reduced compared to wild type and Y2R KO also for shaft and distal BMC compared to Y2R KO. In the Y2R/ob cross, despite shaft BMC being reduced compared to Y2R KO, shaft cortical area and thickness and distal BMC were similar to wild type levels. Due to comparable changes in BMC and bone area, BMD did not differ between groups at either site. Endocortical osteoblast activity was similar in Y2R KO and Y2R/ob and greater than in wt and ob/ob. Increased cortical osteoblast activity was also observed 5 weeks after hypothalamic ablation of Y2R in adult mice compared to controls (0.23 ±0.01 vs 0.17 ±0.02, p<0.05).

	wt	Y2R KO	ob/ob	Y2R/ob
Body weight (g)	26.7 ± 4	29.8 ± 1	52.4 ± 4 a b	47.3 ± 5 a b
Shaft BMC (mg)	7.8 ± 1	8.8 ± 1a	6.7 ± 1 b	6.6 ± 1 b
Shaft Cortical Area (mm^2)	0.9 ± 0.03	1.1 ± 0.04 a	0.7 ± 0.06 a b	0.9 ± 0.02 b
Shaft Cortical Thickness (μm)	191 ± 6	221 ± 7a	151 ± 13 a b	175 ± 7 b
Distal BMC (mg)	10 ± 1	12 ± 1a	9 ± 1 b	11 ± 1
Distal Endocortical MAR (μm/d)	0.21 ± 0.03	0.31 ± 0.03 a	0.24 ±0.01b	0.32 ±0.01a c

a p< 0.05 vs wt, b p< 0.05 vs Y2R KO, c p<0.05 ob/ob vs Y2R/ob.
Therefore, the Y2 receptor pathway represents an adult-inducible stimulator of cortical as well as cancellous bone formation. Y2R deficiency increased BMC and osteoblast activity in Y2R/ob mice, in part counteracting the leptin-deficient reduction in cortical bone. These data clearly delineate opposing effects of Y2R and leptin pathways on cortical bone.

Disclosures: ***P.A. Baldock****, None.*

M423

Low-Dose Strontium Increased the Formation of New Lamellar Bone at the Periosteal Surface, with Normal Mechanical Competence. H. Oxlund, J. S. Thomsen*, T. T. Andreassen. Dept of Connective Tissue Biology, University of Aarhus, Inst of Anatomy, Aarhus, Denmark.

Low-dose strontium (Sr) has been shown to possess bone anabolic properties. In the present study the effects of Sr chloride on tibia cortical bone of intact and ovariectomised (OVX) rats were studied. Seventy-five Wistar female rats, 6 months old, were allocated to five groups: 1. Baseline control, 2. Sham operated group, 3. OVX, 4. Sr, 5. OVX + Sr group. Sr chloride, 4 mmol/day, was given in the drinking water for 140 days. The rats were injected with Alizarin Red 7 days after start of the experiment, and calcein 14 days and tetracycline 4 days before killing by exsanguination. The mechanical properties of the tibia diaphysis were studied by a 3-point-bending test. No differences were found in the stress, stiffness and deflection parameters between the groups. Mid-diaphyseal transverse sections were cut and dynamic histomorphometry was performed on the basis of the fluorochrome labels. The periosteal bone formation rate (BFR) was increased (P=0.01) in the Sr group (0.74 ± 0.13, mean ± SEM) compared with the control group (0.53 ± 0.09 μm^3 x 10^3/day). The periosteal BFR was increased (P=0.001) in the OVX group (1.74 ± 0.20 m^3 x 10^3/day) compared with the sham group (0.53 ± 0.09 μm^3 x 10^3/day). Likewise, the periosteal BFR was increased (P=0.02) in the OVX + Sr group (2.47 ± 0.21 m^3 x 10^3/day) compared with the OVX group. Sr did not increase the mid-diaphyseal endocortical bone formation. In conclusion, low-dose Sr given perorally increased the periosteal bone formation, but did not stimulate endocortical bone formation. The new cortical bone exhibited a normal lamellar structure, and the mechanical competence of the cortical bone seemed to be preserved.

Disclosures: ***H. Oxlund****, None.*

M424

Strontium Ranelate Effects on Osteoblasts: A Potential Role of Endogenous Prostaglandins. S. Choudhary[1], C. Alander*[1], P. Halbout*[2], L. Raisz[1], C. Pilbeam[1]. [1]UConn Center for Osteoporosis, University of Connecticut Health Center, Farmington, CT, USA, [2]Groupe Servier, Courbevoie Cedex, France.

Strontium ranelate is a new anti-osteoporotic treatment with a dual effect on bone formation and bone resorption. Previous studies have shown that strontium can increase prostaglandin (PG) production in osteoblasts by increasing the expression of the inducible cyclooxygenase (COX)-2. The present study was designed to assess the role of endogenous PG production in the anabolic response to strontium ranelate. Bone marrow stromal cells (MSC) from 7 to 8 wk old mice and primary calvarial osteoblast (POB) cells from neonatal mice were cultured under differentiating conditions for 10-14 d. Strontium was tested at 1 and 3 mM, mixed with ranelic acid at 10 and 30 μM, respectively, in order to reflect the ratio found in the human plasma of patients after dosing. Measurements included bone alkaline phosphatase (ALP) activity, normalized to total protein, and alizarin red staining for mineralization. In MSC cultures, continuous treatment with strontium ranelate (3 mM Sr^{2+}) for 10 and 14 d significantly increased ALP activity by 92% (p<0.01) and 63% (p<0.01), respectively, as well as mineralization. In the presence of NS-398 (0.1 μM), a selective inhibitor of COX-2 activity, there was no significant increase in ALP activity with strontium ranelate in MSC cultures. When MSC cultures were treated with strontium ranelate for varying intervals (first 3, first 7 or last 7 d) during 14 d of culture, treatment for the first 7 d with strontium ranelate (1 and 3 mM Sr^{2+}) increased ALP activity by 69% (p<0.05) and 110% (p<0.01), respectively. Cumulative PGE_2 levels in MSC cultures measured after a 7 d treatment with strontium ranelate (1 and 3 mM Sr^{2+}) were respectively elevated 2.4-fold (p<0.01) and 11.0-fold (p<0.01). NS-398 blocked all significant increases in PGE_2 production. In POB cells cultured for 14 d, treatment with strontium ranelate (3 mM Sr^{2+}) for 0-3, 0-7, or 0-14 d of culture significantly increased ALP activity by 186% (p<0.01), 95% (p<0.05) and 92% (p<0.05), respectively. NS-398 also inhibited the increase in ALP activity in this system (preliminary data). To summarize, strontium ranelate stimulated the anabolic response of MSC and POB as shown by the increased ALP activity and mineralization in both models, which was coupled to an endogenous PG production. We conclude that the ability of strontium ranelate to stimulate endogenous PG production represents one attractive hypothesis to support the beneficial effect of strontium ranelate on bone formation.

Disclosures: ***S. Choudhary****, Servier, Courbevoie Cedex, France 2.*

M425

A Peptide Derived from Chemokine CXCL7 Stimulates Bone Formation and Increases BMD in OVX Rats. S. A. F. Peel, G. O. Ramirex-Yañez*, D. Squires*, L. T. Malek. Osteopharm Inc, Oakville, ON, Canada.

It was previously reported that a fragment of the human CXC chemokine neutrophil activating peptide-2 (NAP-2, CXCL7) stimulated bone mineral apposition in rats (Tam 2004, US Pat.No. 6,693,081). The purpose of this study was to determine whether the modified 8 amino acid peptide Ac-TTSGIHPK-amide derived from CXCL7 (OSB) would increase bone formation in ovariectomized (OVX) rats. Virgin SD rats were OVX or sham operated at 23 weeks of age and placed into groups (N=12) on the basis of DEXA measurements of bone and body composition. Eight weeks after surgery the rats in 3 groups received injections for 5 days a week of PBS (sham and OVX groups) or OSB (300 nmoles/kg) in PBS. Changes in bone were monitored monthly by DEXA (regional and whole body) and bimonthly by pQCT. After 26 weeks of treatment the rats were sacrificed, serum samples were collected for biomarker analysis and bones were collected for terminal analysis by pQCT or fixed for histology. Rats treated with OSB had significantly increased whole body BMC and BMD compared to OVX control by DEXA (p<0.03). Terminal analysis of the lumbar spine (L3) by pQCT demonstrated that OSB significantly increased total BMC and BMD (p<0.02) and cortical area and BMC (p<0.006) relative to the OVX control. For tibia and femur, the OSB treated group had significantly higher trabecular BMC and BMD (p<0.03) at metaphyseal sites, and higher cortical area and BMC (p<0.04) at diaphyseal sites. The OSB group had significantly higher total BMC and BMD (p<0.03) at the femoral neck than the OVX control. Analysis of blood samples for markers of collagen degradation showed no differences between OVX and OSB treated animals. Samples analyzed for osteocalcin showed an increase with OSB treatment that neared significance (p = 0.09). Based on these results we conclude that OSB stimulates bone formation and increases BMD in OVX rats. These results suggest that OSB may be efficacious as bone anabolic drugs for the treatment of osteoporosis.

Disclosures: ***S.A.F. Peel****, Osteopharm Inc 3.*

M426

Novel Peptides Stimulate Bone Formation *In Vitro* and *In Vivo*. G. O. Ramirez-Yañez*, S. A. F. Peel, D. Squires*, L. T. Malek. Osteopharm Inc, Oakville, ON, Canada.

The novel peptide OSA was derived from the sequence of a cDNA clone in a fetal human liver library. A series of structurally related 10 amino acid peptides were developed based on the active region of the original peptide (Tam 2004 patent WO2004/050701). The aim of the present study was to test the efficacy of these novel peptides in bone formation. Neonatal rat calvaria (3-5 days old) were either treated with OSA peptides (10^{-9} M), or IGF-1 (50 ng/ml) or left untreated. Mineralization and matrix synthesis were evaluated based on incorporation of ^{45}Ca and ^{3}H-proline. Calvaria treated with OSA peptides had a significantly higher incorporation of ^{45}Ca and ^{3}H-proline compared with the untreated control (p<0.05), similar to the IGF-1-treated calvaria. Virgin SD rats were OVX or sham operated at 23 weeks of age and placed into groups (N=12) on the basis of DEXA measurements of bone and body composition. Eight weeks after surgery the rats received injections for 5 days a week of PBS (sham and OVX groups) or 1 of 3 OSA peptides (117M, 153M or 155M; 300 nmoles/kg) in PBS. Changes in bone were monitored monthly by DEXA (regional and whole body) and bimonthly by pQCT. After 26 weeks of treatment the rats were sacrificed, serum samples were collected for biomarker analysis and bones were collected for terminal analysis by pQCT or fixed for histology. OSA treated rats showed significant increases in whole body BMC and BMD after 3 and 6 months of treatment as compared with the OVX control (p<0.05). Terminal pQCT analysis indicated that rats treated with one OSA peptide analog (155M) had significantly higher trabecular BMD at lumbar vertebrae (p<0.05) and at metaphyseal sites in the tibia (p<0.007) and femur (p<0.03) than the OVX rats treated with vehicle. Total BMD of the femoral neck was significantly higher (p<0.04) in the group treated with OSA 155M than in the OVX control. Serum samples tested for markers of bone resorption (RatLaps) or formation (osteocalcin) showed no significant differences between the groups, possibly due to the large variations observed within each group. These results demonstrate that OSA peptides stimulate bone formation *in vitro* and *in vivo*. Further these peptides may be useful in the treatment of osteoporosis.

Disclosures: ***G.O. Ramirez-Yañez****, Osteopharm Inc 3.*

M427

Characterization of Osteogenic Oxysterols and their Molecular Mechanism(s) of Action. J. A. Richardson*[1], C. M. Amantea*[1], K. Nguyen*[2], M. E. Jung*[2], T. J. Hahn[1], F. Parhami[1]. [1]Medicine, UCLA, Los Angeles, CA, USA, [2]Chemistry, UCLA, Los Angeles, CA, USA.

Identification of anabolic agents that enhance bone formation is critical for the better management of bone fractures and osteoporosis. We previously reported that specific oxysterol compounds, products of cholesterol oxidation, used in combination have potent osteogenic properties when administered to osteoprogenitor cells *in vitro* and to neonatal mouse calvarial organ cultures *ex vivo*. The oxysterol combinations with osteogenic

activity consisted of 22(*R*)- or 22(*S*)-hydroxycholesterol with 20(*S*)-hydroxycholesterol (RS and SS, respectively). Recently we found that the oxysterol 20*S*, when used alone at doses of 5-15 μM in cultures of marrow stromal cells, M2-10B4 (M2), induced the formation of mature osteoblastic cells demonstrated by the induction of alkaline phosphatase (ALP) activity, Runx2 DNA binding and protein expression, osteocalcin (OCN) mRNA expression, and mineralization. Pre-treatment or co-treatment of cells with 22*S* or 22*R* oxysterols greatly enhanced the osteogenic effects of 20*S* suggesting that 22*S* and 22*R* prime the osteoprogenitor cells for better responsiveness to 20*S*. We have identified other oxysterols with osteogenic properties when used alone or in combination with 22*R* or 22*S*. These newly identified oxysterols are 5-cholesten-3β, 20α-diol 3-acetate, 24(*S*),25-epoxycholesterol, 24(*S*)-hydroxycholesterol (also known as cerebrosterol), and 26-hydroxycholesterol, all of which induced the markers of osteogenic differentiation in M2 cells. In contrast, 4β-hydroxycholesterol and 7α-hydroxycholesterol did not have osteogenic properties suggesting that the carbon side chain of the sterols and the position of the hydroxyl groups are important characteristics of the osteogenic sterols. Structurally similar molecules to oxysterols, estren, estrone, and β-estradiol, which do not have the carbon side chain and have differences in the position and number of their double bonds, did not have any osteogenic properties. Pretreatment of M2 cells with the hedgehog signaling inhibitor, cyclopamine (1-10 μM), significantly inhibited SS-induced ALP activity, Runx2 protein and OCN mRNA expression, and mineralization. In addition, pretreatment of cells with the inhibitor of Wnt signaling, DKK-1 (1 μg/ml), significantly inhibited SS-induced ALP activity, OCN expression and mineralization but not Runx2 protein expression. These results suggest that the oxysterol-induced osteogenic differentiation of cells is mediated through hedgehog- and Wnt-dependent mechanisms. Oxysterols form a new class of osteoinductive agents that may be useful in the enhancement of local and/or systemic bone formation.

Disclosures: ***F. Parhami****, None.*

M428

Systemic Administration of Thyroid Stimulating Hormone (TSH) Prevents and Restores Bone Loss in Rats Following Ovariectomy. K. T. Sampath[1], P. Simic*[2], R. Sendak[1], N. Draca*[2], S. Schiavi*[1], J. McPherson*[1], S. Vukicevic[2]. [1]Genzyme Corporation, Framingham, MA, USA, [2]Laboratory for Mineralized Tissues, Zagreb Medical School, Zagreb, Croatia.

Thyroid stimulating hormone (TSH) affects bone remodeling as demonstrated by reduced bone mass in TSH receptor knockout mice (Abe E, et al Cell, 115: 130-140, 2003) and in post-menopausal euthyroid patients following single administration of human TSH (Mazziotti G, et al., JBMR 20: 480-486, 2005). In the present study, we examined whether the systemic administration of TSH could prevent and restore bone loss in an osteoporosis animal model. Female SD rats were ovariectomized (OVX) at 6 months and the TSH therapy was started immediately (prevention mode), and 3 months following OVX (restoration mode). Animals were divided into six groups (12 rats/group): (1) Sham, (2) Ovariectomized (OVX), (3) OVX + TSH (low dose), (4) OVX + TSH (medium dose), (5) OVX + TSH (high dose) and (6) OVX + 17-ß estradiol. Recombinant human TSH (Thyrogen®) (0.7, 7.0 and 70 μg/rat) or rat pituitary-derived native TSH (0.01, 0.1, 0.3, 1, 3 and 10 μg per rat) were administered i.p., three times per week. The whole body, lumbar spine and hind limbs bone mineral density (BMD) were measured at 2 week intervals in vivo, following 8-16 weeks of therapy. Results show that TSH significantly increased BMD at all measured sites both in the prevention and restoration mode. Doses of 0.1 and 0.3 μg/rat of TSH significantly increased the BMD of the hind limbs whereas the dose of 0.01 μg/rat of TSH did not have an effect on BMD. High doses of human and rat TSH had lesser effect on BMD. Ex vivo BMD values of excised femur, tibia and lumbar spine confirmed the in vivo measurements. Compared to OVX, OVX plus TSH increased cortical thickness of femurs by 14.5% as measured by pQCT, and increased BV/TV by 163%, trabecular thickness by 21.1% and trabecular number by 125% as determined by μCT analyses. Serum biochemical analyses suggest that TSH suppresses the ovariectomy-induced bone turnover by decreasing osteocalcin and C-telopeptide levels to sham values. Importantly, low TSH doses had no effect on serum T3 and T4 values, suggesting TSH at these levels may have a direct effect on bone without affecting the thyroid axis. In vitro studies demonstrate that TSH inhibits RANKL induced-osteoclast formation, osteoclast-mediated resorption, promotes osteoblast differentiation and provides protection against apoptosis in osteoblast enriched cultures. These results demonstrate for the first time that systemically administered TSH can suppress high bone turnover following ovariectomy and exert both anti-resorptive and anabolic effects on bone remodeling, resulting in both prevention and restoration of bone loss in aged OVX rats.

Disclosures: ***S. Vukicevic****, None.*

M429

Characterization of Bone and Cartilage Stimulating Peptide Derived from the Collagen Type I Alpha I Chain. D. Sindrey, E. Plawinski*, R. R. Simon*, J. Auluck*, J. Terryberry*. Millenium Biologix Corp, Mississauga, ON, Canada.

We have previously reported on the bone stimulating effects of Bone and Cartilage Stimulating Peptides for the treatment of bone trauma and other bone diseases like osteoporosis. Originally extracted from bovine bone as BCSS (Bone Cell Stimulating Substance, Clark I. et al 1988) we have demonstrated through extensive purification and structure function studies, one of the active components as being a fragment of procollagen 1A1 1156-1174. Purification of BCSS bovine extracts by RP-LC, ion exchange and size exclusion chromatography, identified an active collagen fragment that was confirmed by identification from digests of collagen Type I. Several overlapping candidate peptides ranging from a 4mer to 16mer were synthesized with different N and C terminal truncations and/or additions to optimize biological activity in a rat tibia model of bone formation. No ectopic bone formation was noted in any of the animals. Histologically, the change in bone size was a result of periosteal bone proliferation and was peptide related. A noticeable intra-medullar bone proliferation was also seen in rats treated with the most active peptides and was considered peptide-related as it was not observed in any rats treated with carrier or Saline. Typical increases in local BMD were 10-12% above controls with peptides as small 450 kD. In vitro testing demonstrated two basic cellular responses in rat calvaria cells of increased proliferation and differentiation as seen by the 3 fold increase in WST assay; a 36% increase in the number of bone nodules and a 71% increase in bone area formed in calvaria cultures. These data corresponded well with observations in vivo of a rapidly proliferating periosteum and its subsequent mineralization. Bioactivity was sequence dependent and resided in two novel and complimentary sequence motifs in the BCSP-1 molecule. The synthesis of these molecules by solid phase peptide chemistry and their subsequent testing in a rat tibia model of bone formation validated the collagen origin of the original BCSS bovine extracted material. The actions of BCSP peptides on cell interactions in osteogenesis demonstrated specificity toward bone cells present in the periosteum as noted by the absence of heterotopic or ectopic bone formation. The rapid onset of the stimulatory effect and the absences of ectopic bone formation make the BCSP peptides a potent site-specific candidate for the treatment of bone fracture and trauma. Osteoinductive BCSP is currently in development for the treatment of local bone repair and systemic treatment of various skeletal diseases.

Disclosures: ***D. Sindrey****, Millenium Biologix 1, 3.*

M430

Retrovirus-Mediated Gene Transfer of RANK-Fc Ameliorates Bone Resorption in Ovariectomized Mice. D. Kim*[1], S. Her*[2], S. Cho*[2], S. Kim[2], S. Kim*[2], C. S. Shin[2]. [1]Department of Internal Medicine, Dankook University College of Medicine, Cheon-An, Republic of Korea, [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

Postmenopausal osteoporosis is characterized by increased bone resorption due to estrogen deficiency. RANK-Fc, a fusion protein that specifically blocks RANKL binding to RANK, has been known to be efficient and well-tolerated in animal models of osteoporosis. Here we show that cell-based gene therapy with RANK-Fc effectively prevented bone loss in ovariectomized (OVX) mice. Twenty-four young adult female C57B mice were used and repeated intraperitoneal injection of mesenchymal stem cells(MSCs) transduced with retrovirus was performed as follows: (1) Sham-operated mice (SHAM, n=6) (2) OVX mice treated with PBS (OVX-P, n=6) (3) OVX mice injected with MSCs cells transduced with control retrovirus (OVX-GFP, n=6) (4) OVX mice injected with MSCs transduced with RANK-Fc (OVX-RANK-Fc, n=6). Cellular expression of RANK-Fc was confirmed by Western blot analysis of cell lysates and conditioned medium, and also by ELISA for the mice serum. Measurement of BMD by dual energy x-ray absorptiometry (PIXImus) revealed that OVX-RANK-Fc group showed significantly higher BMD ($p<0.05$) than either the OVX-P group or OVX-GFP group after 8 weeks. The expression of GFP, which is co-expressed with RANK-Fc was observed in the liver, spleen, and intra-abdominal fat of mice but not in femur or freshly isolated bone marrow. Our results suggest that expression of RANK-Fc by genetically modified MSCs may be a feasible option for ameliorating the OVX-induced bone loss.

Disclosures: ***D. Kim****, None.*

M431

Lumbar Spinal Mobility and Back Extensor Strength Are Important Factors for Quality of Life in Patients with Osteoporosis. N. Miyakoshi, M. Hongo, S. Maekawa, Y. Ishikawa*, Y. Shimada*, E. Itoi*. Orthopedic Surgery, Akita University School of Medicine, Akita, Japan.

We have recently demonstrated that quality of life (QOL) in patients with osteoporosis is affected by the total spinal mobility and that the back extensor strength is the most significant contributor to the total spinal mobility. However, how much thoracic and lumbar spinal mobilities affect QOL has not been clarified. In this study, we evaluated the relation between QOL and thoracic and lumbar spinal mobilities and their related factors in patients with osteoporosis. A total of 174 postmenopausal women with osteoporosis aged over 50 years (mean, 68 years) were included in this study. Their QOL was evaluated using the Japanese Osteoporosis QOL Questionnaire (JOQOL) proposed by the Japanese Society for Bone and Mineral Research. JOQOL contains six domains with higher scores indicating higher levels of QOL. Bone mineral density (BMD) of the lumbar spine, proximal femur, and whole body were measured with dual-energy X-ray absorptiometry. The kyphosis angle and range of motion (ROM) of thoracic and lumbar spine were measured in the upright position and at maximum flexion/extension with a computer-assisted device (SpinalMouse®). The number of vertebral fractures was evaluated with lateral radiographs of the spine. Bilateral grip strengths and isometric back extensor strength were evaluated with dynamometers. JOQOL showed significant correlation ($p<0.05$) with age ($r=-0.303$), back extensor strength ($r=0.455$), grip strengths of dominant and non-dominant hands ($r=0.273$ and $r=0.255$, respectively), number of vertebral fractures ($r=-0.282$), BMDs of proximal femur and whole body ($r=0.200$ and $r=0.157$, respectively), lumbar kyphosis angle ($r=-0.296$), and lumbar spinal ROM ($r=0.345$). Among these factors, the multiple regression analysis revealed that the back extensor strength and lumbar spinal ROM were the significant contributors to the JOQOL. We conclude that back extensor strength and lumbar spinal mobility (but not thoracic mobility) are the important factors for QOL in patients with postmenopausal osteoporosis.

Disclosures: ***N. Miyakoshi****, None.*