promoter-GFP selected osteoprogenitor maturation profiles, but is not enriched in macrophage, osteoclasts, APC dendritic cells, T cells, B cells. The MLO-Y4 selective gene expression signature can now serve as a basis for further comparison with primary isolated osteocytes and for characterizing osteocyte responses to mechanical signaling.

Disclosures: ***W. Yang****, None.*

SA292

Heel and Hip Bone Densitometry in Postmenopausal Women with Hip Fractures. J. D. McCrea, T. Hewer*. North Cumbria Osteoporosis Service, North Cumbria Acute Hospitals NHS Trust, Whitehaven, Cumbria, CA28 8JG, United Kingdom.

Reduced heel bone mineral density (BMD) is a risk factor for hip fracture yet there is scant information on heel BMD in patients with hip fractures and no consensus on the levels of reduced heel BMD at which treatment might be indicated. The aim of this study was to compare heel BMD results in postmenopausal female patients with recent hip fractures with those from 429 elderly community dwelling ladies studied previously. All postmenopausal women with low trauma hip fractures requiring surgery admitted to the West Cumberland Hospital in 2004 were included (n=120). After discharge, hip and heel BMD scanning was perfomed using Lunar Prodigy and Pixi scanners (GE Medical Systems) following our standard protocol. Thirty patients (25%) died prior to scanning and a further 43 (36%) did not attend leaving 47 patients (39%) who were scanned (9 heel only, 5 hip only, 33 both sites). Mean ± SD results were: total hip BMD = 0.647 ± 0.132 g/cm^2, T -score = - 2.94 ± 1.10; heel BMD = 0.323 ± 0.10 g/cm^2, T -score = - 2.19 ± 1.22. Results for the hip fracture and community dwelling patients are compared below:

Mean ± SD	Community dwelling patients: Heel T > - 1.6 (n=301)	Community dwelling patients: Heel T ≤ - 1.6 (n=128)	Hip fracture patients (n = 47)
Age (years)	72.0 ± 5.3	74.7 ± 6.0	79.2 ± 8.2 [a,b,c]
Weight (kg)	71.5 ± 14.3	58.4 ± 11.0	54.3 ± 12.0 [a,b,c]
Osteoporosis Screening Tool [OST]	- 0.12 ± 3.23	-3.21 ± 2.69	- 5.28 ± 2.66 [a,b,c]
Heel BMD (g/cm^2)	0.496 ± 0.08	0.312 ± 0.05	0.323 ± 0.10 [a,b]

Statistical comparisons (independent samples t tests) between hip fracture patients and patients with heel T ≤ -1.6 are as in the table; for all comparisons between hip fracture patients and patients with heel T > - 1.6 p was < 0.001. 89% of patients scanned were advised to start antiresorptive treatment. Of the 33 patients with both hip and heel BMD results, 22 had hip T -scores ≤- 2.5, 19 had heel T -scores ≤- 1.6 and 17 had heel T scores ≤- 2.0. This latter device specific figure (90% sensitivity & specificity for hip/spine osteoporosis) was adopted following the 2005 UK National Osteoporosis Society (NOS) Position Statement on peripheral DXA. These results from a small group of patients indicate no significant difference between heel BMD in hip fracture patients and the subgroup of primary care patients who had T -scores ≤- 1.6 in our previous community study. Adopting the device specific threshold, however, would have excluded 34% of the community dwelling ladies from treatment which may not be appropriate since BMD is a continuously distributed variable. We conclude that in the absence of central dxa, measurement of heel BMD with the Lunar Pixi, may offer a potentially valuable therapeutic intervention level for elderly women at risk for low trauma hip fractures.

Disclosures: ***J.D. McCrea****, John McCrea 2, 8.*

SA293

See Friday Plenary number F293.

SA294

Failure to Motivate Orthopedic Surgeons and Neurologists to Refer High Risk Patients for DEXA Exam. D. R. Mandel, P. L. Scott*, J. A. Holmes*. David R. Mandel, MD, Inc., Mayfield Village, OH, USA.

Osteoporosis patients with important clinical risk factors are often not evaluated in a timely way with bone mineral density (BMD) studies for possible medical therapy. Two large groups at risk are those who have had recent fragility fractures and those who have had a hemiplegic stroke. We initiated a study to educate local orthopedic surgeons and neurologists about these high risk patients. They were provided newsletters and medical articles about the benefits of BMD studies. At educational conferences we invited 40 orthopedic surgeons and 20 neurologists to refer at least 6 patients over a 3-month period of time who had a fragility fracture or a hemiplegic stroke in the past year. Each physician was provided with 6 vouchers which would enable their patients to receive a free DEXA BMD study. Results of the study would then be sent to both referring physicians and the patient's primary care physician. Only two patients were referred for BMD during the first 3 months. A follow-up letter informing the referring physicians of the small number of referrals was sent. Additional medical information and articles were also sent to encourage and motivate them. The study was continued for an additional 3 months with no additional patients referred. These results would indicate that physicians who come in contact with patients at risk for fracture do not refer for BMD studies. Additional study is required to understand what factors encourage and impede physicians to refer their patients who are at high risk for fracture.

Disclosures: ***D.R. Mandel****, The Alliance for Better Bone Health, P&GP Pharmaceuticals.,Sanofi-Aventis Pharmaceuticals 2.*

SA295

Fracture Patients Have Low Bone Mineral Density. A Consecutive Study of 239 Patients. J. Åstrand*, M. Tägil*, K. G. Thorngren*. Orthopaedic department, Lund University, Lund, Sweden.

Purpose: We screened fracture patients at our orthopaedic department for osteoporosis during one year to evaluate a relatively simple routine and identify patients with low BMD that might benefit from treatment. **Methods**: We included all patients between 50-75 years of age visiting our department with a distal radius-, proximal humerus-, vertebrae- or hip fracture from 1 November 2002 to 1 November 2003. A nurse identified patients who recieved a questionnaire concerning riskfactors for osteoporosis and were admitted for a DEXA-scan. Results of the scan were then evaluated together with the questionnaire and the diagnosis "normal", "osteopenia" or "osteoporosis" was established. **Results**: A total of 338 patients (84 males) were contacted, 87 hip fractures (38 males), 196 distal radius fractures (36 males), 27 vertebral fractures (5 males) and 28 with proximal humerus fractures (5 males). 99 patients were excluded: 33 were considered to have had high-energy fractures, 8 could not participate due to illnesses (1), dementia (4), poor language skills (2) or living far away (1). 28 did not want to participate or did not respond to the questionnaire. In total, only 13 patients were already examined and under treatment for osteoporosis. 256 patients were referred for a DEXA-scan (54 males). 16 patients never showed up at the DEXA-scan (3 males) and one woman died. Consequently, 239 patients (51 males) underwent DEXA scans. Of these, 13 % had a normal bone mineral density (<- 1 S.D.), 45 % had osteopenia (-1-2.5 S.D.) and 42 % had osteoporosis (<- 2.5 S.D.). **Conclusions**: In this study, fracture patients between 50-75 years of age had low bone mineral density and needed further investigations and treatment. If patients with low BMD are identified already after their first fracture, treatment can prevent or postpone subsequent fragility fractures. These patients appear at the orthopaedic clinic and orthopaedic surgeons have the opportunity not only to treat the fracture but also identify patients most at risk for future fractures. We organized our routine screening in a low resource demanding way using a team with a doctor, nurse and a secretary.

Disclosures: ***J. Åstrand****, None.*