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Abstract No.	Topic
P1-27	Poster abstracts (Authors marked with an asterisk (*) are Recipients of the Alice L. Jee Travel Award)

P-1

Regulation of Osteocyte Lifespan by Mechanical Forces *In Vivo* and *In Vitro*

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Osteocytes, former osteoblasts entombed in the bone matrix, form an extensive cell communication network that is thought to detect microdamage and mechanical strains and to transmit signals leading to repair and compensatory bone augmentation or reduction. We report that mechanical forces control the integrity of this network by regulating osteocyte survival *in vitro* and *in vivo*. Specifically, mechanical stimulation by biaxial stretching of MLO-Y4 osteocytic cells activates the extracellular signal regulated kinases (ERKs), which in turn are responsible for attenuating osteocyte apoptosis. The effect of osteocyte stretching is transmitted by integrins and a signalsome comprising actin filaments, microtubules, caveolae, and Src kinases. Stretch-induced anti-apoptosis also requires ERK nuclear translocation and new gene transcription. Furthermore, knock-down or knock out of the estrogen receptor (ER) α and β abolishes ERK activation and survival induced by mechanical stimulation, indicating the requirement of a ligand-independent function of the ER for the transduction of mechanical forces. Consistent with these *in vitro* studies, bone unloading by tail suspension of 4 -month-old Swiss Webster mice increases the prevalence of osteocyte apoptosis both in cancellous and cortical vertebral bone as early as 3 days, and this event precedes the decrease in bone mineral density and compression strength observed at 18 days.

These findings are consistent with the contention that physiologic bone loading provides continuous survival signals that preserve osteocyte viability, and that lack of these survival signals in states of low or absent mechanical loading induces apoptosis and disruption of the osteocyte network, and hence increased bone fragility.

The authors have no conflict of interest.

P-2

Combination of Disuse and Orchidectomy Has Additional Effect on Bone Loss in the Rat

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Risk factors for osteoporosis include disuse and hormonal depletion. The orchidectomized (ORX) rat is a model for male osteoporosis due to hypogonadism[1]. An IM injection of botulinum neurotoxin (BTX) induces a paralysis and a localized bone loss[2]. ORX and BTX models were combined to see the preventive effect of risedronate or the curative effect of a calcium-phosphate biomaterial (IBS1).

Aged male rats were randomized into 4 groups: SHAM operated, ORX-BTX (right hindlimb) ORX-BTX-RISE with injection of risedronate. One month after surgery, ORX-BTX rats received an intramedullary injection of IBS1 (ORX-BTX-IBS1). Animals were euthanazied after one (RISE) or two months (IBS1). Histomorphometry was done on the tibia to measure BV/TV and trabecular characteristics (Tb.Th, Tb.N, and Tb.Sp). Microarchitecture was analyzed by X-ray microtomography on the femur; BV/TV_{3D} and Structure Modeling Index (SMI) were measured.

The effects of ORX+BTX were cumulative: histomorphometric changes were maximized on BV/TV, BV/TV_{3D}, Tb.N, Tb.Sp and SMI on the paralyzed side. After risedronate therapy, BV/TV and Tb.Sp remained at the SHAM levels and Tb.N became significantly higher than SHAM value. Risedronate appeared to have early effects while testosterone failed to prevent this massive and acute bone loss.

After IBS1 implantation, bone parameters were elevated vs. SHAM but osteoclast number was markedly increased. IBS1 seems to restore bone temporarily since both IBS1 and the apposed woven bone were removed secondarily.

The ORX-BTX model induces a bone loss making it suitable to evaluate the preventive effect of new drugs as well as to evaluate the restorative effects of biomaterials.

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The authors have no conflict of interest.

P-3

Multiple Signaling Pathways Mediate *Osx*, a Zinc-fiinger Transcription Factor

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Osx-deficient mice do not form a bony skeleton, forming only cartilage (Nakashima, 2002), similar to the phenotype observed in *Runx2*-deficient mice (Ducy, 1997). Recently, several lines of research suggested the possibility of *Runx2*-independent ossification, suggesting that additional pathways may act in parallel to, or independent of *Runx2* to regulate *Osx* expression during osteogenic lineage progression. We tested the hypothesis that alternate signalling pathways mediate *Osx* expression in addition to the BMP-2/Smad signalling. Using real-time RT-PCR, we showed upregulation of *Osx* in response to BMP-2 in human mesenchymal stem cells (hMSC) and mouse fibroblasts (NIH3T3). The BMP-2-mediated effect was not direct; protein synthesis was required downstream of the BMP-2 signal to induce *Osx* expression, despite the overexpression of *Runx2*. We tested the effects of various bone-related growth factors in mediating *Osx*. hMSC were treated with recombinant human (rh)BMP-2, FGF-2, IGF-I, PGDF-BB, and TGF-β1 for 48 hrs. BMP-2 and IGF-I alone induced an increase in *Osx* expression 48 hrs post-treatment. Further, BMP-2 and IGF-I had a synergistic effect on the induction of *Osx*. While BMP-2 can promote *Runx2* upregulation, IGF-I did not have an effect.

BMP-2 and IGF-I synergy may be due to the convergence of downstream signalling components such as MAPK. Chemical inhibitors U0126, SB203580 were used to block specific MAPK components, ERK1/2 and p38, respectively. Treatment with Erk1/2 inhibitor abolished matrix mineralization, while p38 inhibitor did not have an effect on mineralization. *Osx* expression was abolished in response to a block in p38 and Erk1/2 signalling. BMP-2 mediated induction of *Osx* was down-regulated due to inhibition of p38 signalling. The results from the MAPK inhibition experiments with chemical inhibitors were further confirmed via a genetics approach using dominant negative p38 and Erk constructs.

Recently, evidence was presented for the involvement of *Wnt3a* on proliferation and differentiation of hMSC. We conducted studies to determine the effect of recombinant mouse *Wnt3a* (*rmWnt3a*) protein on *Osx* expression. *rmWnt3a* did not induce *Osx* expression in hMSC maintained under basal, or osteogenic conditions. hMSC were transduced with adenoviral constructs for *Wnt1* and *Wnt5A*. Neither *Wnt1*, nor *Wnt5A* induced *Osx* expression, but there was an upregulation of Alp in response to *Wnt1* expression 7 days post-transduction. Further studies will need to be conducted to resolve the involvement of *Wnt* signalling in hMSC and their possible effect on *Osx* expression during osteoblastic lineage progression.

We conclude that during osteogenic lineage progression, in addition to the BMP-2/Smad pathway, IGF-I and MAPK signalling may mediate *Osx* and a possible involvement of *Wnt* signalling in *Osx* expression parallel to or independent of these signalling components requires further clarification.

The authors have no conflict of interest.

P-4

Analysis of Variation in Expression of ADO2: Searching for Modifier Genes

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Autosomal Dominant Osteopetrosis type II (ADO2) is an osteosclerotic disorder due to heterozygous mutations in the ClCN7 gene. Analysis of ADO2 in our pedigrees indicates that the penetrance is 66%, with a highly variable phenotype even between family members with the same mutation. To identify genes that modify disease severity, we performed a 10cM genome-wide scan using 400 microsatellite markers in 8 ADO2 families. Parametric linkage analysis was conducted with the MLINK program, using both dominant and recessive models. Using the recessive model, evidence of linkage was detected with marker D9S283 (LOD=2.03) and with marker D2S347 (LOD=1.52). Additional microsatellite markers were then genotyped on chromosomes 9 and 2 to better delineate the position of the putative modifier locus. Based on the key recombinants in the families, a candidate region was identified on chromosome 9q21-22. Of note, candidate genes in this region include cathepsin L, which is secreted by osteoclasts and processed to an active enzyme by acid, surface activation, or proteolysis. We are currently analyzing cathepsin L as well as other genes on chromosomes 9q22 to identify genes that modify phenotypic expression.

Since the linkage search strategy pursued above would be invalid in the chromosome 16pter region due to confounding of the disease mutation and putative modifier effects, we have also considered the hypothesis that genetic variation at other sites within the ClCN7 gene might influence disease expression. DNA sequence data has shown that at position 418 of the ClCN7 protein sequence there is a missensce polymorphism resulting in an amino acid change from valine to methionine (V418M). To test the hypothesis that this polymorphism affects disease severity, we genotyped this polymorphism in our ADO2 families. We found that, on the chromosome with the wild-type ClCN7 allele, 95% (53/56) of affected and 75% (24/32) of carriers had the V allele while 5% (3/56) of the affected and 25% (8/32) of carriers had the M allele (p=0.007). Further analysis indicated that most of the evidence of association came from the 4 families with the G215R mutation. In these families, 100% (16/16) of affected and 43% (3/7) of carriers had the V allele on the non-disease carrying chromosome, while none of the affected and 57% (4/7) of the carriers had the M allele (p=0.0009).

In conclusion, regions of 9q21-22 may harbor modifier genes that affect disease status and severity. Additionally, we find statistical support for the hypothesis that the V418M polymorphism, on the non-mutant ClCN7 gene allele, influences the tendency to become an asymptomatic carrier.

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P-5

Mechnical Properties of Subadult Manatee Bone

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The Florida manatee *(Trichechus manatus latirostris)* is considered one of the most endangered marine mammals found in U.S. waters. Watercraft-related mortality, caused by propeller wounds or impact, accounts for 24% of all deaths from 1976-2003, and comprises 80% of human-related deaths. The proportion of deaths due to impact has increased, and now accounts for more than half of all watercraft-related deaths. Approximately two-thirds of animals killed by impact suffered broken or luxated ribs; death is primarily attributed to these injuries. Reducing watercraft-related mortality is a high priority in state and federal manatee recovery efforts, which focus primarily on regulating boating activities. However, with the ever-increasing number of humans utilizing the large amount of waterways along the Florida coastline, as well as advances in boat technology that are allowing greater access to these waterways, the threat to manatees by watercraft continues to increase substantially. In order to establish safe boat speeds for manatee protection, an estimate of the forces required to fracture their bone is needed. This study represents the first attempt to quantify the biomechanical effects of boat strikes on manatees.

In contrast to most marine mammals, manatee bone is pachyostotic, characterized by thickening of bone tissue, replacement of cancellous with compact bone, and absence of free medullary cavities. We investigated the material properties of subadult manatee bone. Flexural strength, modulus, and toughness were measured for six male and six female subadults. Parallelpipeds were machined from ribs in the cranial, middle, and caudal body regions, and fractured in three-point bending to calculate flexural strength and modulus. By applying principles of fracture mechanics, we can relate the size of the initiating crack and stress at failure to the toughness of the material. Unlike typical bone, manatee bone behaves as a ceramic. Therefore, we were able to apply fractographic analysis to fracture surfaces to calculate toughness. Mean flexural strength by animal ranged from 91–163 MPa, mean toughness from

1.9–2.9 MPa·m$^{1/2}$, and mean modulus from 8–15 GPa. Material properties increased with body size up to 265 cm total length. In comparison, typical flexural strengths for human and bovine bone tested in 3-point bending are 209 MPa and 224 MPa, respectively. Toughness of human and bovine bone ranges from 2.2–6.3 MPa·m$^{1/2}$, indicating that manatee bone is on average less strong and tough. Material properties are likely correlated to the mineral content (67-70%) and plexiform organization. This project is part of a larger study that examines the ontogenetic changes in mechanical properties of manatee bone. This information will contribute significantly to our understanding of manatee-boat interactions, and will be critical in establishing boat speed zones adequate to minimize the chance of fatal impacts.

The authors have no conflict of interest.

P-6

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones – I. Effects of Hypophysectomy and Recombinant Human Growth Hormone

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Aiming to analyze the musculoskeletal effects of hypophysectomy (Hx) and a partial substitutive treatment with recombinant human growth hormone (rhGH), we determined the intrinsic stiffness (elastic modulus, E) and volumetric BMD (vBMD) of cortical bone; the periosteal and endosteal perimeters, area and moment of inertia (CSMI) of the cross-sections, and the structural stiffness and pre- and post-yield strength of femur diaphyses (pQCT and mechanical tests), and the gastrocnemius weight of rats, either intact (n=9) or Hx at 15 days of age (20), otherwise untreated (Hx controls, 4) or given 0.4 (8) or 2.0 (8) IU/kg/d sc of rhGH since 15 days after surgery during 45 days.

The Hx delayed the musculoskeletal development (gastrocnemius weight, bone geometric properties), thus affecting the diaphyseal stiffness and strength. It also reduced the cortical vBMD through an undefined mechanism, paradoxically increasing E. The Hx also affected the correlation between bone geometric and material properties (CSMI *vs* E), suggesting an anti-anabolic interaction with the biomechanical control of bone modeling. As an integrated result, Hx reduced the stiffness and the post-yield and ultimate strength of the diaphyses.

These effects should reflect changes in bone tissue microstructure associated with crack generation and progress, unrelated to bone mineral mass. Results are compatible with a delayed collagen turnover with associated increases in fibers' diameter and crystals' size, resulting from the suppression of other hormones, presumably thyroid.

The assayed doses of rhGH tended incompletely to prevent the negative Hx effects on bone and muscle development correlatively. However, rhGH treatment failed to prevent the curious, demineralizing/stiffnening effect of Hx on bone tissue and the unusual effects observed on the post-yield strength (less clearly related to muscle development than the former).

The effects of larger rhGH doses and the interaction of other hormones with the described effects remain to be investigated. These findings point out a novel feature in rhGH effects on bones and challenge the prevailing view that in endocrine-metabolic bone-weakening diseases the bone matrix always shows a normal composition.

The authors have no conflict of interest.

P-7

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones – II. Effects of Recombinant Human Growth Hormone on Bones and Muscles After Ovariectomy

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Doses of 150 IU/kg/d of human recombinant growth hormone (rhGH, *BioSidus, Buenos Aires*) were sc given during 3 months to 3-month old rats, either intact or OX at that age. At the end of the study their diaphyses were scanned by pQCT and tested in bending. The fresh gastrocnemius muscles were weighed.

OX reduced bone tissue's mineralization and stiffness. A significant enhancement of bone growth in width improved significantly the diaphyseal architecture (cross-sectional moment of inertia, CSMI). This geometric improvement overcompensated the negative impact of the OX-induced impairment on bone material's mineralization and stiffness, thus the diaphyseal strength was increased.

The assayed rhGH dose rhGH was little effective in intact rats. However, it prevented the OX-induced impairment in bone tissue's mineralization (not stiffness) and improved additively the OX-enhanced geometric variables.

These effects of OX and rhGH were correlative with additive increases in muscle mass. Simple regression analyses showed that the impact of muscular improvement was more evident on bone architecture than on bone strength.

The positive OX and rhGH effects on cortical bone mass and architecture may have derived from the induction of an "anabolic" shift of bone *mechanostat* threshold for triggering bone modeling during growth, with a positive biomechanical impact on the diaphyses (larger CSMI and fracture load than controls). The apparent incongruence between the repercussion of the additive improvement in muscle mass induced by OX and rhGH on bone geometry (large impact) and strength (relatively low impact) can be explained because rhGH did not prevent the OX-induced impairment in bone material's stiffness because rhGH did not act on the microstructure of mineralized tissue.

Based on original arguments, this evidence suggests the ability of rhGH to improve human postmenopausal osteopenias in which a relatively large impairment in cortical bone mass and/or distribution occurs. However, the actual benefit of the positive rhGH effects on bone mass and architecture in any species would remain uncertain as long as the nature of rhGH effects on the OX-impaired bone's material stiffness is unknown.

These results are interesting because they defy the prevailing view that the remaining bone tissue in metabolic osteopenias is normal.

The authors have no conflict of interest.

P-8

Experimental Effects on Material's Properties and Pre- and Post-Yield Behavior of Rat Bones – III. New Insights on the Effects of Bisphosphonate (Olpadronate) Administration

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Doses of 45-90 mg/kg/d of olpadronate (OPD, IG-8801, *Gador, Buenos Aires*, carcinogenicity dose-range finding study) were orally given during 3 months to 20 male and 24 female rats 4-5-week-old (7 & 9 controls). The cortical vBMD, cross-sectional perimeters (PM), area (CSA) and moments of inertia (MI) of femur diaphyses and their structural stiffness (load/deformation ratio) and strength (ultimate load) during the successive "elastic", reversible (*pre-yield*, no microcracks) and "plastic", irreversible (*post-yield*, microcrack accumulation) deformation periods were determined by pQCT and bending tests. The pre-yield stiffness of cortical tissue (elastic modulus, E) and a Bone Strength Index, BSI = vBMD*MI (which can predict ultimate strength but does not capture any microstructural indicator of cortical tissue) were calculated from those data.

No effects on growth were observed. Treatment significantly improved CSA and MI by increasing both endosteal and periosteal PMs, more evidently in male than female rats (probably a size-related difference), with no effects on cortical vBMD and E. As a result, mild increases in diaphyseal stiffness and strength at yield (only significant in males) were observed. Diaphyseal ultimate strength was substantially enhanced (males, +68.1%, $p<0.001$; females, +21.7%, $p<0.01$) chiefly because of a large increase in the post-yield fraction of ultimate load (a correlate of bone "toughness"; males, +344%, $p<0.001$; females, +101%, $p<0.05$). The BSI failed to

predict ultimate load in treated animals.

The positive effects of the assayed OPD doses on pre-yield bone behavior would reflect an anabolic improvement in diaphyseal geometry induced independently of bone material's mineralization and elastic stiffness (i.e., beyond the homeostatic control of bone structure as predicted by bone *mechanostat* theory). The large effects on bones' post-yield behavior and ultimate strength should be assigned to changes in some "creeping" factors not determined in the study, affecting crack progress within cortical tissue ("plastic" deformation period) previously to fracture. Failure of BSI to predict ultimate strength suggests that the observed bone strengthening would have been determined chiefly through changes in some mineralization-unrelated, microstructural factors.

These results point out some novel bisphosphonate effects on bone strength and mechanism of fracture with no apparent involvement of bone mineralization.

The authors have no conflict of interest.

P-9

Experimental Effects on Material's Properties and Pre- and Post-Yield Behavior of Rat Bones – IV. Novel Effects of Ovariectomy and Alendronate

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Effects of bisphosphonates on bone mineralization and strength are not necessarily correlative. To analyze ALN effects on bone strength, forty 3-month-old rats were OX and given immediately 0 (OX-C, n=13), 5 (OX-5, n=13) or 25 ug/kg sc (OX-25, n=14) 2/wk for 6 months, keeping a further 15 as sham controls. Their femurs were scanned by pQCT and tested in bending.

Despite not affecting bone mineralization (cortical vBMD) and cross-sectional diaphyseal geometry (diameters, moment of inertia -MI-), OX impaired the intrinsic stiffness of cortical tissue (elastic modulus, E) and the structural stiffness of femur shafts (load/deformation ratio), and reduced yield and fracture loads (W_y, W_f). The post-yield fraction of W_f ($Wp = W_f - W_y$) was significantly enhanced by OX, perhaps because of the naturally inverse relationship between the tissue's ability to prevent crack generation (impaired) and progress (improved). Effects of ALN were dose-dependent. The highest ALN dose prevented all negative effects of OX and improved W_f over sham values. No changes in W_y were observed in treated rats (no effect on crack generation). However, Wp (bone toughness) was enhanced in a similar proportion than it was in OX rats. The naturally negative "distribution/quality" curves (correlations between cortical architecture, MI and intrinsic stiffness, E) shifted to the "anti-anabolic" region (lower-left) in the graphs for OX rats and to the "anti-catabolic" region (upper-right) for ALN-treated rats with respect to sham controls. This would indicate negative or positive interactions of OX and ALN, respectively, with the feed-back control of bone architecture as a function of bone stiffness and mechanical usage of the skeleton (bone *mechanostat* theory).

In agreement with previous observations in intact rats treated with Olpadronate, lack of effects on bone mineralization and geometry in this study suggests that both OX and ALN treatment would have improved Wp (and additionally ALN would have improved W_f) by affecting some microstructural determinant(s) of bone material's stiffness and toughness (creeping factors) independently of bone mineralization. These novel effects of bisphosphonates may explain the striking dissociation observed between induced improvements in BMD and fracture incidence in large studies with post-menopausal osteoporotic women.

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P-10

Experimental Effects on Material's Properties and Pre- and Post-Yield Behavior of Rat Bones – V. Chronic Effects of Aluminum Accumulation on Cortical Bone

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In order to analyze the effects of Al accumulation on pre- and post-yield behavior of cortical bone, 14 rats aged 90 days received ip doses of 27 mg/d of elemental Al as $Al(OH)_3$ during 26 weeks while another 14 remained as controls. Their femur diaphyses were studied tomographically (pQCT) and tested in bending. The load/deformation curves obtained showed the successive, linearly elastic (Hookean, pre-yield) and non-linear, "plastic" (non-Hookean, post-yield) deformation periods of bones, separated by the yield point.

No effects on body weight were observed. Aluminemia and bone histological and ash data confirmed Al accumulation. Treatment reduced cortical bone mineralization (volumetric cortical BMD, $p<0.01$) with a negative impact on the intrinsic stiffness of cortical tissue (Young's elastic modulus, $p<0.05$). Despite the absence of any cortical mass increase (cross-sectional area), an improvement of the spatial distribution of the available cortical tissue (cross-sectional moment of inertia, MI, $p<0.05$) occurred through a directional modulation of the *modeling drifts* during growth. Up to the yield point, neither the strength, strain, or structural stiffness (load/deformation ratio) of the diaphyses were affected by treatment. However, Al intoxication significantly reduced the ultimate load, W_{max} and the "post-yield" fraction W_p of that load (an estimation of bone "toughness", $p<0.01$). A positive correlation between W_{max} and W_p for all the studied animals as a whole was observed.

The presumably adaptive response of bone modeling (as assessed by the MI) to the induced impairment of the *intrinsic stiffness* of bone tissue should have proved adequate for maintaining a normal *structural stiffness* (load/deformation ratio) of femur diaphyses according to the bone "mechanostat" theory, but not so as to provide a complete neutralization of the impaired diaphyseal *strength* (W_{max}). Although a relative inhibition of bone formation could not be discarded, an Al-induced impairment of bone "toughness" (W_p) should have caused the striking disruption observed between effects on bone stiffness and strength.

In addition to describing an unusual finding, these results suggest that the microstructural elements affecting the *post-yield* behavior of cortical bone in these conditions ("creeping factors") ought to be further investigated as a promising field in skeletal research.

The authors have no conflict of interest.

P-11

Tomographic and Mechanical Evaluation of Muscle-Bone Interactions in Mice Artificially Selected for Body Conformation

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Antagonistic artificial selection of adult male and female mice with wide variation in body conformation produced animals with light body/long skeleton (CBi/L) or heavy body/short skeleton (CBi/C) from a parental line CBi. On changing the natural proportions between body and skeletal size/shape, this procedure provided an interesting model for analyzing correlations between the body and gastrocnemius weight and indicators of material, geometric and mechanical properties of cortical bone of femur diaphyses (as assessed by pQCT and bending tests) avoiding the natural, allometric associations which normally blunt the biomechanical interrelationships between muscles and bones.

As expected, the selection procedure determined a wide inter-strain variation of the natural proportions between gastrocnemius mass, body weight (bw) and femur length, and between femur length and diaphyseal cross-sectional moment of inertia (CSMI). Analyzing all the animals as a whole, it was observed that a. the CSMI correlated closer with gastrocnemius weight than it did with bw; b. diaphyseal strength correlated significantly with CSMI, gastrocnemius weight and bw, and c. correlation of CSMI with gastrocnemius weight was closer than with bw and was the only graph describing the studied association as a single (linear) function for all the 3 strains as a single group.

Results suggest that (1) muscle mass would not depend alometrically on bw in any circumstance; (2) geometric proportions between long-bone length and cross-sectional properties would not be independent determinants of bone structure or strength; (3) muscle development would

not depend on bone development; (4) the diaphyseal design would be adapted to muscle ability to directionally deform the skeleton rather than to the weight of the supported biomass; and (5) the biomechanical adaptation of bone strength to customary mechanical usage as allowed by the biochemical and microstructural constitution of the skeleton would be determined more closely by the dynamic influence of muscle contractions than by the static, gravitational load of the bw.

Those relationships, difficult to assess in natural conditions, are crucial for interpreting the biomechanical homeostasis of the skeletal structure and the etiopathogenesis of all osteopenias and osteoporoses. This knowledge could be extrapolable to the pathogenetic analysis of many human bone-weakening diseases.

The authors have no conflict of interest.

P-12

DEXA Assessment of Muscle-Bone Relationships in Humans – I. Reference Study of the Lean Mass / BMC Relationships in Whole Body and Limbs of 2,512 Normal Men and Pre- and Postmenopausal Women

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A whole-body (WB) DEXA study of 1,450 normal Caucasian individuals [Bone 1998; 22:683] found that mineral mass, either crude (BMC) or statistically adjusted to fat mass (FA-BMC) correlated linearly with lean mass (LM, proportional to muscle mass), showing similar slopes but decreasing intercepts in the order: pre-MP women > men > post-MP women > children. This supported the control of bone status by muscle strength in humans (bone "mechanostat" theory) and the positive interaction of sex hormones with that control. Now we study those relationships in 2,512 normal Hispanic adults (307 men, 753 pre-MP women, 1,452 post-MP women), including determinations in upper and lower limbs (UL, LL).

In all studied regions the slopes of the BMC or FA-BMC vs. LM relationships were parallel. However, the intercepts of the curves showed regional differences. In WB, the BMC/LM relationships showed the same intercept differences observed previously. In LL, those differences were smaller but highly significant, showing the order: pre-MP women>men=post-MP women. In UL, the decreasing intercept order was: men>pre-MP women>post-MP women. After fat-adjustment of the BMC, the intercept order in both limbs was men>pre-MP women>post-MP women. Parallelism of the curves was always maintained. A larger independent influence of LM than FM, body weight or age on these results was shown.

Parallelism of the curves further supports a common biomechanical control of bones by muscles in humans. Results suggest that the sex-hormone-associated differences in the DXA-assessed muscle-bone proportionality in humans could vary in different regions because of the different weight-bearing nature of the musculoskeletal structures studied. Besides the obvious anthropometric associations, FM would exert a mechanical effect as a component of body weight, evident in the LL, while muscle contractions would induce a dynamic effect in both limbs. Multiple regression tests showed that muscles exert a significantly larger influence than FM, body weight and age on BMC in WB and LL, regardless of gender and reproductive status of the individual.

In order to ease the clinical evaluation of these bone-muscle relationships, specific reference charts and a special software utility were developed from normal data, as reported separately.

The authors have no conflict of interest.

P-13

DEXA Assessment of Muscle-Bone Relationships in Humans – II. Validation Study for a Novel Differential Diagnosis Between "Disuse" and "Systemic" Osteopenias

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DEXA assessment of bone mass (BMC) can be complemented by measuring *lean mass* (LM, proportional to *muscle* mass) in whole body (WB) or limbs. This little profitted resource may provide a differential diagnosis between "disuse" and "systemic" osteopenias. We have shown linear, parallel BMC(y)-vs.-LM(x) relationships in WB and limbs of 1,450 Argentine and 3,000 Colombian normal individuals. Parallelism of the curves would reflect the biological control of bones by muscles by bone *mechanostat*. However, the intercepts of the graphs differed between groups, in the order: boys/girls<post-MP women<men<pre-MP women, suggesting a positive modulation of that control by sex hormones.

These results allowed performing *z-scored* graphs, specific for gender, reproductive status, region studied, race, and DEXA equipment employed, suitable for evaluating the bone/muscle mass proportionality. They may allow evaluating whether an eventually low BMC value is or not adequately proportionate to an individual's WB or regional muscle mass. "Disuse-related" osteopenias (as well as small or lean, normally active individuals) should show a normal z-score for the BMC-vs.-LM relationship. "Systemic" osteopenias caused by alterations of bone cells (either primary or secondary to endocrine-metabolic changes) should show low BMC-vs.-LM z-scores. Such cases should be further studied employing other technologies to determine whether bone strength is or is not affected; i.e., for diagnosing an *osteoporosis* as a metabolic or systemic "osteopenic fragility" (NIH criterion), which is outside the DEXA scope.

This report shows some studies in which we tested the ability of our WB or lower-limb BMC-vs.-LM percentile or z-score reference charts and application software to detect "metabolic" osteopenias in a. haemodialysed men (37) and pre- and post-MP women (71), in which the validation was achieved by showing that z-scores decayed as time on dialysis or serum PTH increased; b. obese hyperinsulinemic euglycemic men (30) and pre- and post-MP women (110), in which z-scores diminished as BMI or fasting plasma insulin increased or insulin sensitivity decreased; c. professional young adult female ballet dancers (20), in which z-scores decayed as calciuria increased, presumably because of a disturbed estrogen metabolism, and d. hypopituitary men (14) and women (15) before and after treatment with rhGH, in which z-scores improved as serum IGF-I levels increased.

The authors have no conflict of interest.

P-14

DEXA Assessment of Muscle-Bone Relationships in Humans – III. Application Study: Differential Diagnosis Between "Systemic" and "Mechanical" Osteopenias in Fractured Pre- and Postmenopausal Women

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DEXA could analyze the bone/muscle (BMC/lean mass, LM) proportionality and distinguish between osteopenias of "mechanical" (disuse) and "systemic" (primary or secondary) etiologies based on z-scores of the corresponding correlations. To validate this hypothesis we compared whole-body and lower-limb (WB,LL) data of 623 pre- and post-MP Hispanic women fractured in specific locations (hip, spine, wrist, arm, leg; *Type-I Fx*, n=396) or other sites (*Type-II Fx*; n=227). Individual z-scores for the BMC-vs.-LM correlations were calculated with respect to reference BMC-vs.-LM curves previously determined in 814 pre- and 1,656 post-MP healthy Hispanic women of comparable ages.

The BMC-LM z-scores were similar to controls in the whole Type-II-Fx group and significantly lower than that in the Type-I-Fx group. The BMC-vs.-LM curves for pre-MP women with Type-I or *Type-II Fx* and for post-MP women with *Type-II Fx* were linear and similar to controls in WB and LL, showing a high prevalence of normal BMC-LM z-scores. Instead, post-MP women with *Type-I Fx* showed nonlinear BMC-vs.-LM relationships, with BMC and BMC-LM z-scores rapidly decreasing toward low LM values.

Variance of the data was lower in LL than in WB.

This indicates that (1) "systemic" osteopenia (low BMC-LM z-scores) predominated in women with *Type-I Fx* while "mechanical" osteopenia (normal BMC-LM z-scores) did in those with *Type-II Fx*; (2) Post-MP women had more fractures associated to "systemic" than "mechanical" osteopenias compared with pre-MP women, in which the former were practically absent; (3) in post-MP women, "systemic" etiology tended to predominate as LM decreased, perhaps because the lack of estrogen reduced the sensitivity of bone cells to mechanical stimuli, and (4) determinations in LL are more reliable than those in WB for this purpose.

Results show that DEXA could differentiate osteopenias with different treatments (physycal intervention in "mechanical" cases, pharmacological resources in "systemic" cases), thus allowing monitoring therapeutic effects according to biomechanical criteria with no additional costs over the standard WB determinations.

Nevertheless, the BMC/LM proportionality can not distinguish by itself between individuals more or less exposed to fractures in any instance. Neither DEXA could be used as a single resource to diagnose osteoporoses (defined as an "osteopenic fragility" by the NIH).

The authors have no conflict of interest.

P-15

DEXA Assessment of Muscle-Bone Relationships in Humans – IV. Impact of Parathyroid Status and Ca and Vitamin D Supplementation on Muscle-Bone Relationships in 208 Belarussian Children After Thyroidectomy Because of Thyroid Carcinoma

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This observational study analyses Ca-P metabolism and its impact on bone mass accrual and density and the muscle-bone mass/mass relationships in male and female children and adolescents who were parathyroidectomized because of thyroid carcinoma.

Two hundred and eight children and adolescents (119 girls and 89 boys) from Gomel City (Belarus) and its rural surroundings were referred to the Würzburg Clinic after having undergone total thyroidectomy for the treatment of advanced papillary thyroid cancer. A subgroup of children with demonstrated primary hypoparathyroidism received dihydrotachysterol (AT-10) and/or Ca supplementation. Among routine procedures over a maximum follow-up period of 5 years (average 3.7 years, maximum 8 visits), whole-body DXA scans were taken at each visit in order to determine whole-body bone mineral content (TBMC), projected "areal" bone mineral density (TBMD), total lean mass (TLM) and total fat mass (TFM).

The average serum Ca, P and AP concentrations over the whole observation period were significantly different between the groups; however, TBMC z-scores for all studied children were statistically similar in all visits. In girls, supplementation exerted no effect on height- and weight-adjusted TBMC and TBMD or the TBMC/TLM ratio, suggesting that the total bone mass accrual was not impaired by PTH deficiency in the studied conditions. However, non-supplemented boys showed lower values of the TBMC/TLM ratio than girls, and supplementation normalized these values in direct correlation with the induced improvement in serum P availability to bone as assessed by serum P concentration.

Results indicate that the primary impairment in parathyroid function and bone metabolism indicators in the thyroidectomized children was unrelated to any measurable change in crude bone mass values. However, in boys this condition impaired the TBMC/TLM ratio in such a way that the administered supplementation could normalize it as a function of improved P availability. Girls' skeletons seemed to have been naturally protected against the negative metabolic effect of the studied condition. An estrogen-induced enhancement of the biomechanical impact of muscle contractions on bone mass and structure could not be excluded in this group.

The authors have no conflict of interest.

P-16

Skeletal Consequences of Paclitaxel Treatment in a Nude Rat Xenograft Model

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The incidence and diagnosis of prostate cancer continues to rise each year due to an aging population, increased awareness of the disease and better diagnostic tools, such as the prostate specific antigen test (PSA). Prostate cancer starts as an androgen-dependent tumor[1] which may progress to a hormone-independent adenocarcinoma. Androgen ablation therapy slows tumor progression, but also has significant impact on quality of life. Secondary effects of therapy include muscular atrophy and osteoporosis that can lead to bone fractures[2]. Chemotherapy and radiation therapy, currently used to treat malignancies, target rapidly dividing cancer cells. In addition to malignant cells, this type of therapy also affects rapidly dividing normal cells that reside in the bone marrow, gastrointestinal regions and hair follicles.

Paclitaxel, an anti-cancer agent isolated from the bark of *Taxus brevifolia*, has a broad range of anti-neoplastic activity. Recently, paclitaxel has been reported to inhibit human prostate cancer cell growth *in vitro*[3] and *in vivo*[4]. However, clinical data in prostate cancer patients has not confirmed this pre-clinical data, instead suggesting that taxen-based regimens are not curative and have not been proven associated with substantial survival benefits in prostate cancer patients.

The main objective of this study was to determine the effectiveness of intravenous delivery of paclitaxel on human prostate cancer growth and bone metabolism utilizing a nude rat xenograft model. This pre-clinical model closely mimics disease conditions in humans and allows for the determination of efficacy and safety of the i.v. formulation of paclitaxel against localized prostate tumors.

Results of our study confirmed clinical study data demonstrating that chemotherapy is ineffective in both testosterone dependent (22Rv1) and independent (PC3) human prostate tumors. Treatment with paclitaxel negatively affected bone formation and longitudinal growth, causing deterioration of cancellous bone mass and structure. Paclitaxel also caused a decrease in testosterone levels, which may have been a contributing factor to observed decrease in bone formation and longitudinal growth.

Unopposed human prostate cancers, implanted subcutaneously, caused a decrease in bone formation regardless of their osteoblastic (22Rv1) or osteolytic (PC3) properties. This probably resulted from secretion of catabolic factors and cytokines by the tumor that caused cachexia and decreased testosterone production. Cancer bearing rats treated with paclitaxel exhibited severe deterioration of bone structure. This is likely the result of tumor growth that was not curbed by paclitaxel, combined with the compromising effects of the chemotherapy. Our data indicate that the skeletal effects of prostate cancer and chemotherapy aimed against this malignancy are serious. Currently, there exists no effective therapy for advanced prostate cancer that is not associated with skeletal disabilities and severe deterioration of quality of life.

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P-17

Effect of Vitamin K2 on Serum Cholesterol Level in Postmenopausal Osteoporotic Women Treated With Alendronate

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Background and aims. Vitamin K2 is reported to reduce serum total cholesterol levels in patients on continuous peritoneal dialysis. The purpose of the present study was to examine the effect of vitamin K2 on serum cholesterol levels in postmenopausal osteoporotic women treated with alendronate. Methods: Fifty postmenopausal women with osteoporosis (mean age, 69.8 years; range, 54-83 years) were randomly divided into two groups with 25 patients in each group: alendronate (5 mg daily) alone group and alendronate plus vitamin K2 (menatetrenone, 45 mg daily) group. The duration of the study was 12 months.

Results. There were no significant differences in any baseline characteristics between the two groups. The reductions in urinary cross-linked N-terminal telopeptides of type I collagen and serum alkaline phosphatase levels and the increase in lumbar bone mineral density (BMD) did not differ significantly between the two groups. Although the changes in serum low-density lipoprotein cholesterol and triglyceride levels were similar in the two groups, serum total cholesterol (TC) level was significantly reduced at 12 months in the alendronate plus vitamin K2 group (2.5% reduction) than in the alendronate alone group (6.7% increase).

Conclusions. The results of the present study suggest that vitamin K2 showed no recognizable beneficial effect in addition to that of alendronate on the increase in lumbar BMD with reduced bone turnover, but showed a benefit in the reduction in serum TC level in postmenopausal women with osteoporosis during a one-year period.

The authors have no conflict of interest.

P-18

Effects of Bisphosphonates on Periosteal Bone Formation in Rat Ulna

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Recent studies demonstrate that bisphosphonates suppress bone resorption by leading to apoptosis of the osteoclast and inhibiting the differentiation to mature osteoclasts. The influence of bisphosphonates on bone formation is unknown, although it has been hypothesized that bisphosphonates inhibit osteoblast apoptosis, leading to increased bone formation. This is difficult to test on remodeling surfaces where resorption and formation are coupled because suppression of bone turnover naturally reduces both bone resorption and bone formation. We hypothesized that bisphosphonates enhance periosteal bone formation, and tested this hypothesis by evaluating the effects of alendronate and risedronate on bone formation on surfaces known to be undergoing modeling formation in rats.

Materials and methods: Thirty male Sprague Dawley rats 7 months old were divided by weight into five groups. Control rats (CNT; n=6) were given a daily subcutaneous injection of saline vehicle. Four groups of rats were injected subcutaneously daily for 17 days with Alendronate (ALN) in a saline carrier at a dose of 0.1 μg/kg per day (ALN-low; n=6), 10 μg/kg per day (ALN-high; n=6), Risedorate(RIS) at a dose of 0.05 μg/kg per day (RIS-low; n=6) or 5 μg/kg per day (RIS-high; n=6). Prior to killing, animals in all groups were double labeled with calcein (1-5-1-3). Histomorphometric measurements for periosteal MS/BS, MAR and BFR were performed on one unstained section per ulna located 1 mm distant from the coronoid process.

Results: Periosteal cortical bone modeling was significantly suppressed by all treatments. MS/BS of ALN was 19% lower in ALN-low and 29% lower in ALN-high (p=NS) than in CNT. Decrease of MS/BS was 10% for RIS-low, 25% for RIS-high (p=NS). MAR of ALN was 27% lower in ALN-low ($p<0.01$) and 32% lower in ALN-high ($p<0.01$) than in CNT. Similarly the decrease was 27% for RIS-low ($p<0.05$) and 31% for RIS-high ($p<0.01$). BFR of ALN was 34% lower in ALN-low ($p<0.01$) and 43% lower in ALN-high ($p<0.01$),RIS was 29% lower in RIS-low ($p<0.05$) and 42% lower in RIS-high ($p<0.01$) than in CNT.

Discussion: Our results show that bisphosphonates at both clinically-relevant and high doses suppress bone formation on the periosteal surface. Although no measurements were made to assess osteoblast apoptosis on this surface, our results lead us to hypothesize that bisphosphonates at these doses do not suppress apoptosis in osteoblasts, but on the contrary may suppress bone formation independently of bone resorption.

K. Iwata has no conflict of interest. D. Burr is a consultant for Procter and Gamble Pharmaceuticals.

P-19

Identifying Risk Factors for Fracture in Patients with Dialysis-Dependent Renal Failure

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Men and women with dialysis-dependent renal failure have a high prevalence of fractures. To assess if bone mineral density (BMD) measurements, tests of muscle strength, and/or markers of bone turnover were associated with fractures we studied 36 men and 16 women, 50 years and older, who had been on hemodialysis for at least one year. We excluded patients with prior renal transplants and women taking hormone replacement therapy. A chart review gave data on medication use and most recent laboratory tests. We asked about risk factors for fracture. Subjects underwent BMD testing at the lumbar spine and total hip with a Lunar DPX-L densitometer. Tests of muscle strength included: timed up and go (TUG), six minute walk, functional reach, and grip strength. We inquired about low trauma fractures since starting dialysis and we obtained lateral and thoracic radiographs of the spine. Incident vertebral fractures were identified by morphometry. We took blood, pre-dialysis, for measurement of bone specific alkaline phosphatase (BSAP) and N-telopeptide (NTx). We used logistic regression to examine the relationships between fracture (incident vertebral, self-reported low trauma and/or both) and BMD, muscle strength tests, and bone turnover markers. Results are adjusted for age and weight and reported as Odds Ratios (OR) per standard deviation increase in the independent variable. The mean age of subjects in our study was 65.9 ± 8.9 years, the mean weight was 72.9 ± 15.2 kg and most (35 of 52 subjects) were Caucasian. The average duration of dialysis was 40.8 (20.4 – 55.2) months. The most common cause of renal failure was diabetes (16 subjects). The mean levels of calcium, phosphate, and alkaline phosphatase were normal. The mean level of PTH was 33.8 pmol/L (12.6 – 57.2). Just under half of the subjects (24) reported having at least one fall in the past year. There were no differences by gender or between subjects with and without fractures. Of the 52 subjects, 12 had incident vertebral fractures, 20 reported a low trauma fracture since starting dialysis, and 27 subjects had either a vertebral fracture or low trauma fracture. Mean lumbar spine BMD was 1.1 ± 0.2 g/cm^2 and total hip BMD was 0.8 ± 0.2 g/cm^2. Mean grip strength was 21.5 ± 9.3 kg, mean TUG was 19.7 ± 25.9 seconds, mean functional reach was 17.3 ± 10.9 cm, and mean six minute walk 108.8 ± 58.1 meters. The mean level of BSAP was 29.2 U/L (20.9 – 39.8) and the mean level of NTx was 57.8 nM BCE/L (52.4 – 62.2). There was no association between fractures, hip BMD, spine BMD, grip strength, or markers of bone turnover. However, fractures were associated with the six minute walk (OR = 0.1; p = 0.001), functional reach (OR = 0.3; p =0.005), and TUG (OR = 6.9; p= 0.004). Adjusting for spine or hip BMD did not change our results. Our findings suggest that simple, inexpensive tests of muscle strength may identify subjects with renal failure who have fractures.

P-20

A Novel Role for Megakaryocytes in the Inhibition of Osteoclast Formation

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A growing body of evidence suggests that megakaryocytes (MK) or their growth factors may play a role in skeletal homeostasis. We have previously identified a novel regulatory pathway that controls bone formation which is mediated by MK. The co-culture of MK with osteoblasts resulted in increased osteoblast proliferation *in vitro*, by a mechanism that required direct cell-to-cell contact. Here we examine a second, heretofore unrecognized, MK mediated pathway that regulates osteoclast (OC) generation.

We have begun examining the unique inhibitory effect of MK on OC formation. Spleen or bone marrow cells ($2x10^6$ cells/ml) from C57BL/6 mice, as a source of OC precursors, were cultured with M-CSF (30 ng/ml) and RANKL (50 ng/ml) to induce OC formation. MK were prepared by culturing fetal liver cells with thrombopoietin for 3-5 days which were then separated into MK and non-MK populations by a 1g BSA sedimentation gradient. The MK fraction was 95% pure, whereas the non-MK fraction was comprised of non-MK, MK precursors, and a small number of MK. MK were titrated into the spleen cells and OC were identified as tartrate resistant acid phosphatase positive giant cells with >3 nuclei. There was a significant, dose-dependent reduction (up to 15-fold) in OC formed when MK (0-0.5%) were added to the spleen cell cultures. To rule out the possibility that the inhibition was non-specific, we cultured spleen cells with either thymocytes or the non-MK fraction. We demonstrated that from 0-0.15%, thymocytes and non-MK did not inhibit OC development, whereas comparable numbers of MK inhibited OC formation by up to 5-fold. We determined that MK conditioned media (CM) inhibited OC formation ($ED_{50} \approx 2\%$) in a dose-dependent manner (up to 10-fold), indicating that a soluble factor(s) is responsible, at least in part, for the inhibition. Next, we examined MK CM for known inhibitors of OC formation, using ELISAs. IL-4 was undetectable in both MK and non-MK CM, whereas IL-10 levels were similar in MK and non-MK CM (21±9 and 21±5 pg/ml), respectively. Interestingly, we found a significant increase in the levels of OPG in MK CM compared to the non-MK CM (450±62 and 10±8 pg/ml), respectively, suggesting that OPG could be responsible for the reduction in OC formation in these cultures. Furthermore, the potential of MKs as a heretofore, unrecognized OPG source, implicates a potential physiologic role for MKs in regulating OPG levels. These studies indicate that MK may play a dual role in skeletal homeostasis by stimulating osteoblast proliferation and simultaneously inhibiting OC formation.

The authors have no conflict of interest.

P-21

High-Resolution Pet Can Image Bone Microdamage *In Vivo*

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Microdamage accumulation in bone is now considered a contributing cause for bone fragility in older women. Although quantitative evaluation of bone microdamage can be done *ex vivo* using basic fuchsin and fluorescien, there is still no method to detect and quantify microdamage *in vivo*. In the present project, a high resolution Positron Emission Tomography (PET) was applied to image bone microdamage *in vivo* in the rat model, and the damaged and non-damaged regions were compared with autoradiographs and histological measurements made on bone stained en bloc in basic fuchsin. The IndyPET II is a high-resolution small field-of-view PET scanner with an axial field of view of approximately 10 cm and dynamic imaging capabilities with Sodium Fluoride 18 F ([^{18}F] NaF) as the tracer. The rat axial ulnar loading model was used to produce bone microdamage in ulnar cortex using a force-controlled loading device. The fatigue loading was applied in a sine wave at 2 Hz with a peak force of 17 –20 N until a 10% displacement increase was reached, equivalent to 10% stiffness loss. In the first experiment, background PET scanning was performed before fatigue loading. Fatigue loading was applied on the right ulnae of the rats. Ten days later, the left ulnae were loaded in the same manner as the right ones. One day after the left ulnae were loaded (11 days after the right ulnae were loaded), the PET scanning was performed again in those animals. Background PET scanning showed there was no tracer accumulation in the midshaft of either right or left ulna. In contrast, accumulation of tracer was seen at the midshaft of both right and left ulnae after fatigue loading. The intensity of the right ulnae (loaded 10 days earlier) was higher than the left ulnae (loaded one day earlier). Comparing basic fuchsin stained serial histological sections microscopically with the PET images, we found a range of microdamage from the ulnar mid-diaphysis to about 3 mm distal of midshaft, consistent with the tracer accumulation area in the midshaft of ulna using PET scanning. Where there is no tracer accumulation in PET images, no microdamage was found under microscope. In the second experiment, right ulnae were loaded until 10% stiffness loss with certain cycles (fatigue loading cycles: 4000 – 8000 cycles). Left ulnae were loaded with half of the fatigue loading cycles (2000 – 4000 cycles) used on the right sides without stiffness loss. PET imaging was performed on the following day. No tracer accumulation on the left ulnae (normal loading side) was observed. Accumulation of tracer was only seen on the right ulnae (fatigue loading sides), which was consistent with autoradiographs and measurements under microscope. These data suggest that PET scanning is specific to the microdamage area. Areas of enhanced [^{18}F] NaF tracer uptake were observed at the midpoint of the forelimbs, coincident with the location of bone microdamages. This study suggests that high-resolution PET can image bone microdamage *in vivo*.

The authors have no conflict of interest.

P-22

Young-Elderly Differences in Periosteal Apposition, Trabecular Bone Mass, and Strength at the Femoral Neck and Trochanteric Region in Caucasian Women

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To discriminate young/elderly changes separately at the femoral neck (FN) and the trochanteric region (TR), and to compare young/elderly differences between FN and TR, 28 young (41±3 years) and 124 elderly (74±3 years) healthy Caucasian-American women underwent volumetric quantitative computed tomography (vQCT) at the hip. Integral bone mineral density and content (iBMD, iBMC) and trabecular bone mineral density and content (tBMD, tBMC) were measured. Geometric parameters obtained included cross-sectional area (CSA), and integral bone volume (iVOL). Structural indicators included compressive strength (Compstr) and bending strength index (BSI). F-test was used to compare mean differences between young and elderly after adjusting for height and body mass index in an ANOCOVA model. Mean values (SD) are tabulated below. F-test was also used to compare logarithmic young/elderly differences between FN and TR after adjusting for height and body mass index in an ANOCOVA model.

On comparing young and elderly separately at the FN and TR, only iVol at FN had no significant difference. On comparing young/elderly differences between the FN and TR, only tBMD and tBMC were significant ($p<001$). These results lead to two conclusions. First, periosteal apposition was more

	iBMD (g/cm^3)	tBMD (g/cm^3)	iBMC (g)	tBMC (g)	iVOL (cm^3)	CSA (cm^2)	Compstr (g^2/cm^4)	BSI (cm^3)
Femoral Neck Region (FN)								
Young	0.351 [a]	0.130 [a]	5.05 [a]	0.350 [a]	14.55 [ns]	8.73 [a]	1.07 [a]	0.51 [c]
	(0.04)	(0.03)	(0.87)	(0.15)	(2.75)	(1.16)	(0.22)	(0.09)
Elderly	0.272	0.086	4.03	0.171	14.96	9.80	0.73	0.45
	(0.04)	(0.03)	(0.97)	(0.20)	(3.49)	(1.65)	(0.23)	(0.11)
Trochanteric Region (TR)								
Young	0.313 [a]	0.130 [a]	19.38 [b]	2.72 [a]	62.24 [b]	23.88 [a]	2.36 [a]	
	(0.04)	(0.03)	(3.56)	(0.69)	(10.7)	(2.72)	(0.58)	
Elderly	0.247	0.086	16.47	2.08	66.63	27.91	1.74	
	(0.04)	(0.03)	(4.03)	(0.84)	(12.0)	(3.39)	(0.58)	

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$, [ns] $p>0.05$

evident at TR. Decreased trabecular bone mass was significantly higher at the FN. This inter-site difference may be due to regional differences in mechanical loading. Second, higher values in the elderly group for geometric parameters are insufficient to completely compensate for reduced BMD, resulting in lower values of estimated strength indices at the FN and TR.

The authors have no conflict of interest.

P-23

Temporary Brittle Bone Disease (TBBD): Analysis of an Additional 39 Cases Associated With Fetal Immobilization

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TBBD was described in 1993 by Paterson as an intrinsic bone disorder with unexplained fractures in the first year of life. In 1999, the analysis of 26 cases of TBBD suggested that fetal immobilization from intrauterine confinement (IUC) as a cause of TBBD (Calcif Tissue Int 64:137-143). Most child abuse protectionists believe that TBBD does not exist and is a ruse for child abuse. Herein is the analysis of an additional 39 cases of TBBD associated with fetal immobilization in which allegations of child abuse were made against the parents. Cases of unexplained fractures in infants were considered TBBD associated with fetal immobilization if they showed the following: (1) parents deny wrongdoing (2) no significant bruising or internal organ injury (3) no history of antecedent trauma (4) no radiographic or laboratory evidence of biochemical bone disease such as OI or rickets, and (5) history/clinical evidence of decreased fetal movement or IUC. The average age of presentation with the fractures was 9 weeks (1 sd = 5 weeks, range = 2-24 weeks). The average number of fractures was 12 (1 sd = 7 fractures, range = 3-26 fractures). The basis of the fetal immobilization was the following: (1) Cephalopelvic disproportion – 20 cases (2) Maternal use of drugs during pregnancy that cause fetal immobilization – 7 cases (3) Twins – 7 cases (4) Breech presentation – 2 cases, and (5) Large maternal fibroids – 1 case. Umbilical cord length was available in 7 cases, and in all 7 it was extremely short, a finding consistent with decreased fetal movement. The striking, early presentation of TBBD is consistent with a prenatal-onset etiology. The large number of fractures without bruising or other extraskeleltal injury suggests this is not child abuse.

The results confirm the previous association of TBBD with fetal immobilization, with the new observation that TBBD can also result from maternal use of medications that can cause decreased fetal movement. These overall findings support the concept that fetal bone loading is an important determinant of fetal bone strength, and that fetal immobilization is the primary risk factor for TBBD. This hypothesis is in accord with the contemporary model of bone physiology and bone strength as posited by Frost in the Utah paradigm (Anat Rec 262:398-419) and explains the transient nature of TBBD.

The author has no conflict of interest.

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Effect of Pamidronate Treatment on Bone Properties in a Murine Model of Osteogenesis Imperfecta

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Introduction. Osteogenesis Imperfecta (OI), the most common heritable bone disease, is caused by abnormal amounts of, or abnormally structured, Type I collagen. OI is characterized by skeletal fragility, multiple fractures, and in some cases blue sclera, hearing loss, short stature, and dentinogenesis imperfecta. Currently there is no cure for OI, however clinical trials involving bisphosphonates have shown ameliorating effects in children with OI. Bisphosphonates are analogs of pyrophosphate, known to inhibit osteoclastic bone resorption. We hypothesize that pamidronate treatment will inhibit ostoclastic resorption on the metaphyseal surfaces of growing OI patients, thereby creating larger diaphyses, and increasing the bone strength and stiffness. We tested this hypothesis in the *oim* mouse model.

Materials and methods. *Oim* mouse breeder pairs were obtained from Jackson Labs (Bar Harbor, ME). The pups were randomly assigned one of 3 treatments. Two pamidronate doses were studied: medium (10 mg/kg/mo) and low (5 mg/kg/mo). The third dose consisted of a phosphate buffered saline control. Treatments were started at age 4 weeks and ended at age 12 weeks, when the mice were sacrificed by CO asphyxiation. The left femurs were tested in 3-point bending such that the posterior surface was in compression, with a 4mm span and a 1mm/s loading rate. Using StatView (Cary, NC) software, the effects of gender, genotype, and treatment on stiffness, yield displacement, yield load, ultimate displacement, ultimate load, total energy, elastic energy, and plastic energy were determined.

Results. All of the results were analyzed using 3 factor (gender, genotype, and treatment) ANOVA and Fisher PLSD for pair-wise comparison. Based on the results, there was a significant effect of genotype ($p < 0.0001$) on all of the measured mechanical properties. Stiffness ($p = 0.0020$), yield displacement ($p = 0.0277$), and yield load ($p = 0.0130$) were affected by treatment. Due to the effect of gender on some of the variables, the males and females were analyzed separately. There was a significant increase in stiffness in the medium dose, heterozygous males (421 ± 78 versus 333 ± 85; $p = 0.0217$). The same was seen in the females (370 ± 75 versus 293 ± 69; $p = 0.0070$). The low and medium dose decreased the yield displacement in wildtype males ($p = 0.0009$ and $p = 0.0045$, respectively), however in the heterozygous, medium dose males there was a significant increase in yield displacement compared to low dose (0.123 ± 0.20 versus 0.088 ± 0.046; $p = 0.0479$). In the females there was a significant increase in yield displacement as compared to control and low dose, in the homozygous mutant, medium dose animals ($p = 0.0164$ and $p = 0.0154$, respectively). Finally, the medium dose produced a significant increase in the yield load in heterozygous females as compared to controls (23.4 ± 7.1 versus 18.1 ± 6.8; $p = 0.0543$). In the mutants the medium dose produced a significant increase as compared to control and low dose ($p = 0.0089$ and $p = 0.0129$, respectively). We are currently examining the effect of pamidronate on the cross-sectional geometry of the right femurs from these animals.

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Estrogen and an ERα Agonist Supress the Osteogenic Response to Exercise

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It is well established that exercise has the potential to increase bone density and strength. It has been proposed that estrogen enhances the osteogenic response to exercise, particularly through estrogen receptor α (ERα). The aim of this study was to determine the combined effect of exercise and estrogen or PPT (an ERα agonist) on bone formation. Forty-three young (8 wks) male Sprague Dawley rats were randomized to one of following groups: 1) high dose 17-α ethynylestradiol (High EE_2), 2) low dose 17-α ethynylestradiol + mechanical loading (Low EE_2), 3) propyl pyrazole triol + mechanical loading (PPT), 4) vehicle treated + mechanical loading (control) or 5) baseline control.

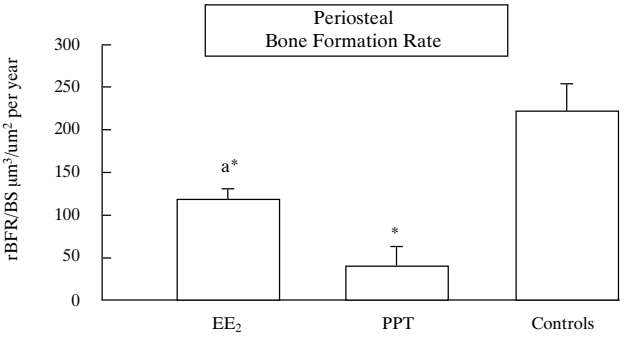

Figure 1. The effect of exercise and Low EE_2, PPT or vehicle (control) treatment on periosteal bone formation rate (loaded versus non-loaded ulna) after 1-2 weeks of mechanical loading in growing male rats.

Low EE_2 was selected to undergo mechanical loading because this dose closely reflects physiological levels of serum estrogen. An axial load was applied to the right ulna for 120 cycles, at 17N, 3 days/wk for 5 weeks. Calcein labels were given on days 5 and 12 of the experiment allowing histomorphometric measurement of bone formation rates of the midshaft ulnae. At the end of the 5 week intervention, High EE_2 and PPT showed reduced gains in body weight, longitudinal growth and cortical area ($p<0.05$). In contrast, High EE_2 also increased trabecular vBMD at the lumbar spine and distal femur ($p<0.05$) but PPT did not. Five weeks of mechanical loading increased cortical area and polar moment of inertia (Ip) in the control group, and only partially increased cortical area in the Low EE_2 and PPT group. Exercise increased BFR/BS of the loaded ulna, however the response was reduced in the Low EE_2 and PPT group versus controls (Figure 1). In conclusion, High EE_2 inhibits gains in longitudinal growth and cortical area, but also increases trabecular bone density. When combined with exercise, Low EE_2 and PPT decrease the osteogenic response to exercise by inhibiting periosteal bone formation.

L.K. Saxon has no conflict of interest. C.H. Turner has consultancies with Lilly and Merck.

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Early Gene Response to Low-Intensity Pulsed Ultrasound Exposure in Rat Bone Marrow Derived Osteoblastic Cells

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Despite its pronounced effects on fracture repair, the underlying mechanisms of low-intensity pulsed ultrasound (LIPUS) in stimulation of bone tissue regeneration remain unclear. The aim of current research was to quantify the response in osteoblastic cells to LIPUS at the gene level during early time points after the ultrasound application.

Rat (Sprague Dawely) bone marrow-derived stromal cells were exposed to LIPUS (operating frequency=1.5 MHz, intensity=30 mW/cm^2) for 20 minutes using sonic accelerated fracture–healing system device (THM-Model 2 A-Collimage type, Exogen Inc.). Sham controls were handled in the same way, except the ultrasound generator was not switched on. Cells were harvested at 0.5, 1, 3, 6, and 12 hr after the end of the treatment. Total RNA was extracted and reverse-transcribed to generate cDNA. Real-time PCR was carried out to quantify the expression of early response genes (c-jun, c-myc, Egr-1, TSC-22 and COX-2), and bone differentiation marker genes (alkaline phosphatase [ALP], Cbfa-1, osteonectin and osteopontin). All data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and presented as fold change relative to sham control.

Compared to sham controls, LIPUS treatment resulted in elevated transient expression of early response genes as well as bone differentiation marker genes osteonectin and osteopontin, at 3 hrs with return to baseline at 6 and 12 hrs. Expression of ALP and Cbfa-1 was not detected throughout the experienced period. The present results suggest that LIPUS may act by stimulating the expression of early response genes as well as extracellular matrix genes in cells of osteoblastic lineage.

The authors have no conflict of interest.

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Improved Imaging and Treatment of Rheumatoid Arthritis With Polymeric Delivery Systems - A Study With AIA Rat Model

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Introduction. Rheumatoid arthritis (RA) is the most common inflammatory arthritis. Current treatments for RA are usually classified as: (a) NSAIDs and (b) DMARDs. While most RA research has been invested in developing new drug targets, little attention has been paid to the pharmaceutical improvement of these treatments.

The hallmark of RA is severe inflammation and extensive angiogenesis with leaky vasculature. We hypothesize that injection of an anti-arthritis drug-polymer conjugate to subjects with severe arthritic inflammation would lead to specific accumulation of the drug conjugate in the arthritic joint. It may greatly enhance the therapeutic effect of many drugs.

To validate this hypothesis, different imaging techniques were used in this study to track the *in vivo* fate of different types of macromolecules on the adjuvant-induced arthritis (AIA) rat model. Successful application of the MR imaging in this study also suggests that the macomolecular contrast agent could improve the imaging of arthritic joints.

Results and discussion. In a preliminary visual examination of AIA rats injected with Evans blue (EB), we found that the hind ankle and paws (where the most severe inflammation normally occurs in this model) developed a pronounced blue color, especially in the ankle joint. This confirmed that there is a higher level of plasma albumin in the inflamed joints, which is probably due to their leaky vasculature. To further support this result, we applied magnetic resonance imaging (MRI) to track the macromolecules injected in AIA rats.

HPMA co-polymer contrast agent was synthesized and injected to both healthy and AIA rats. MR images of the animals were acquired at different time points post-injection of the contrast. While no enhanced MR signal could be observed in healthy rat hind paws, very significant MR signal enhancement was gradually observed in the inflamed hind paws of AIA rats, which may be attributed to the accumulation of HPMA co-polymer contrast agent (Figure 1). Histological examination of the joints showed a correlation between the severity of inflammation and the contrast enhanced MR image. When compared with the common contrast agent OMNISCAN, the macromolecular contrast agent provided greatly improved imaging of the inflamed joints.

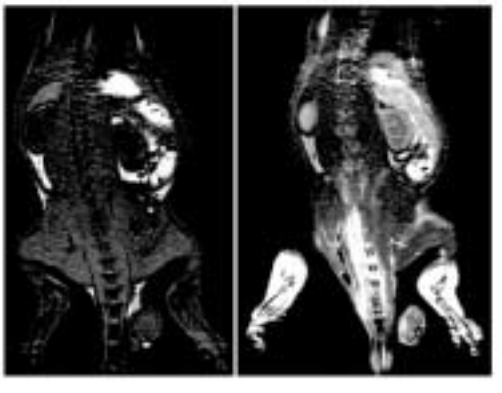

Figure 1. MR images. A. AIA rat before injection of macromolecular contrast agent; B. AIA rat 8 hours after injection of macromolecular contrast agent.

Conclusion. The accumulation of macromolecules in arthritic joints was visualized by MR imaging in the AIA rat model. This finding may provide a novel basis for the design and synthesis of macromolecular drug delivery systems for improved therapies for RA. Additionally, these novel polymeric agents may offer superior imaging contrast for inflammatory sites over traditional low molecular weight contrast agents.

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