IMAGING, HISTOMORPHOMETRY AND BIOMECHANICS OF BONE DISORDERS

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Preclinical Services | Charles River

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DISCLOSURES

Names and the nature of the financial interest or affiliation in commercial firms that I and our immediate families have with any commercial organization that may have a direct or indirect interest in the subject matter of my presentation:

Charles River Laboratories employee

CASE STUDIES

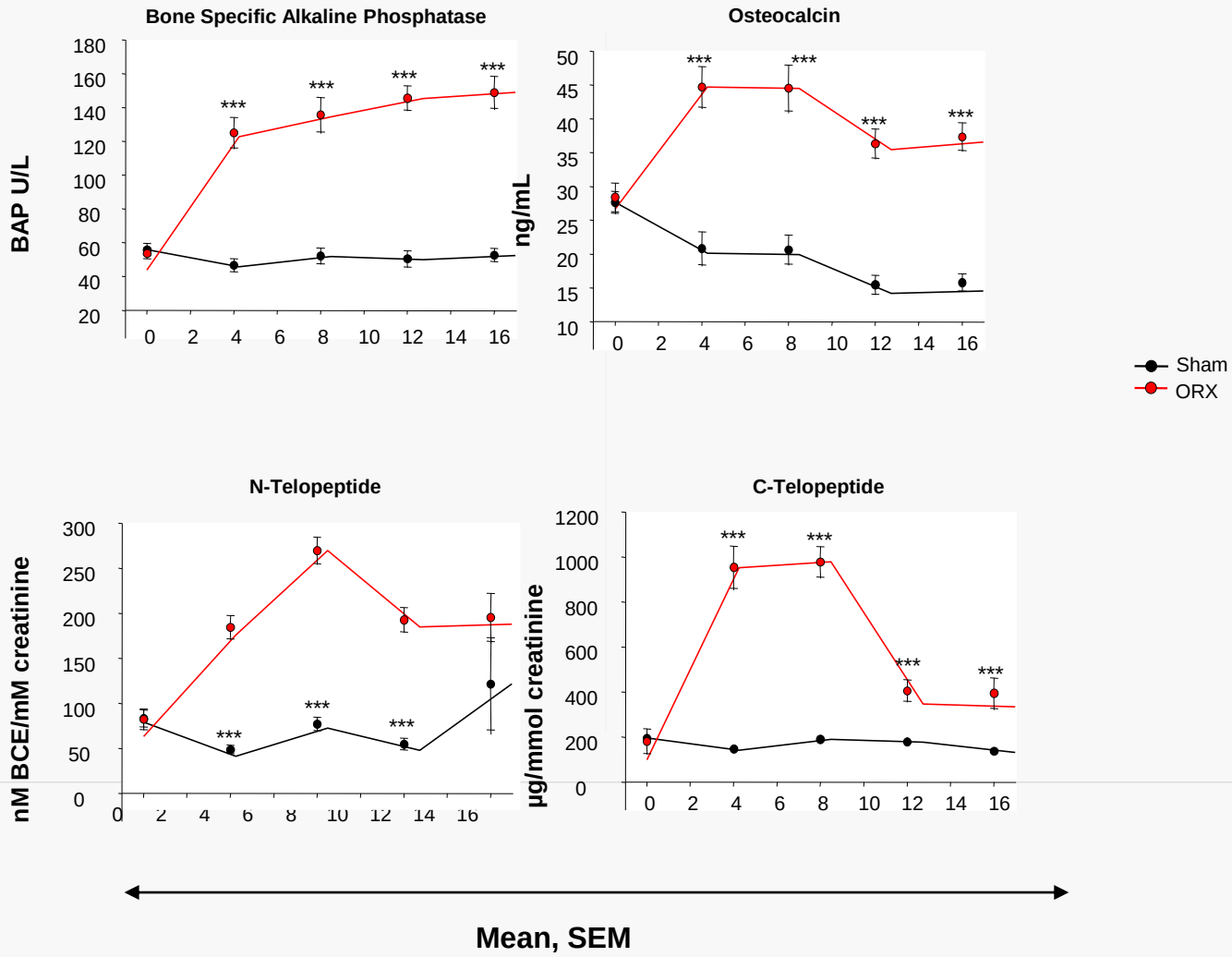
- Orchidectomized and Ovariectomized cynomolgus monkeys of Osteoporosis
- Anti-resorptive agents: Bisphosphonate (Ibandronate)
- Anabolic agents
  - PTH(1-84)
  - Romozozumab
- Juvenile Toxicology
- ePPN study: Denosumab
- Carcogenesis study:
  - PTH(1-34)
  - Romozozumab
- PPAR ɣ agonist

OSTEOPOROSIS MODEL

THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

BIOCHEMICAL MARKERS OF BONE TURNOVER



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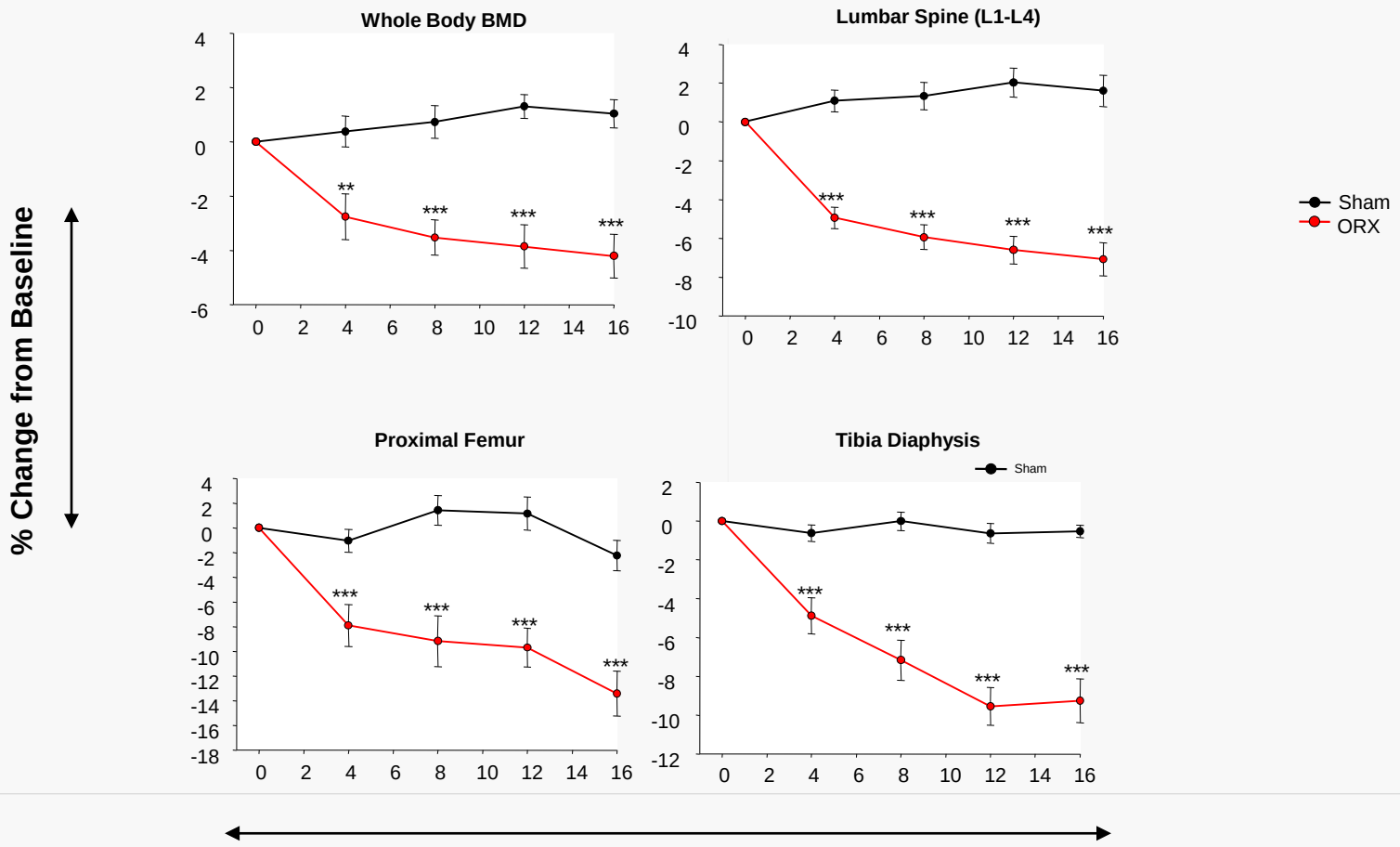
THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

BONE DENSITOMETRY BY DXA



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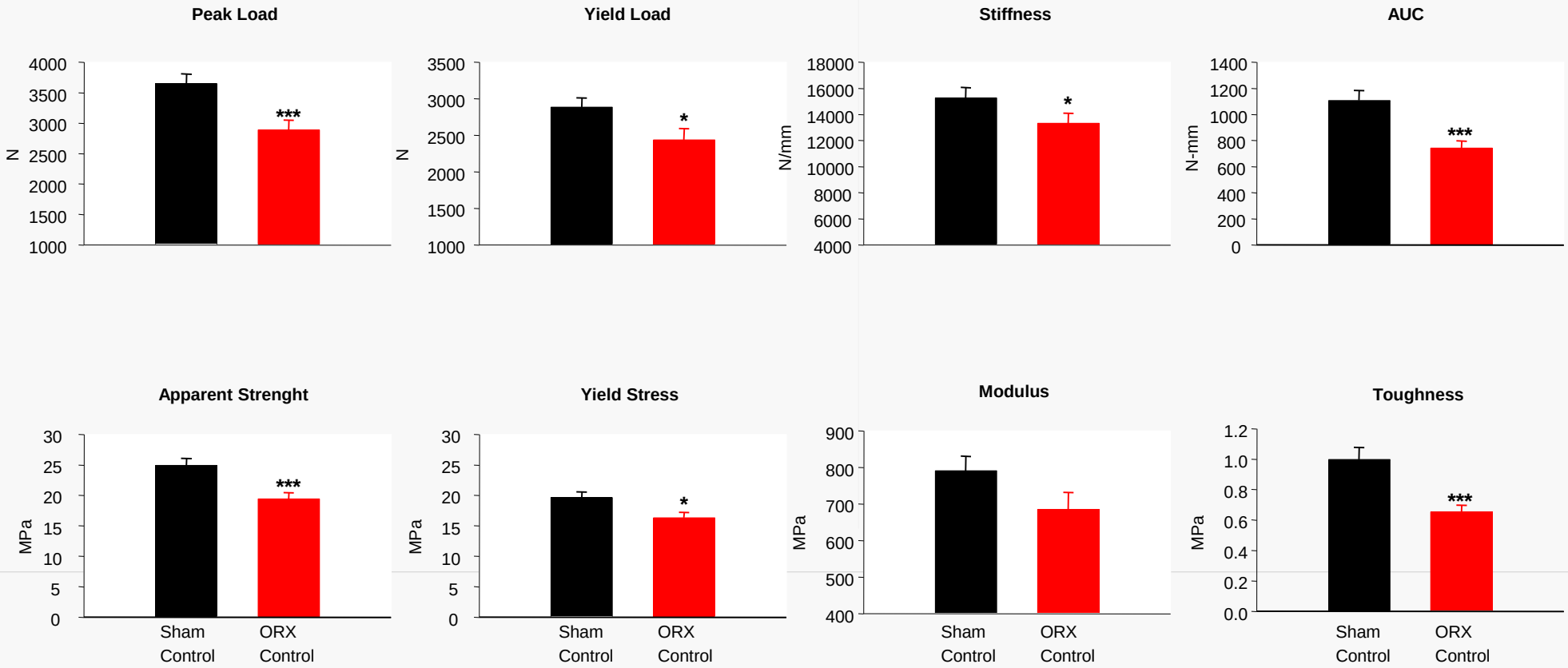
THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

Biomechanical Parameters

Vertebral Body - Compression
Mean (SEM)



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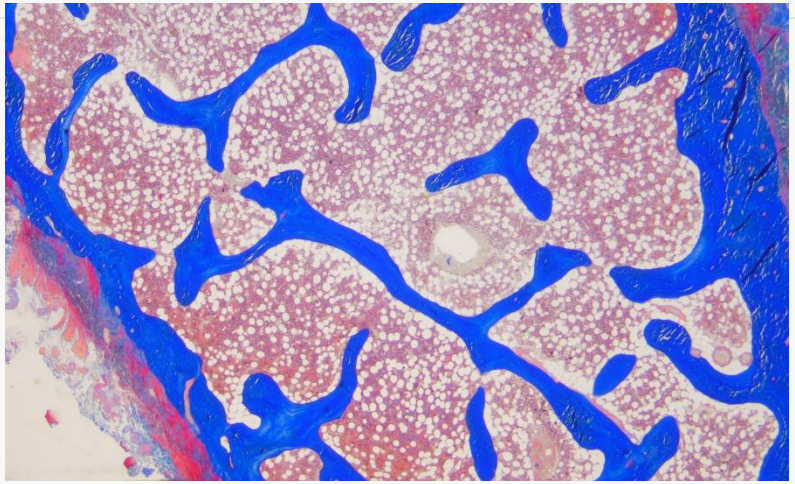
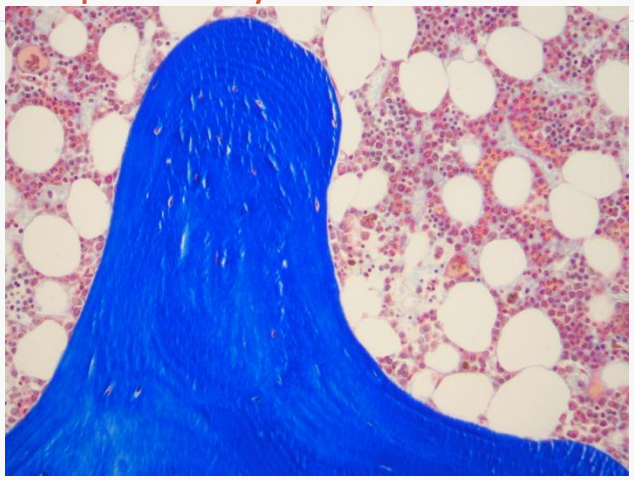
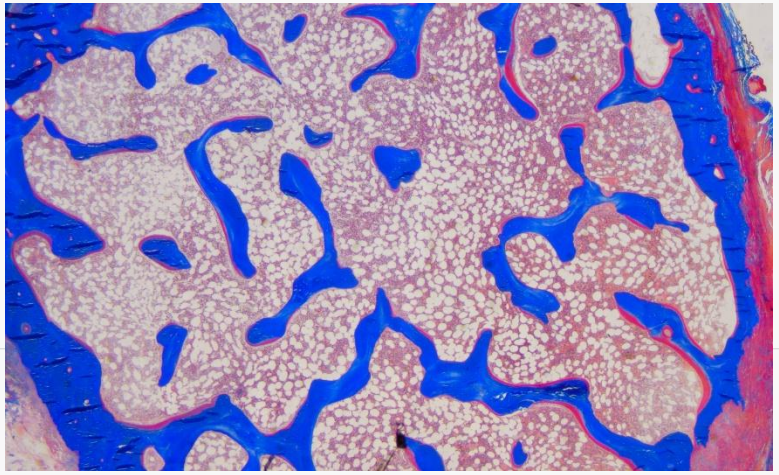
THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

Static Histomorphometry

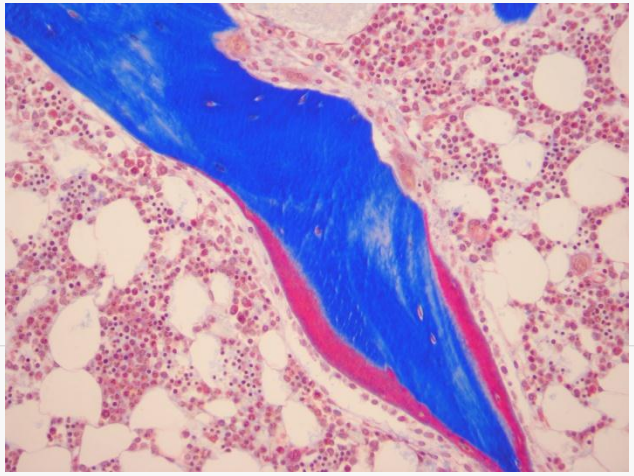


Iliac biopsy: Sham control animal. Goldner's Trichrome. 2X.





Iliac biopsy: ORX control animal six months after castration. Note the high OS/BS compared to the Sham animal. Goldner's Trichrome. 2X.



Iliac biopsy: ORX control animal six months after castration. Note the high Ob.S/BS, Oc.S/BS and ES/Bs compared to the Sham animal. Goldner's Trichrome. 20X.

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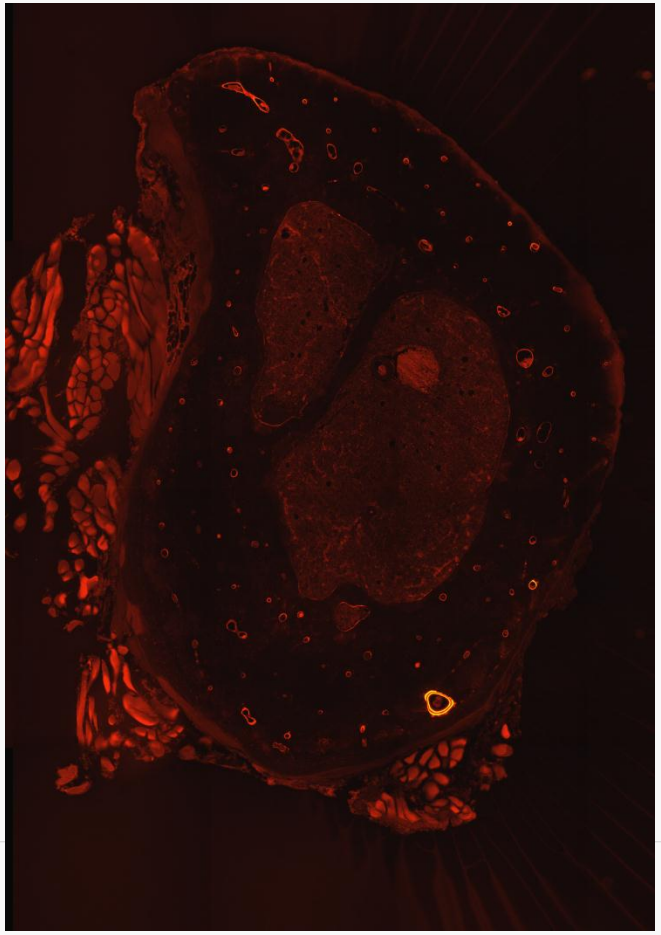
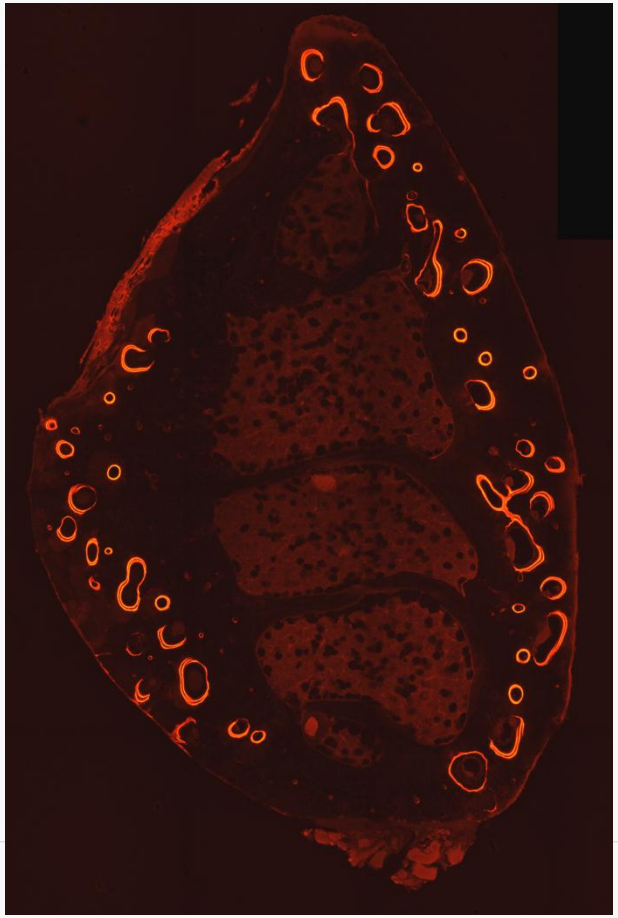
THE NON-HUMAN PRIMATE MODEL OF MALE OSTEOPOROSIS

Dynamic Histomorphometry



Rib biopsy: Sham control. Unstained section. Double alizarin complexon labels. 2X.



Rib biopsy: ORX control animal six months after castration. Note the increased porosity and relative high H.L.Pm/H.Pm compared to the Sham animal. Unstained section. Double alizarin complexon labels. 2X.

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ANTI-RESORPTIVE AGENT

Intermittent intravenous administration of the bisphosphonate ibandronate prevents bone loss and maintains bone strength and quality in ovariectomized cynomolgus monkeys

BIOCHEMICAL MARKERS IN PRECLINICAL RESEARCH

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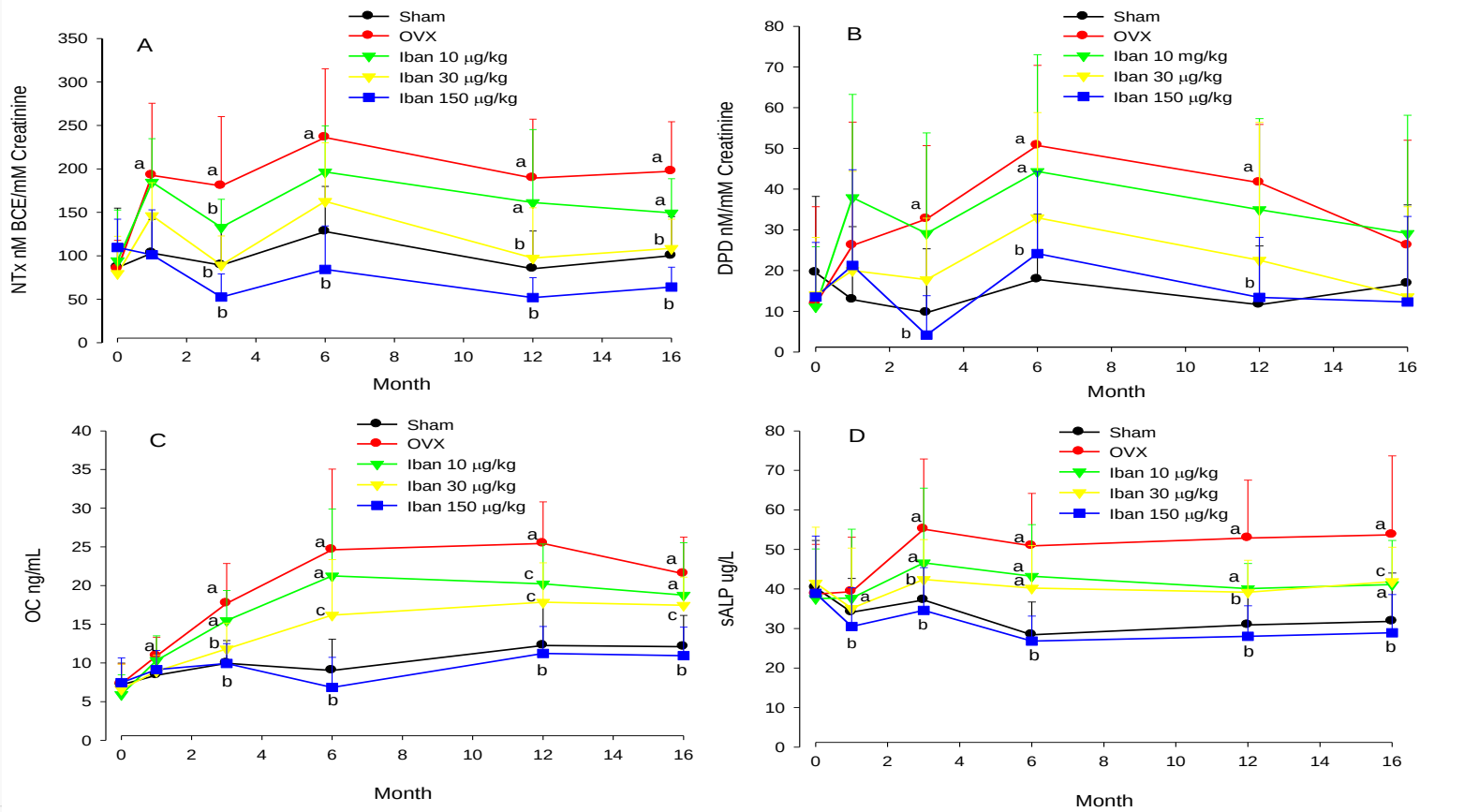

Effect of OVX and treatment with ibandronate (Iban) on biochemical markers of bone turnover in old cynomolgus monkeys: **(A) u-NTX, (B) u-DPD, (C) OC, (D) bone ALP.** Mean % of baseline + SEM. Significances relative to change from baseline ($p<0.05$): different from sham control, a; different from OVX control, b; different from sham and OVX controls, c. (n = 10-15)

Modified from Smith S.Y., Bone 2003;32:45-55.

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IN VIVO DXA IN PRECLINICAL RESEARCH

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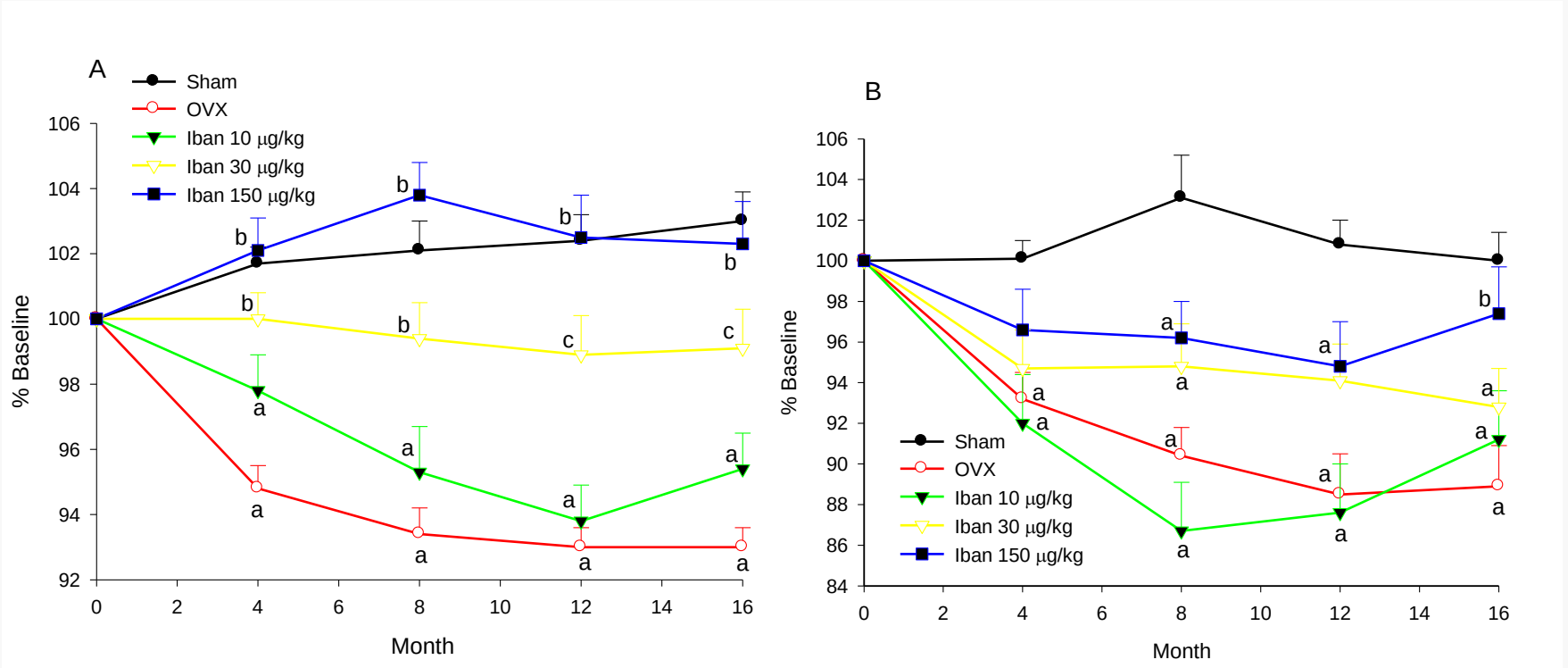

Effect of OVX and treatment with ibandronate (Iban) on BMD measured by **DXA at the lumbar spine L1-L4** (A) and the femoral neck (B) in old cynomolgus monkeys. Mean % of baseline + SEM. Significances relative to change from baseline ($p<0.05$): different from sham control, a; different from OVX control, b; different from sham and OVX controls, c. (n = 10-15)

Modified from Smith S.Y., Bone 2003;32:45-55.

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HISTOMORPHOMETRY IN PRECLINICAL RESEARCH

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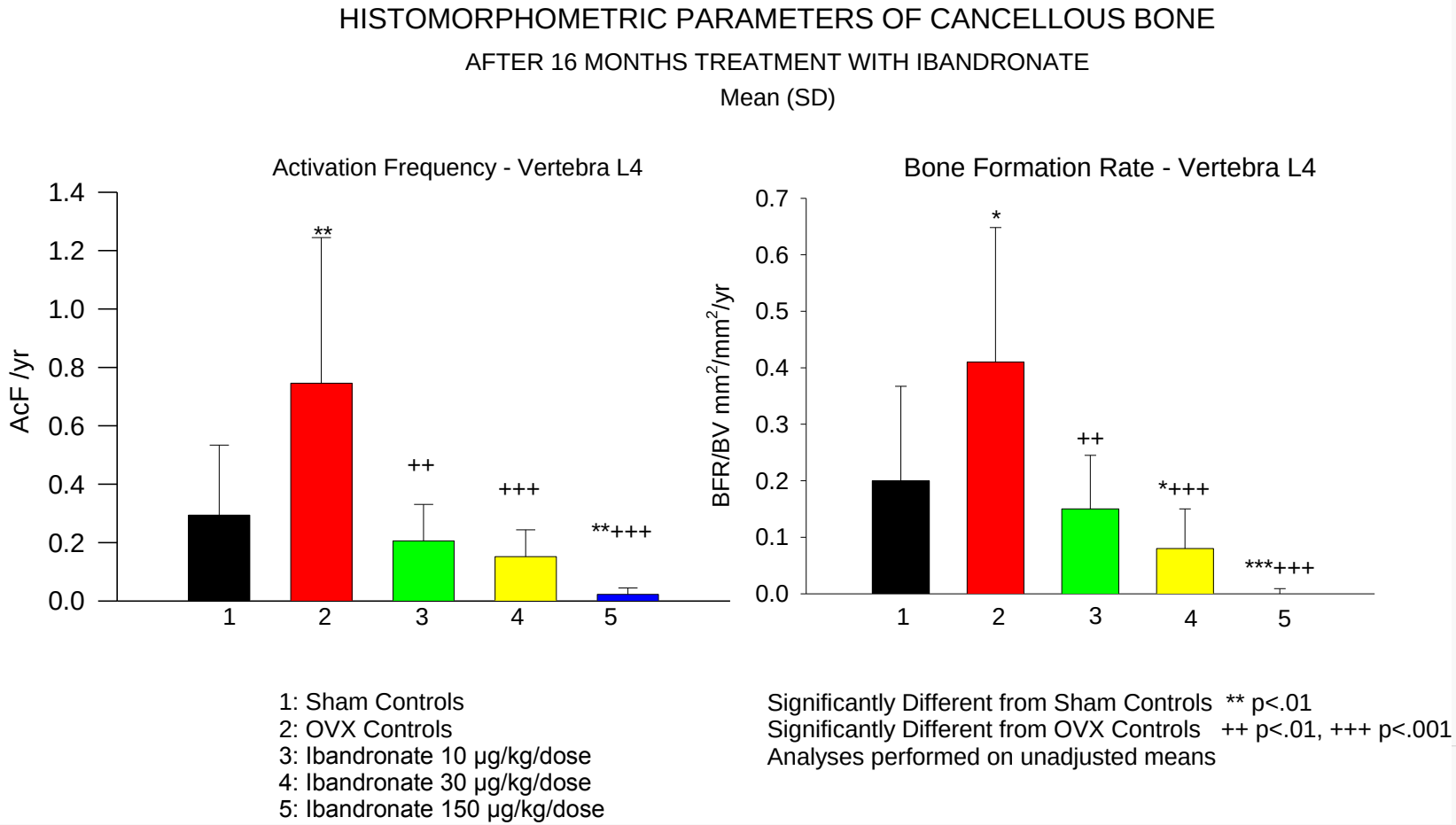

Modified from Smith S.Y., Bone 2003;32:45-55.

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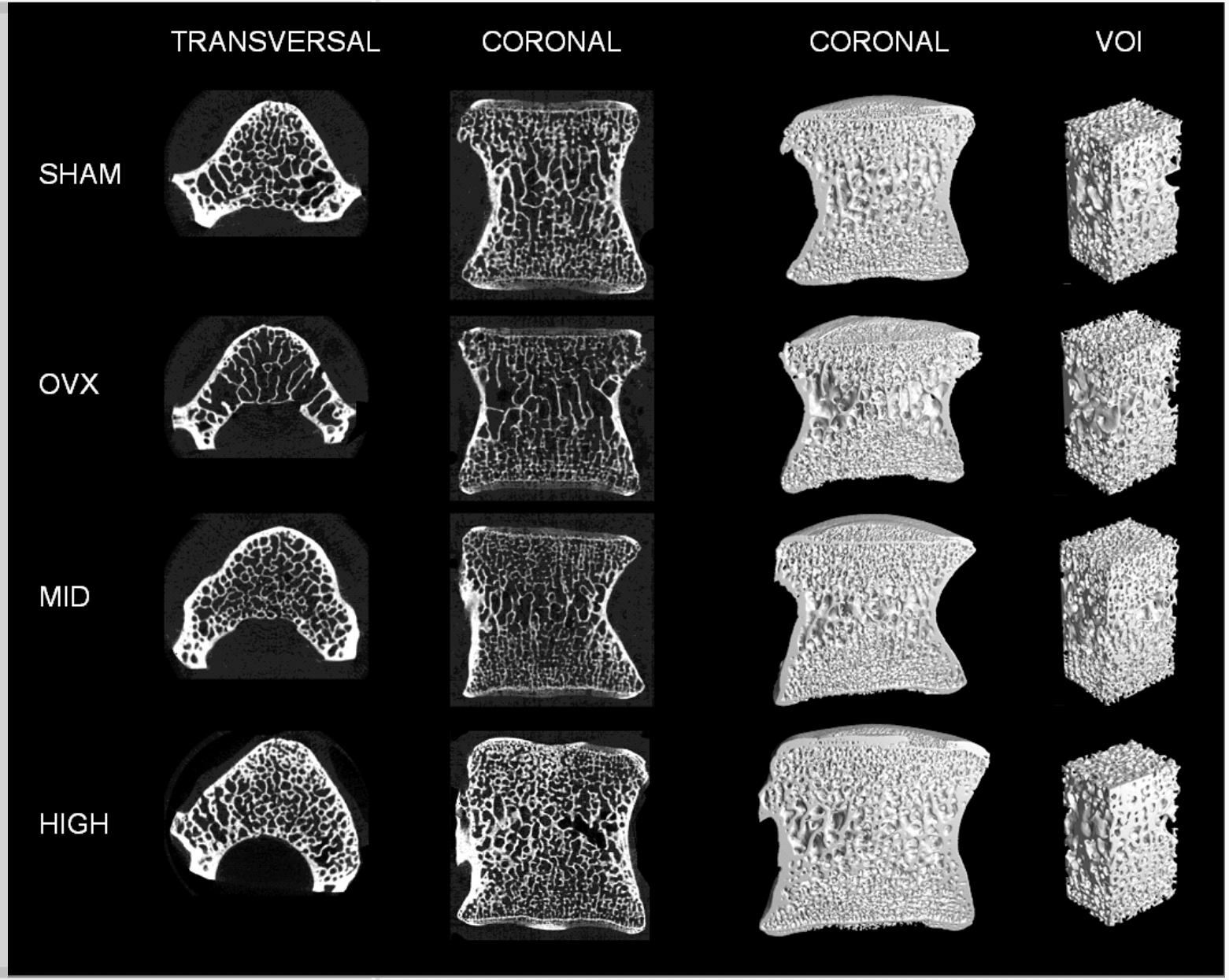
Micro-CT Scans of Monkey Vertebrae Ibandronate

Modified from Muller et al, JBMR, 2004



BIOMECHANICAL TESTING IN PRECLINICAL RESEARCH

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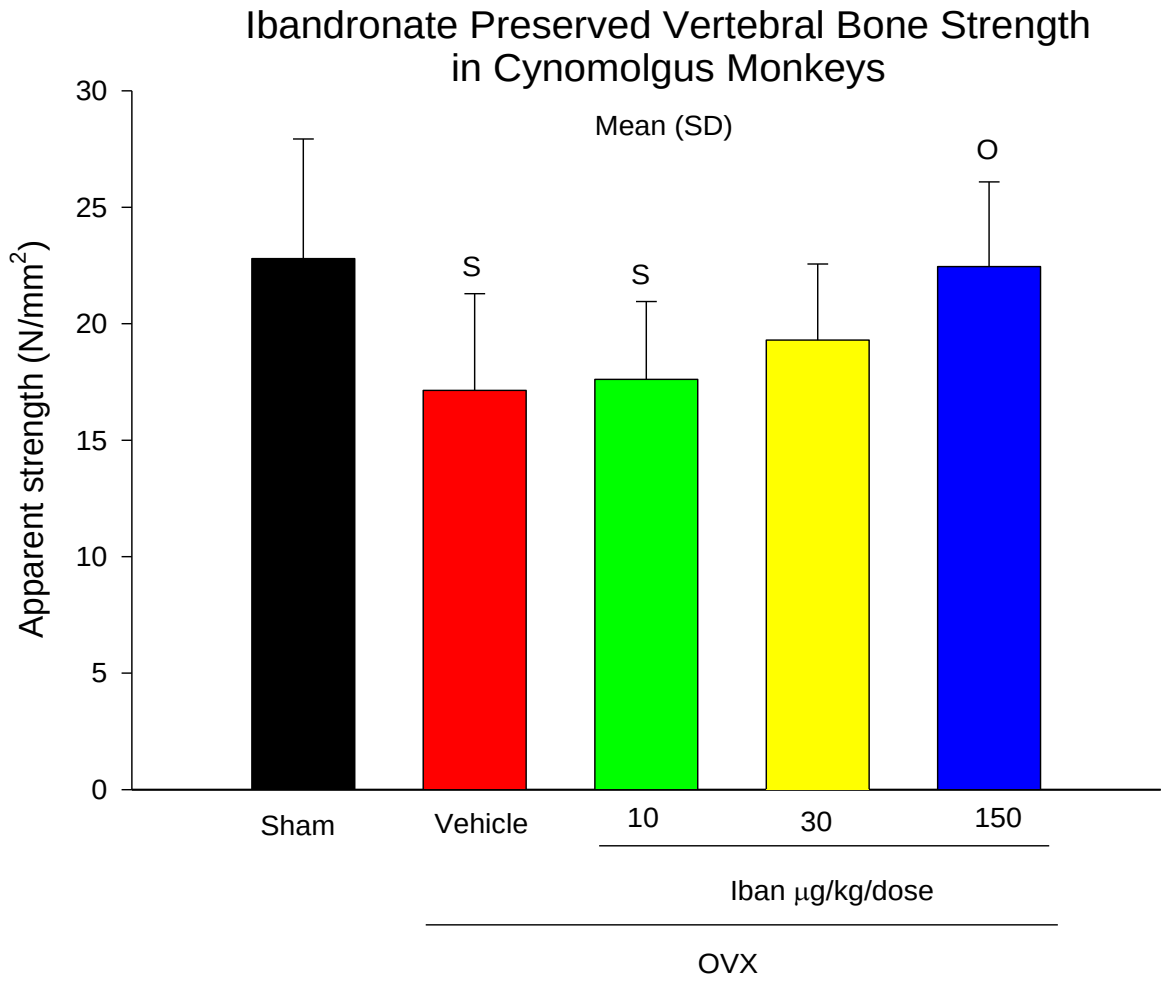

Significantly different from Sham (S) or OVX (O) controls: p<0.05

Modified from Smith S.Y., Bone 2003;32:45-55.

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ANABOLIC AGENT: PTH(1-84)

Treatment of skeletally mature ovariectomized rhesus monkeys with PTH(1-84) for 16 months

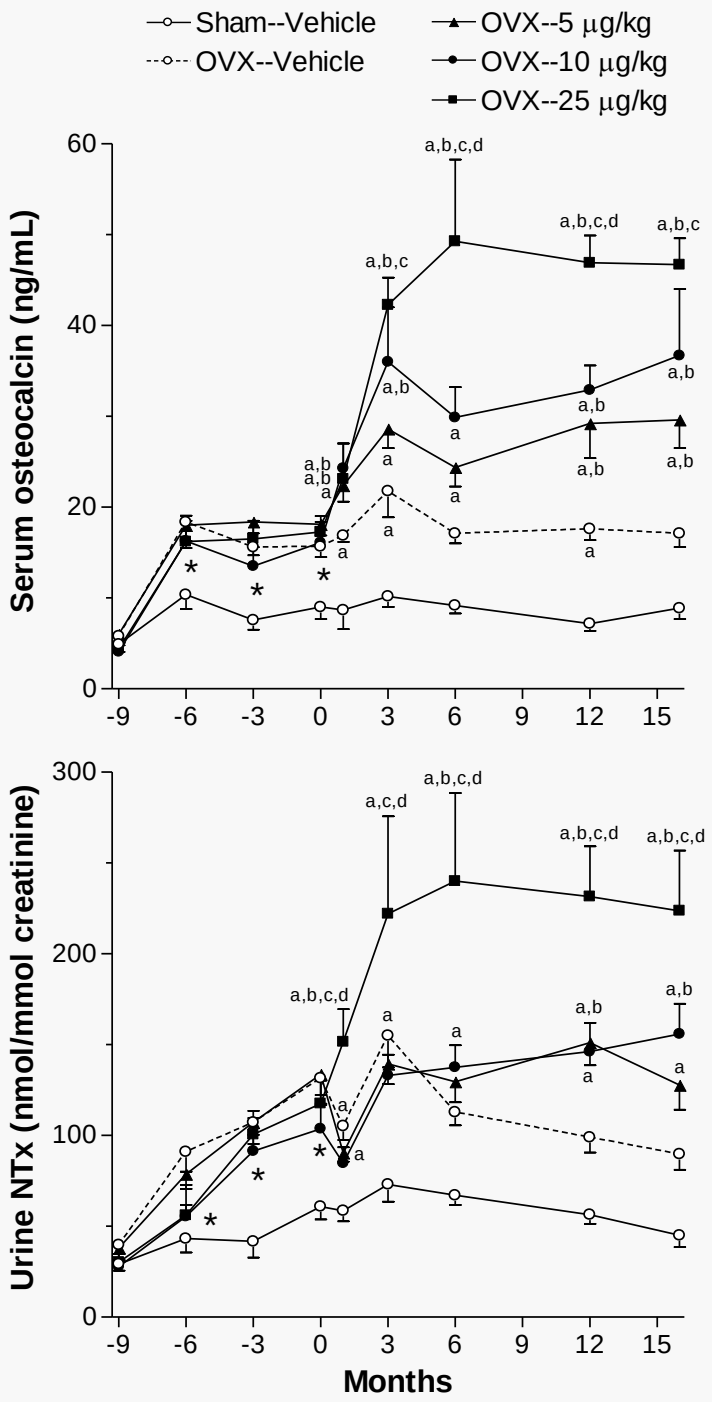
Biochemical Markers in Preclinical Research

PTH(1-84)

Effects of PTH for 16 months in OVX rhesus monkeys on serum levels of **osteocalcin, a marker of bone formation, and urinary N-telopeptides (NTx)**, a marker of bone resorption: increases in formation markers greater than resorption markers.

Ovariectomy or sham surgery occurred at Month -9 and treatment started at Month 0.

Mean ± SE, n = 8-10/group. *pooled OVX and Sham animals significantly different ($p<0.05$). a,b,c,d $P < 0.05$: significance of difference from Sham-Vehicle, OVX-Vehicle, OVX-5 µg/kg, and OVX-10 µg/kg groups, respectively.



Modified from Fox J et al, JBMR 2007

In Vivo DXA in Preclinical Research

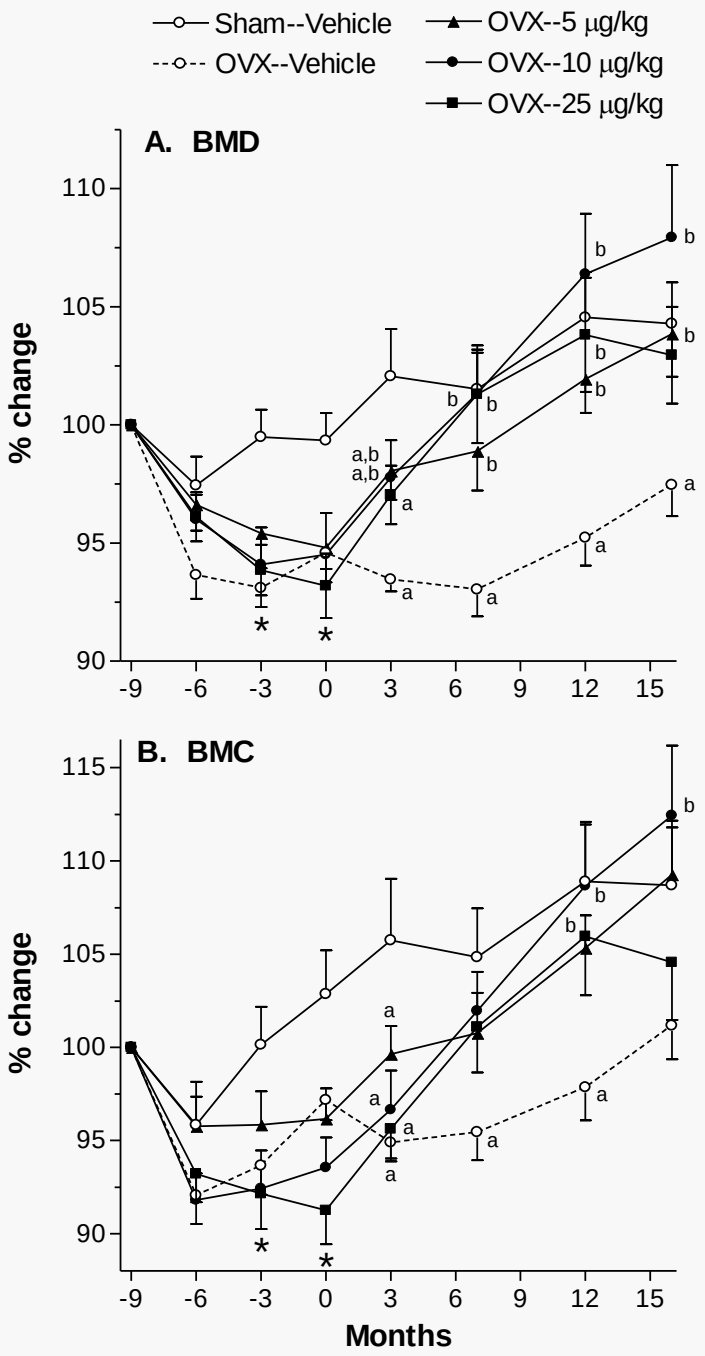
PTH(1-84)

Effects of PTH for 16 months in OVX rhesus monkeys on bone mineral density (panel A) and bone mineral content (panel B) measured by DXA at **lumbar vertebrae 1-4**: BMD and BMC restored to Sham levels or greater. Greatest effect at the mid dose.

Consistent with increases in BV/TV by histomorphometry due to increases in trabecular number by trabecular tunneling.

Ovariectomy or sham surgery occurred at Month -9 and treatment started at Month 0.

Mean ± SE, n = 8-10/group. *pooled OVX and Sham animals significantly different ($p<0.05$). a,b $p<0.05$ = significance of difference from Sham-Vehicle and OVX-Vehicle groups, respectively.



Modified from Fox J et al, JBMR 2007

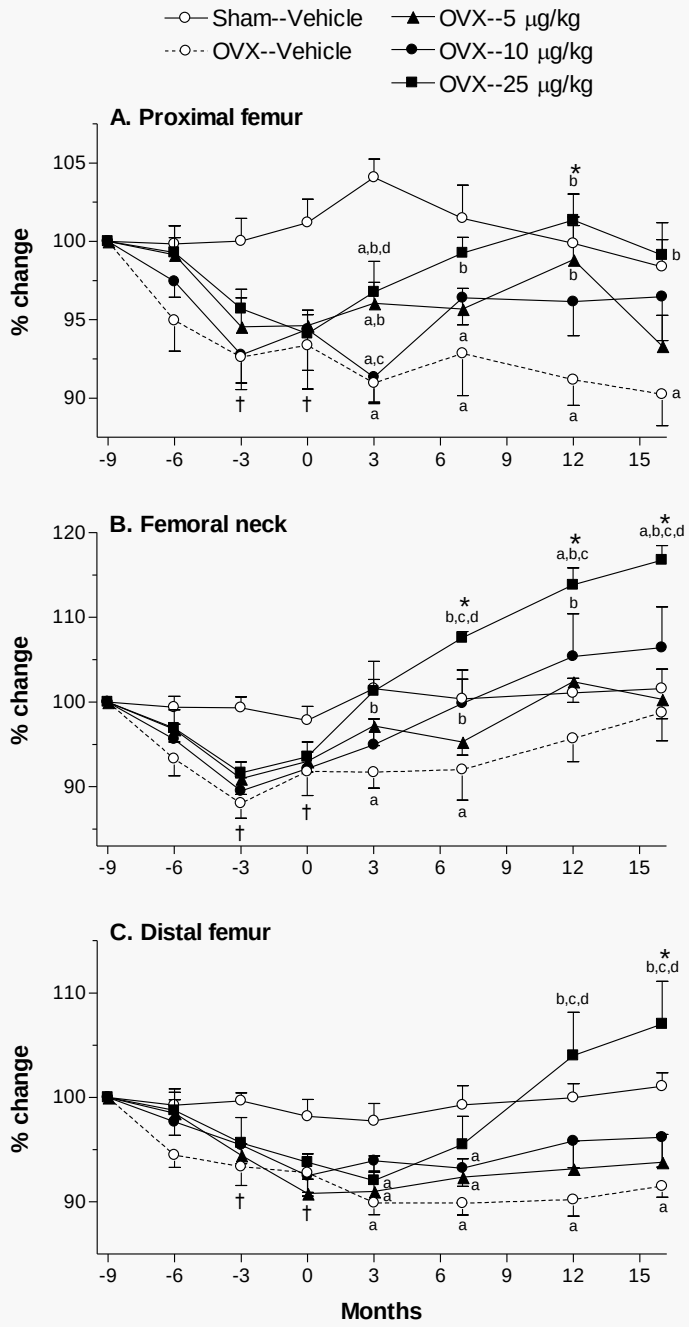
In Vivo DXA in Preclinical Research

PTH(1-84)

Daily s.c. injection of PTH (5, 10, or 25 µg/kg) for 16 months increased BMD measured by **DXA at the proximal and distal femur and femoral neck** compared to sham or OVX rhesus monkeys.

Ovariectomy or sham surgery occurred at month -9 and treatment started at month 0.

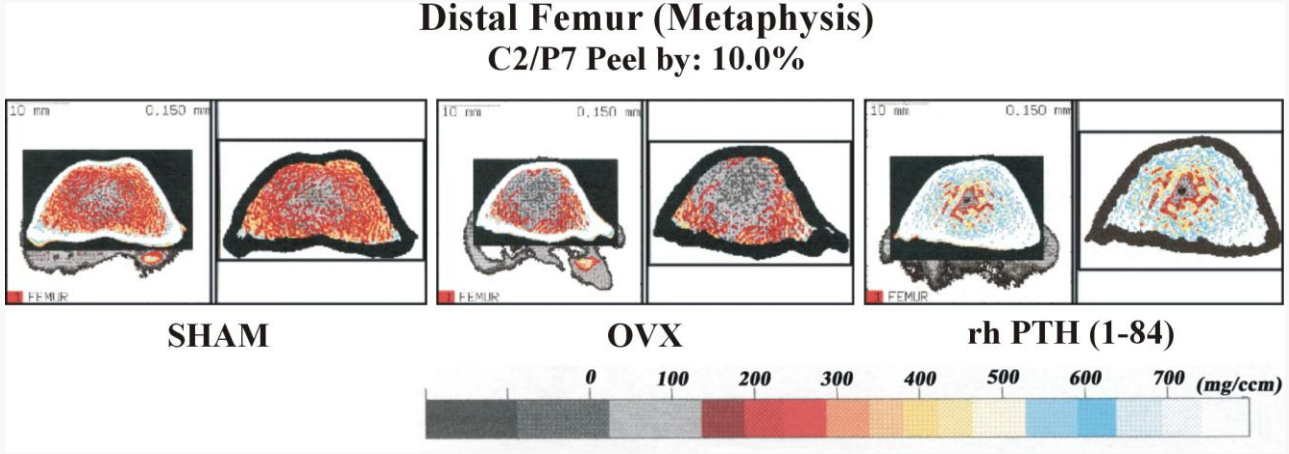
Values are means ± SE, n = 8-10/group. † pooled OVX animals significantly different from sham ($P < 0.05$). a,b,c,d $P < 0.05$: significance of difference from Sham-Vehicle, OVX-Vehicle, OVX-5 µg/kg, and OVX-10 µg/kg groups, respectively.
* significant dose-related trend ($P < 0.05$)

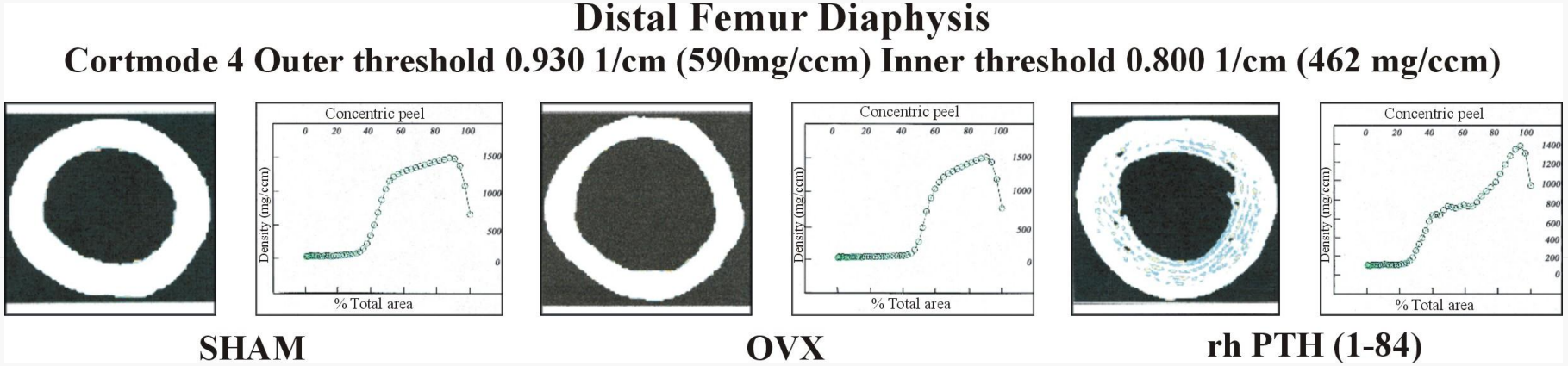


Modified from Fox J et al, JBMR 2007

IN VIVO pQCT IN PRECLINICAL RESEARCH

PTH(1-84)





Modified from Fox J et al, JBMR 2007

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Ex Vivo Micro-CT in Preclinical Research

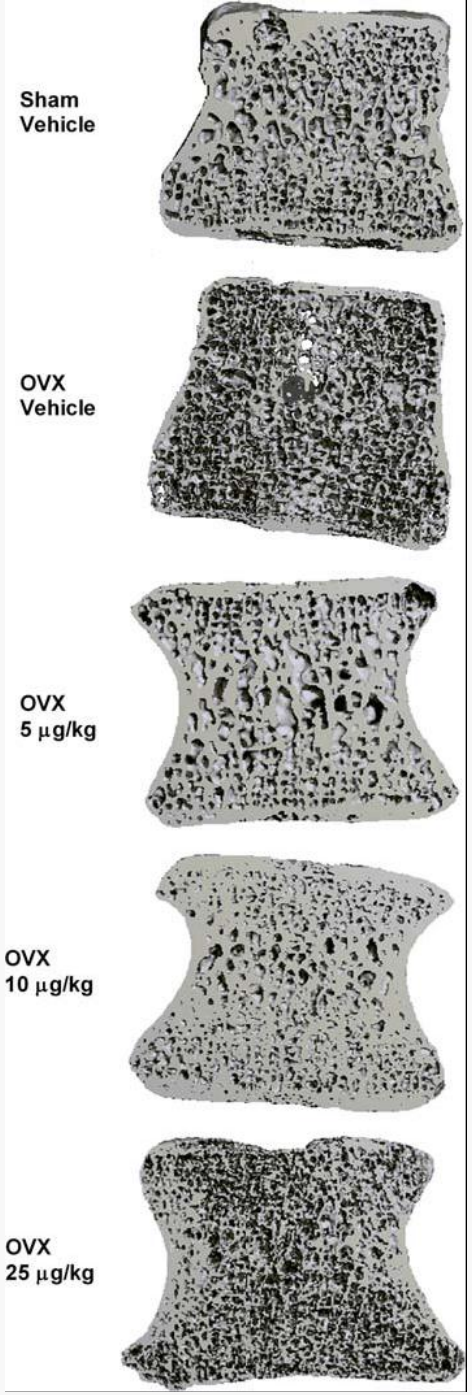
PTH(1-84)

Representative Micro-CT scans from the middle of the **T10 vertebral** body from sham or OVX rhesus monkeys treated daily with vehicle or PTH(1–84) (5, 10, or 25 µg/kg) for 16 months.

Trabecular bone content is lower in the OVX control monkey and increased in PTH(1–84)-treated animals.

Trabecular number and connectivity density increased, particularly in monkeys given the 25 µg/kg dose of PTH(1–84).

BV/TV increases were similar to histomorphometry results at the lumbar spine validating extrapolation of results from lumbar to thoracic spine.

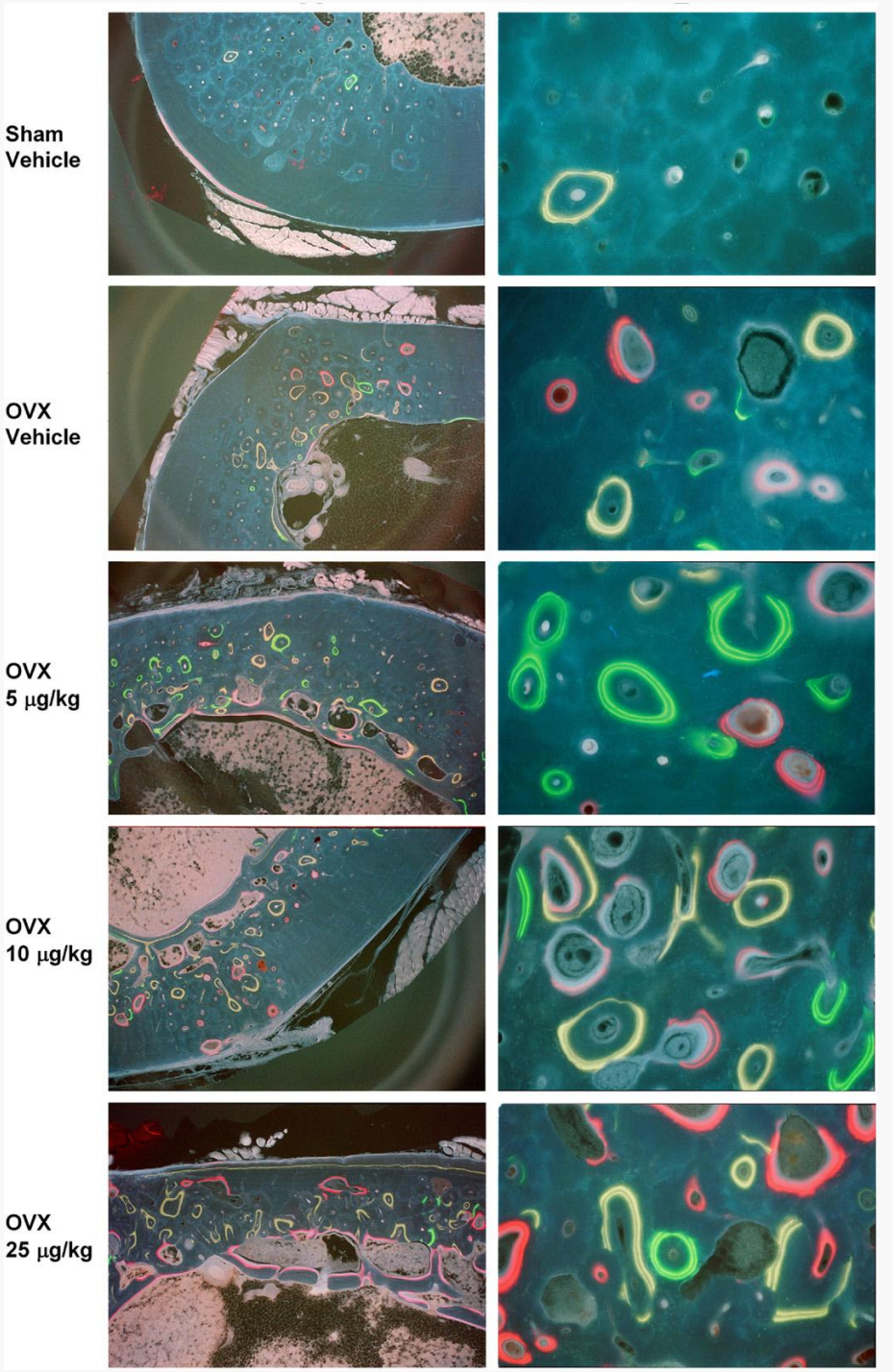


Modified from J. Fox, M. K. Newman, C. H. Turner, R. E. Guldberg, A. Varela, S. Y. Smith, Calcif Tissue Int (2008) 82:212–220

Cortical bone Dynamic Histomorphometry in Preclinical Research

PTH(1-84)

Cortical bone at the tibial diaphysis of sham or OVX monkeys treated daily with vehicle or PTH (5, 10, or 25 µg/kg) for 16 months. The bone-forming surfaces were **double-labeled with calcein (green), oxytetracycline (yellow), and xylenol orange (red)** at baseline, and after 6 and 16 months of treatment, respectively.
Left panel: low-power images with both cortical surfaces.
Right panel: higher power images of Haversian remodeling.
Low level of periosteal, endocortical, and Haversian bone formation in a sham monkey and the higher bone turnover in OVX control and PTH-treated animals, particularly at the endocortical surface.
Note the higher cortical porosity in PTH-treated animals and its location close to the endocortical surface. Also the partial resorption of calcein- and oxytetracycline-labeled osteons formed earlier in the study in PTH-treated animals.



Modified from Fox J et al, JBMR 2007

Ex Vivo Biomechanical Testing in Preclinical Research

PTH(1-84)

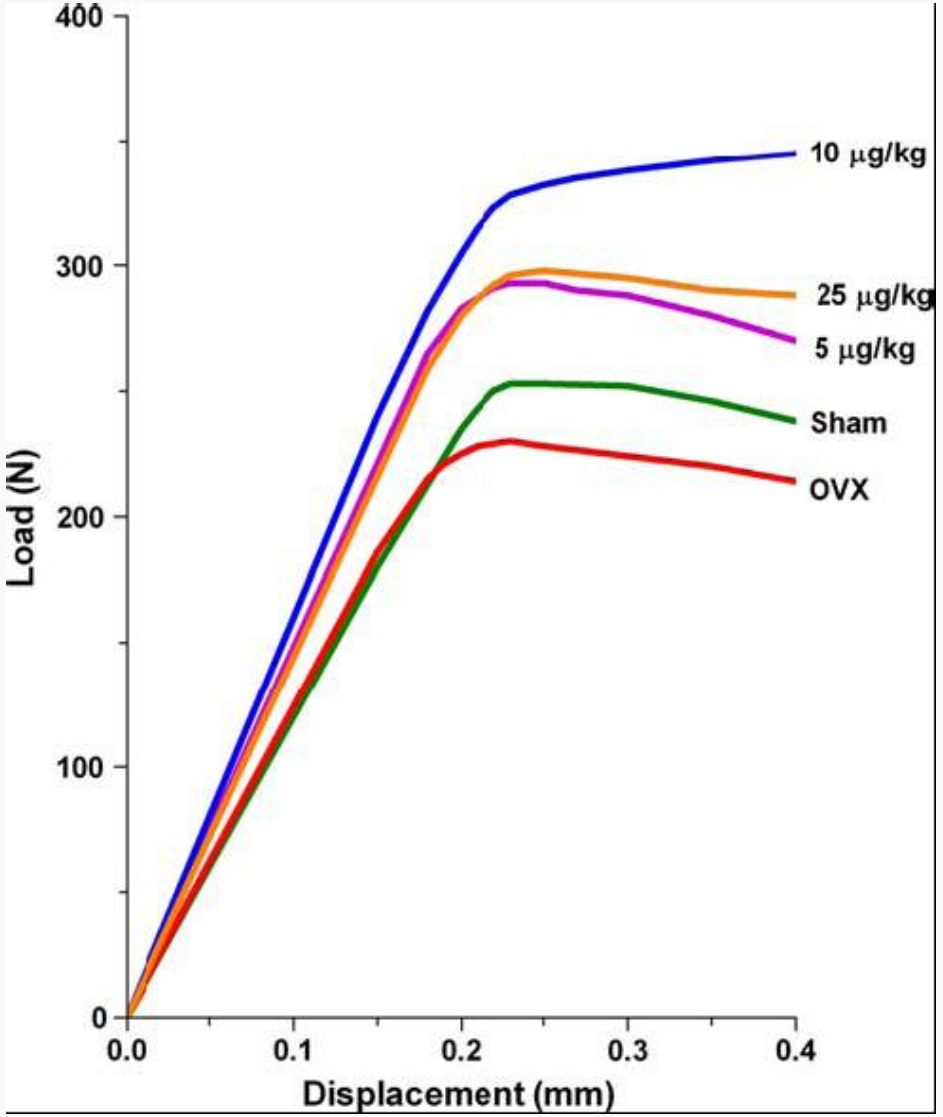
Load-displacement curves from compression testing of trabecular bone cores prepared from a thoracic vertebral body from sham and OVX rhesus monkeys treated daily with vehicle or PTH(1– 84) (5, 10, or 25 µg/kg) for 16 months.

Lower yield load in the OVX vehicle animal compared with the sham-vehicle control and the increased stiffness and yield load in PTH(1–84)-treated animals.

Absence of a clear peak load with the 10 µg/kg dose, which gave the largest increase in stiffness and yield load.

Lower stiffness at 25 relative to 10 µg/kg due to greater turnover and more new bone with reduced mineralization density. High rate of remodeling affected bone strength.

No woven bone formation, osteoid accumulation or marrow fibrosis.



Modified from J. Fox, M. K. Newman, C. H. Turner, R. E. Guldberg, A. Varela, S. Y. Smith, Calcif Tissue Int (2008) 82:212–220

ANABOLIC AGENT: ANTI-SCLEROSTINE ANTIBODY

Romosozumab: A review of scientific weight-of-evidence (chronic toxicity studies in rats and monkeys) and findings in a rat lifetime pharmacology study.

Biochemical Markers in Preclinical Research

Humanized monoclonal antibody that binds and blocks the action of sclerostin

Percent change in serum osteocalcin from baseline in male and female monkeys administered romosozumab. Grey region represents the treatment-free recovery period.

Attenuation of bone formation occurs rapidly with romosozumab

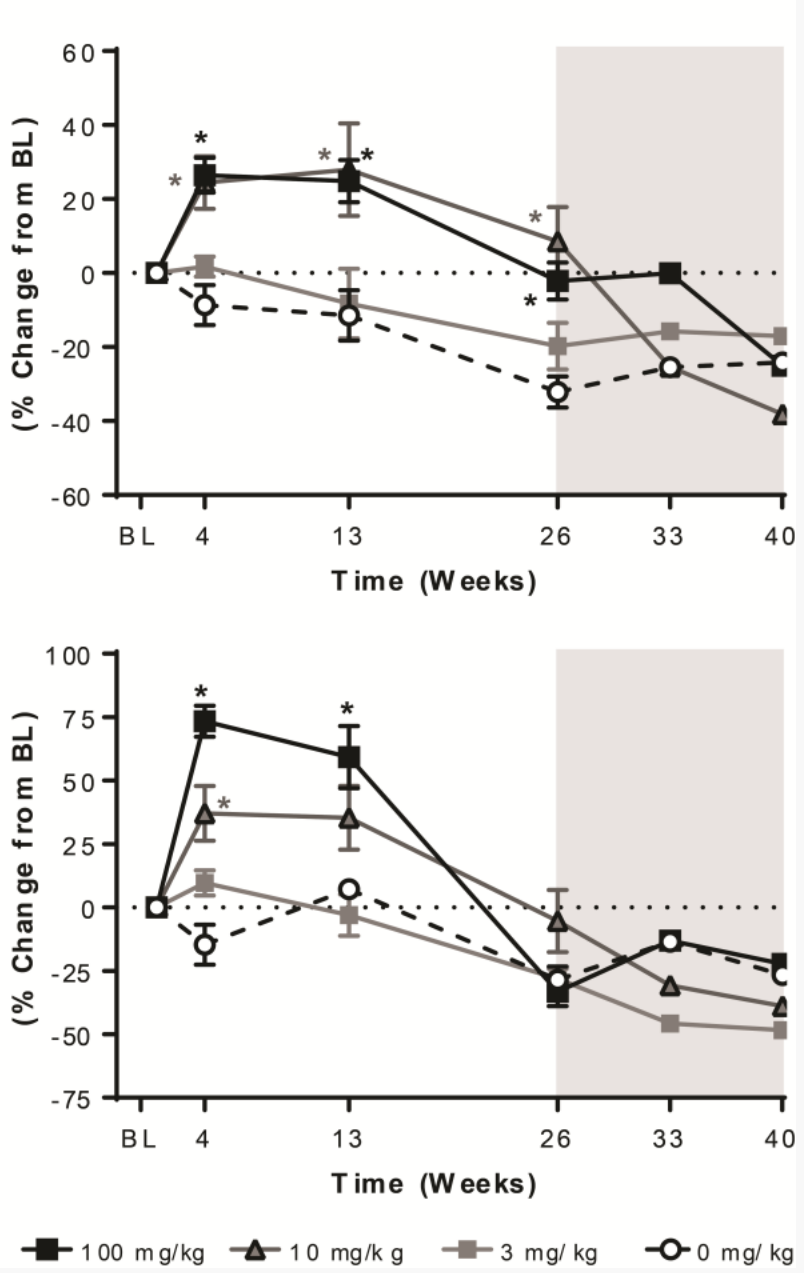
Resorption markers were unchanged (CTx and NTx)

Data represent means ± SEM. * $p < 0.05$ vs 0 mg/kg control; ANOVA followed by Dunnett's t-test of Kruskal-Wallis test. No statistical analysis or group variance values were reported for the recovery phase due to insufficient number of animals. ANOVA = analysis of variance; SEM = standard error of the mean.

Serum Osteocalcin

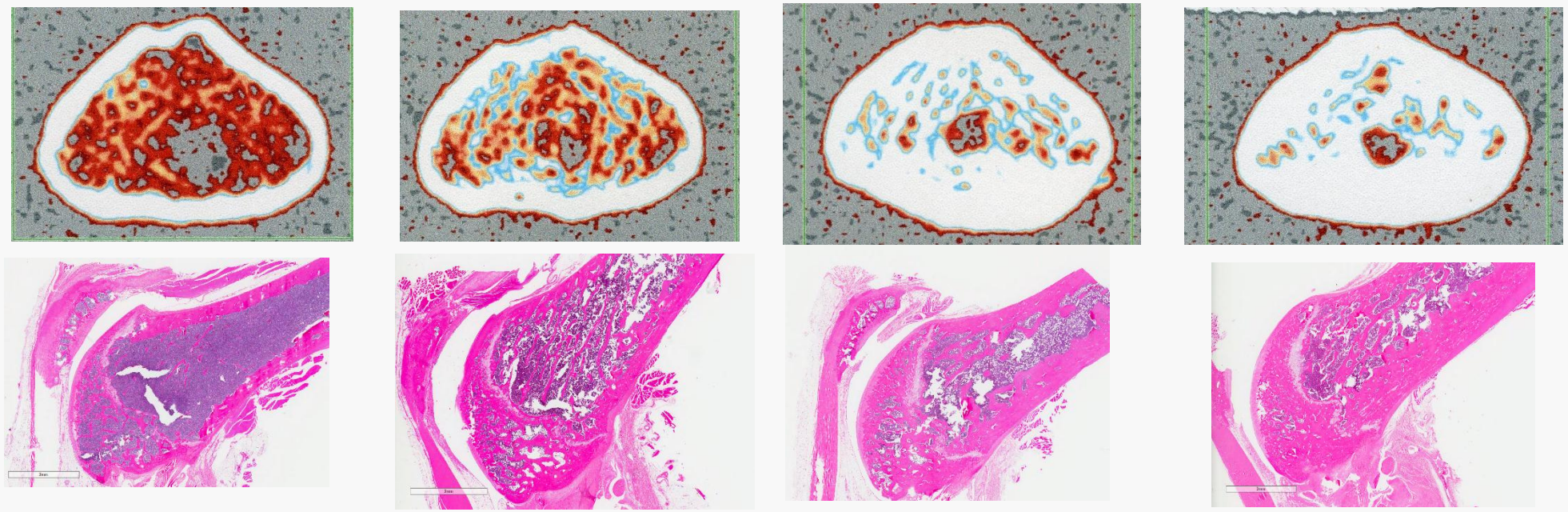
Males

Females



Modified from Chouinard et al. Regulatory Toxicology and Pharmacology, 2016.

EX VIVO DENSITOMETRY AND HISTOLOGY



pQCT images of femur metaphysis from males at 0, 3, 10, and 50 mg/kg (left to right). Metaphyseal slice was obtained at a positi
15% of the total femur length proximal to the distal end of the femur.
Images selected are from animals whose vBMC values were close to the group means.
Correlative histology findings in the femur of males at 0, 3, 10, and 50 mg/kg (left to right).

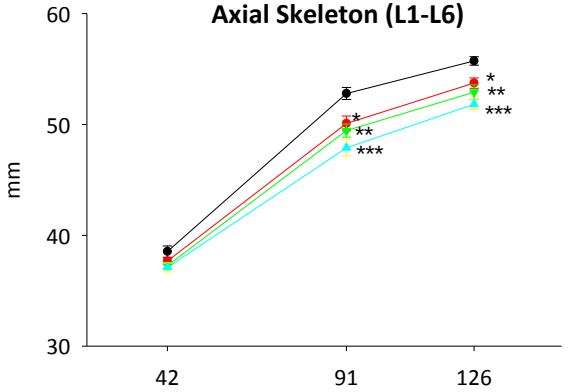
Modified from Chouinard et al. Regulatory Toxicology and Pharmacology, 2016.

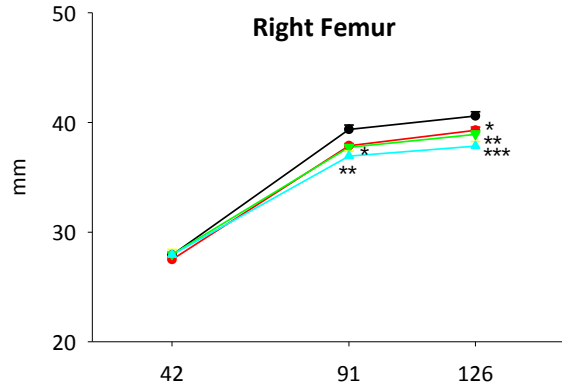
JUVENILE TOXICOLOGY: aromatase inhibitor

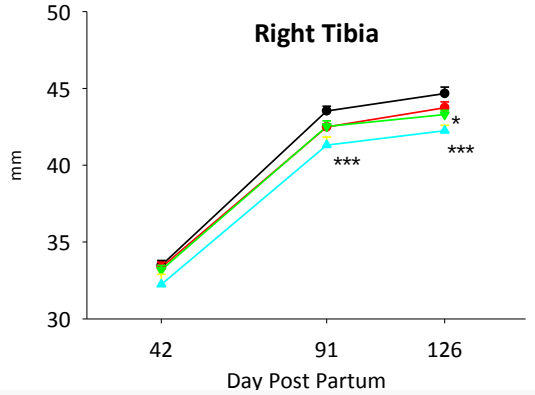
Assessment of a nonsteroidal aromatase inhibitor, letrozole, in juvenile rats.

RADIOLOGY – SKELETAL GROWTH

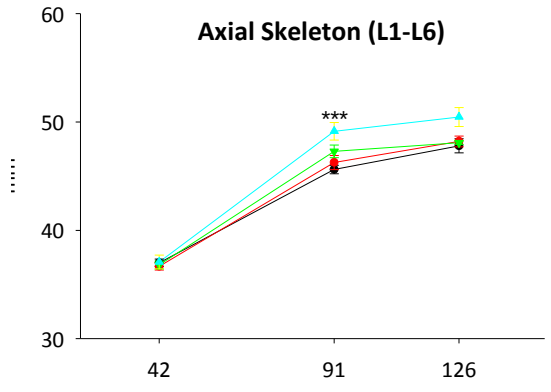
Males

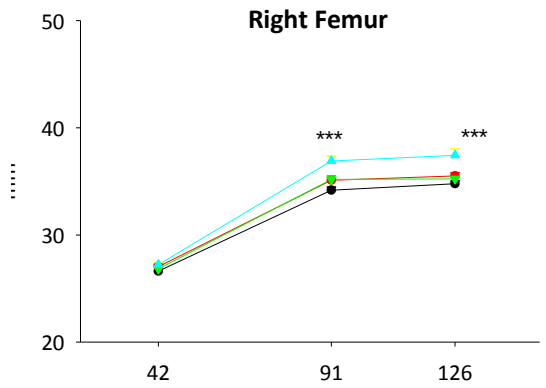


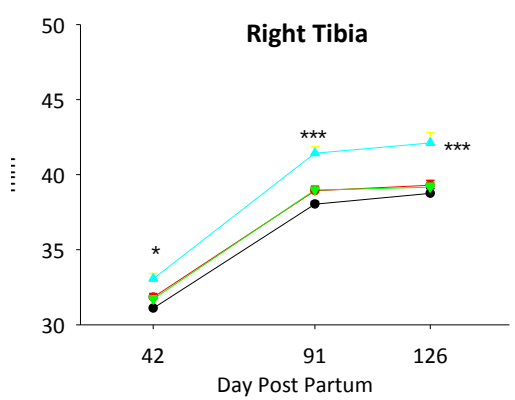




Females







Control
Letrozole 0.003 mg/kg/day
Letrozole 0.03 mg/kg/day
Letrozole 0.3 mg/kg/day

Significantly different from control value:
* - P < = 0.05 ** - P < = 0.01 ***- P < = 0.001

Modified from L Pouliot, M Schneider, M DeCristofaro, R Samadfam, SY. Smith, and DA Beckman. Assessment of a nonsteroidal aromatase inhibitor, letrozole, in juvenile rats. Birth Defects Research (Part B) 98:374–390 (2013)

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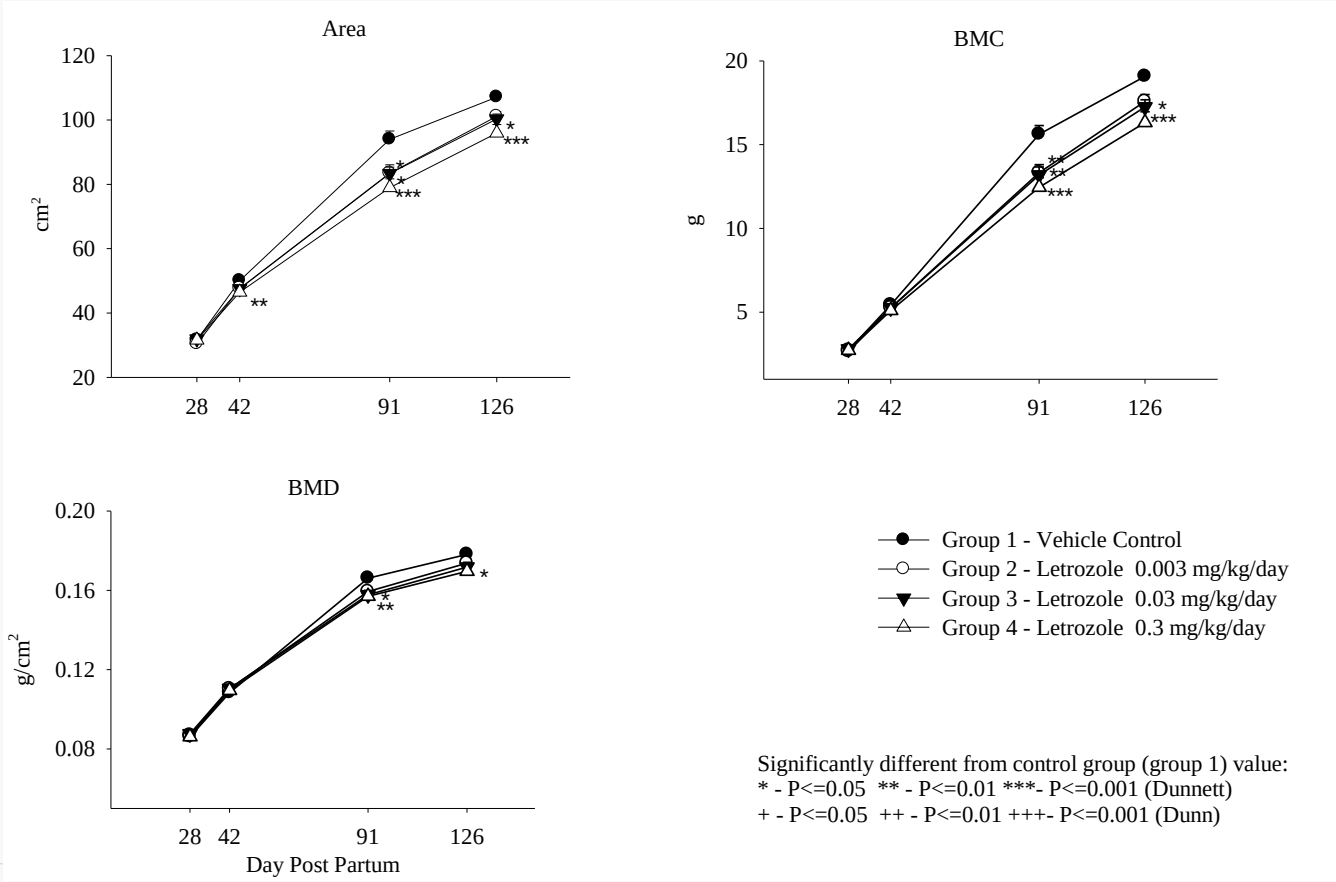
IN VIVO DXA IN PRECLINICAL RESEARCH

Males



Similar results at lumbar spine and femur

Modified from L Pouliot, M Schneider, M DeCristofaro, R Samadfam, SY. Smith, and DA Beckman. Assessment of a nonsteroidal aromatase inhibitor, letrozole, in juvenile rats. Birth Birth Defects Research (Part B) 98:374–390 (2013)

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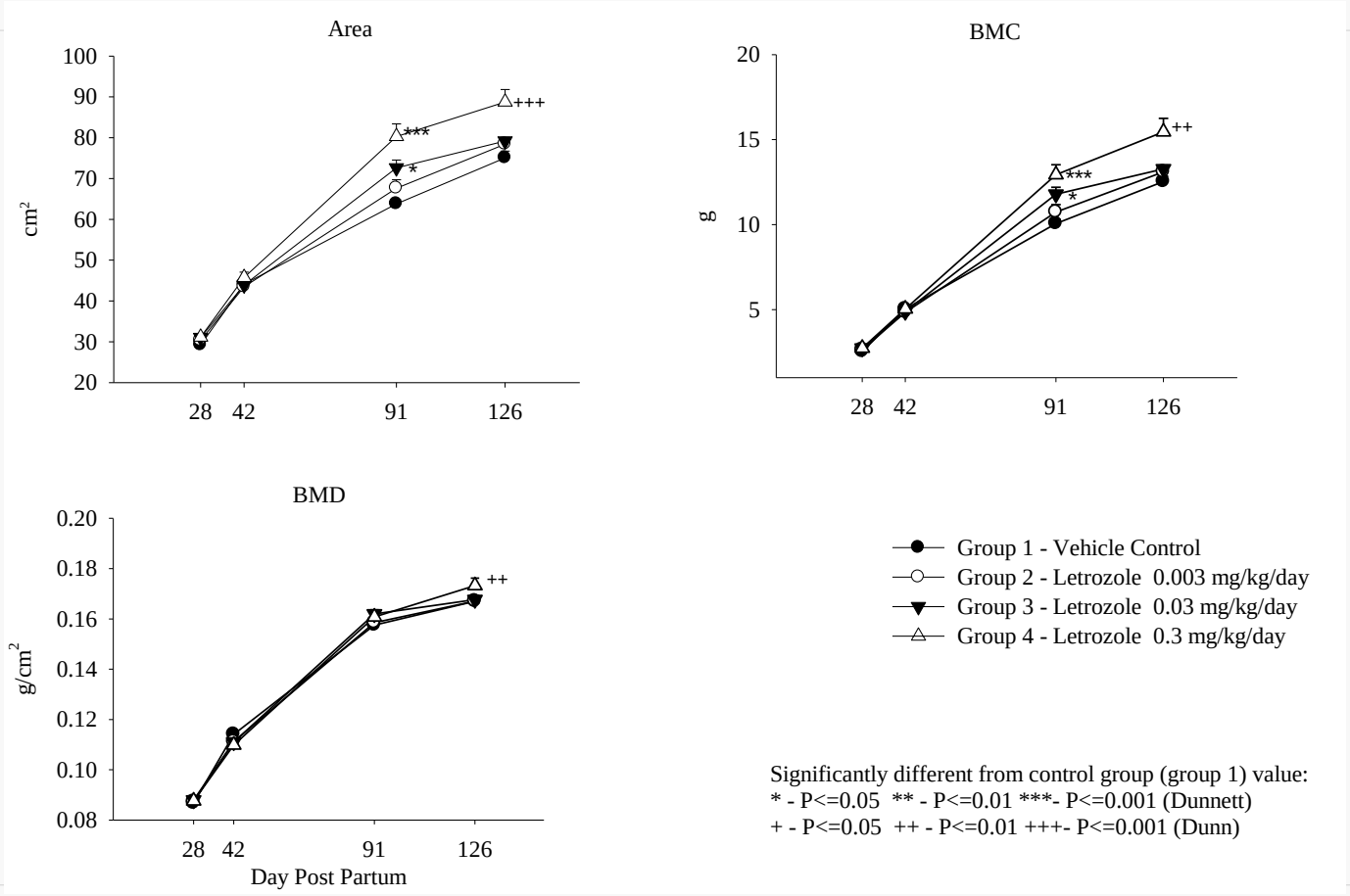
IN VIVO DXA IN PRECLINICAL RESEARCH

Females



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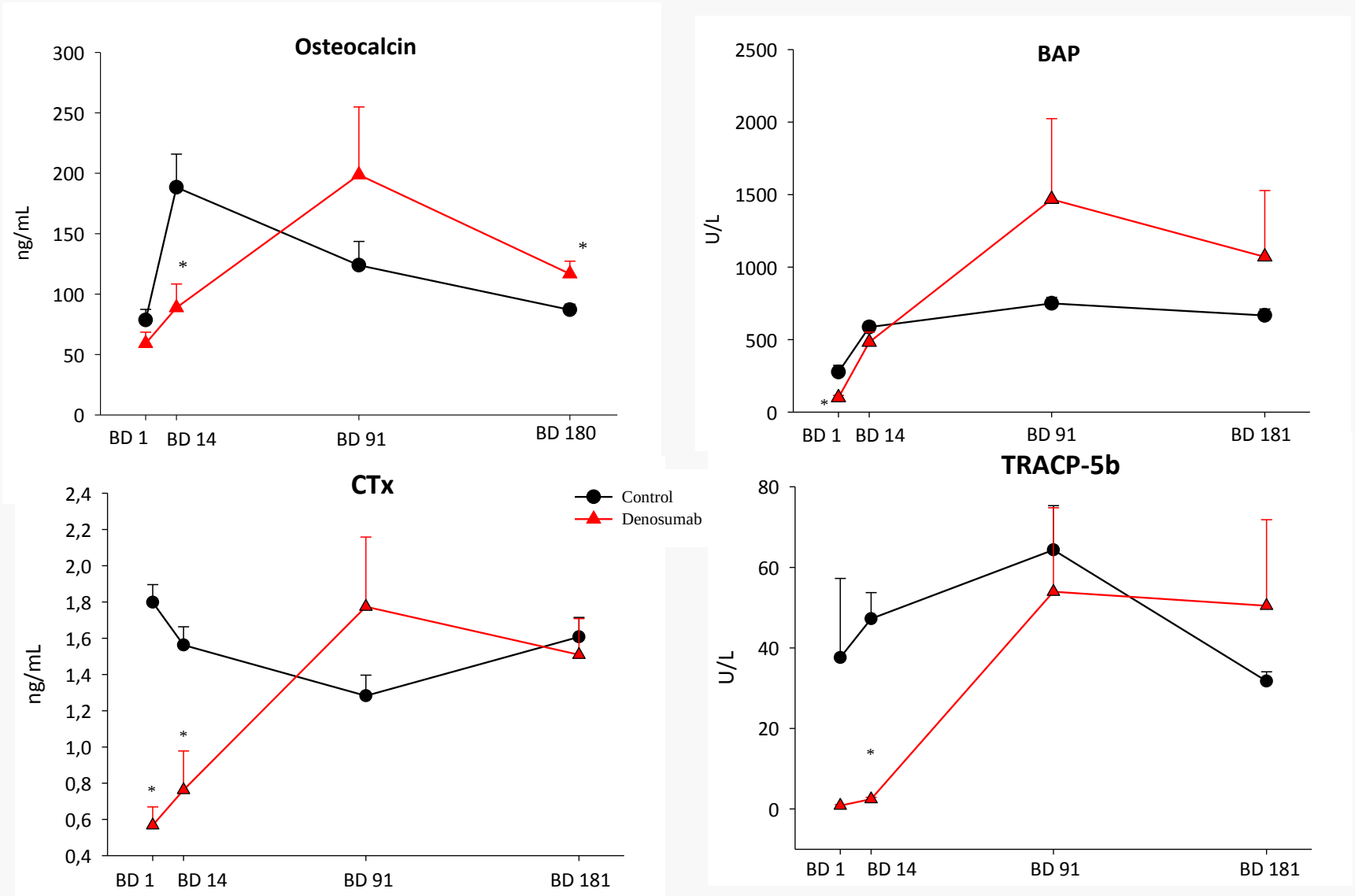
ANTI-RESORPTIVE AGENT: PRE-POSTNATAL STUDY

Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth

BIOCHEMICAL MARKERS OF BONE TURNOVER



Mean (SEM), *p ≤ 0.05.

Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

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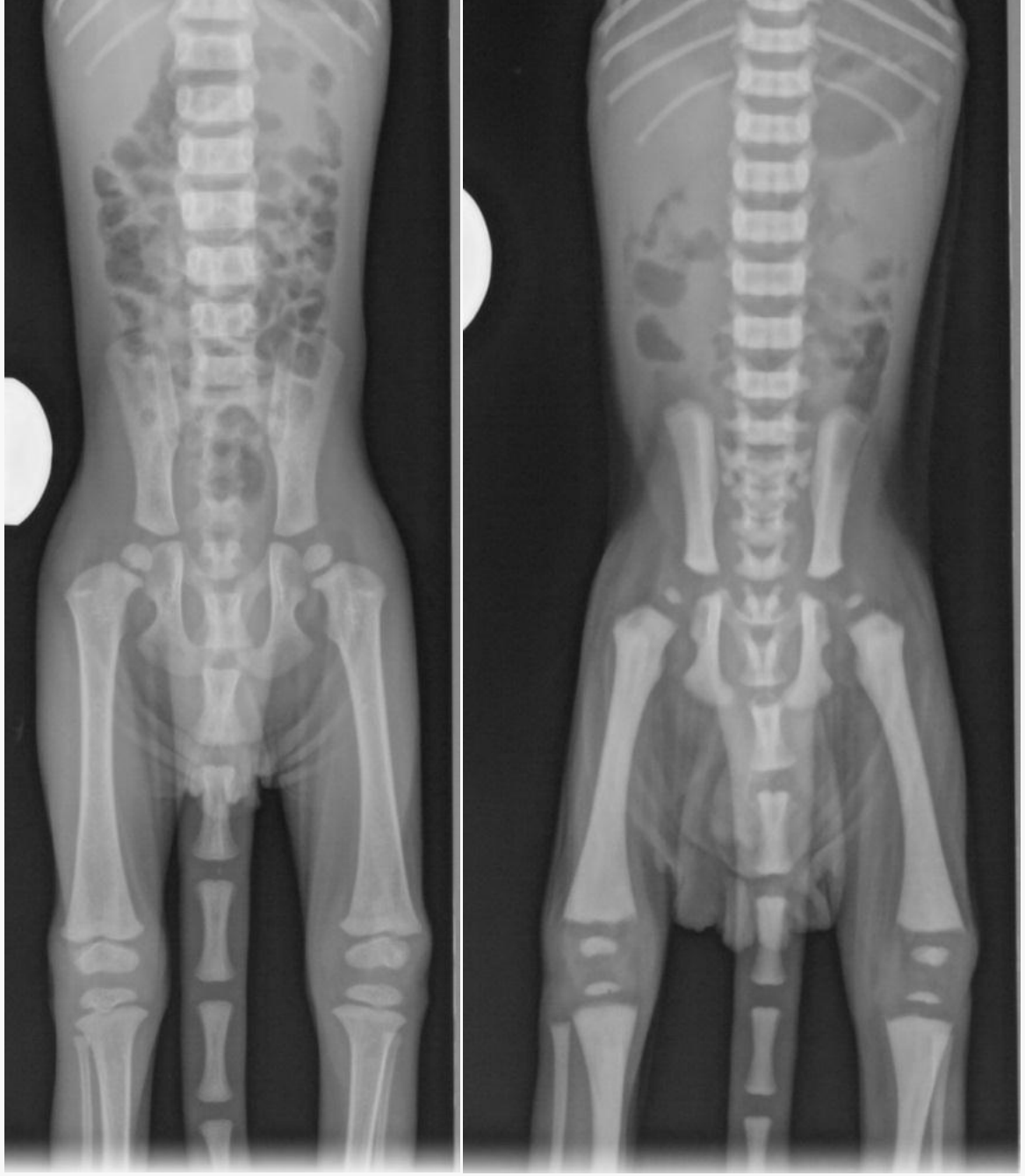
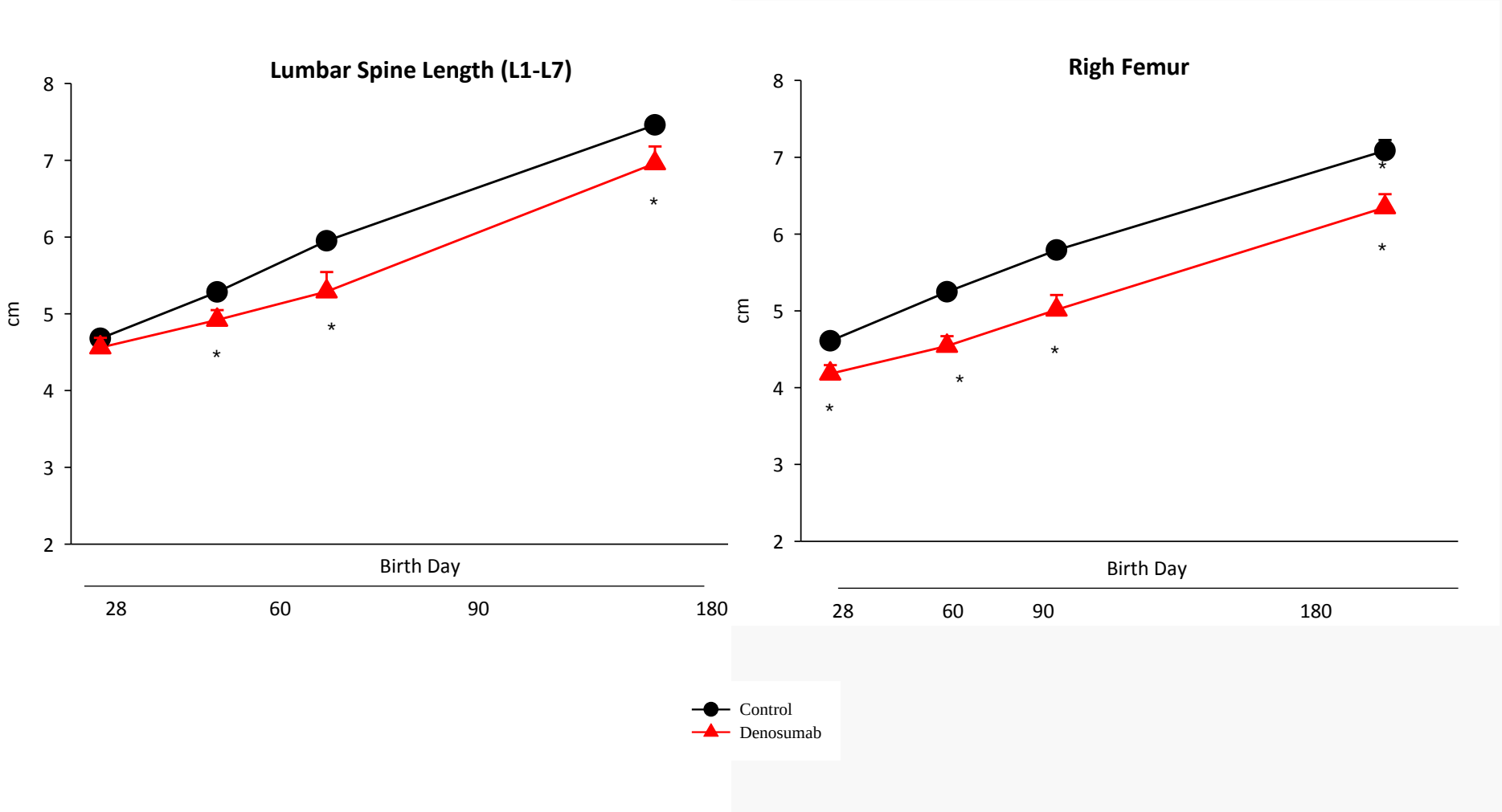


RADIOLOGICAL EVALUATION

- Generalized increased skeletal radio-opacity with widened growth plate and reduced size of secondary ossification in denosumab exposed infant monkey (right) compared with vehicle-exposed infant (left) at at BD28
- Fractures in some infants.

Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

RADIOLOGY – SKELETAL GROWTH



Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

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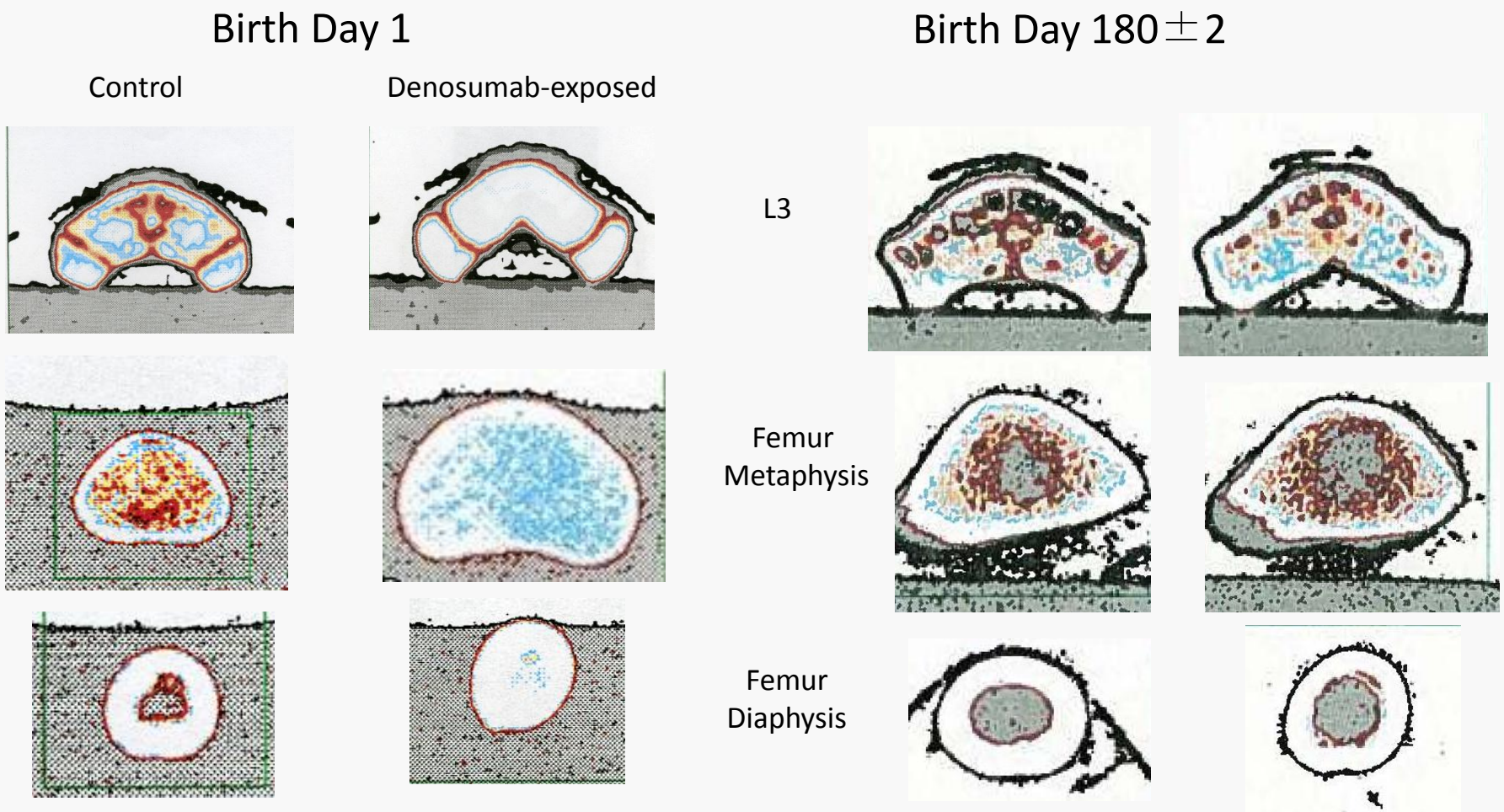
ADVANCED IN VIVO MEASUREMENTS - pQCT



Representative pQCT images

Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

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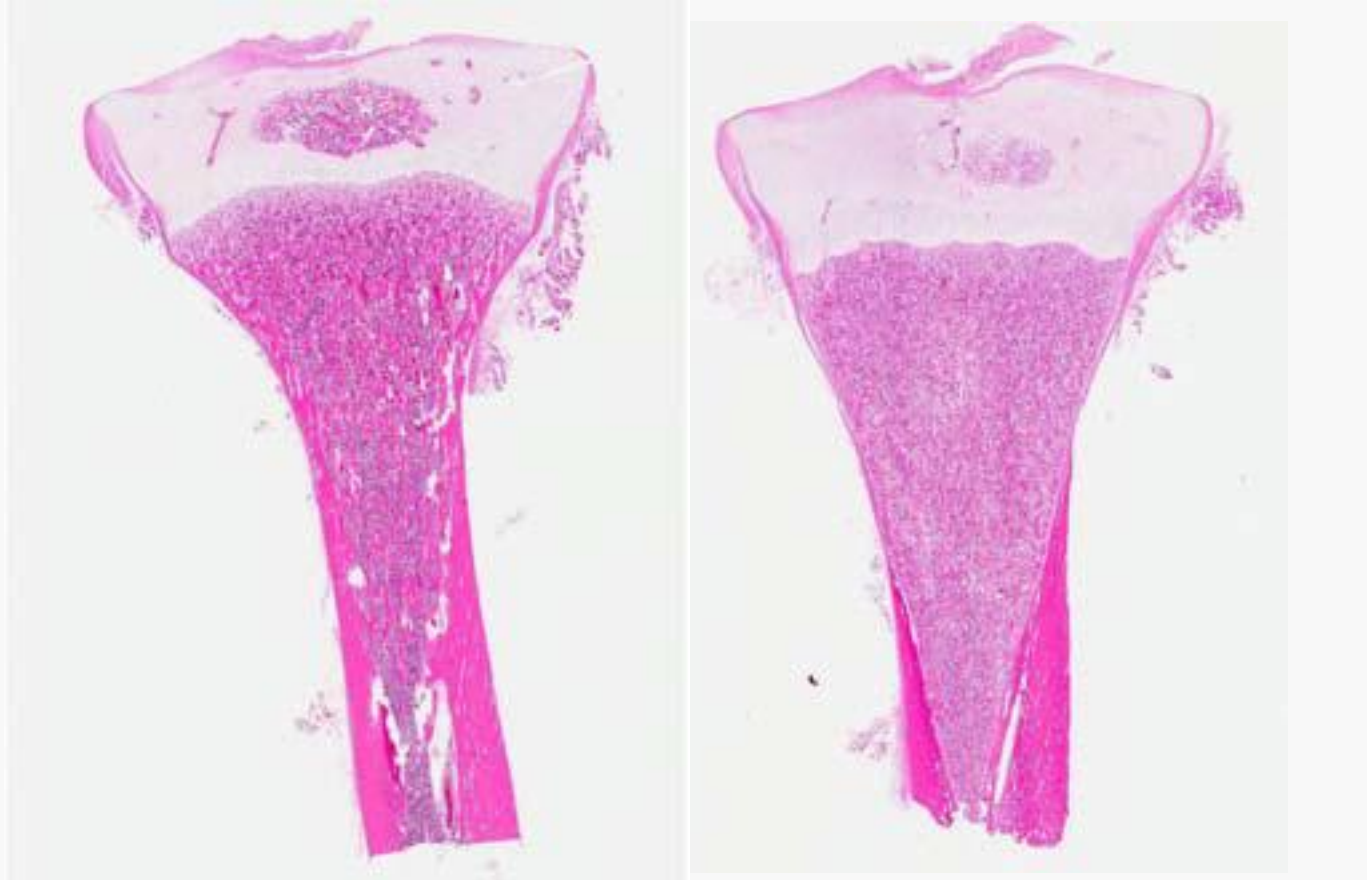

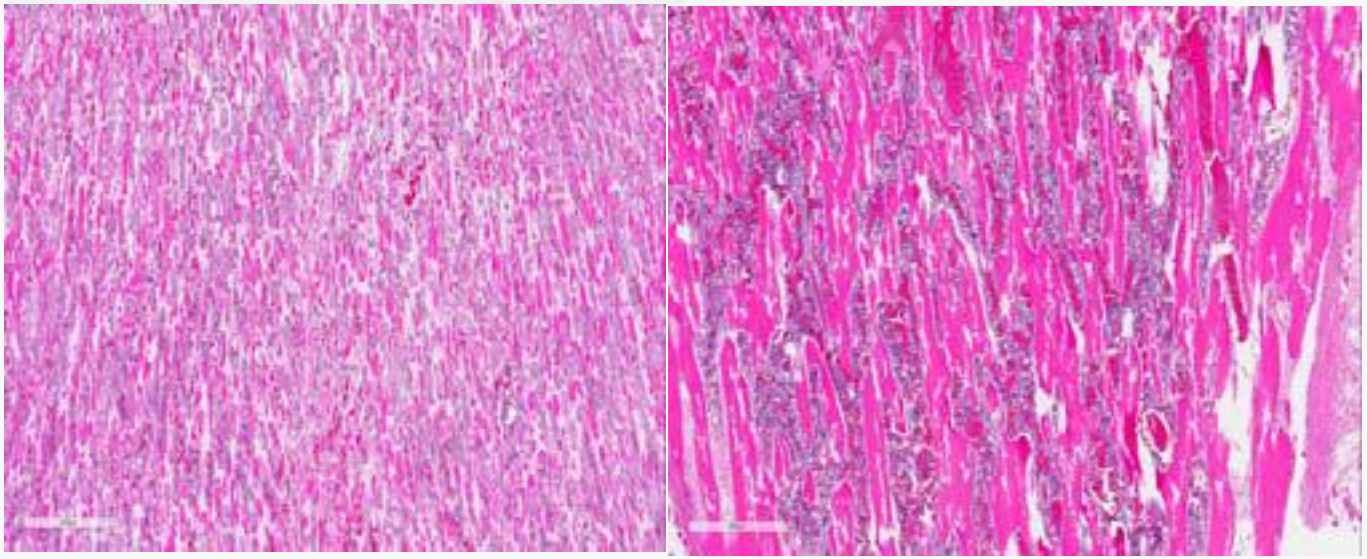

HISTOLOGY

- Marked increased in cancellous bone (nonproliferative hyperostosis) of all examined BD1 infants exposed to denosumab compared with control infants.
-Metaphyseal flaring of long bone due to impaired modeling of the cut back zone.
-Almost complete obliteration of the metaphyseal and diaphyseal marrow spaces by bone trabeculae with prominent residual cartilage consistent with retention of the primary spongiosa (retained primary spongiosa composed of cartilage cores and woven bone)
 -Cortical thickness was reduced in denosumab-exposed infant
-Marked reduction in osteoclast number (osteoclast hypoplasia)

Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

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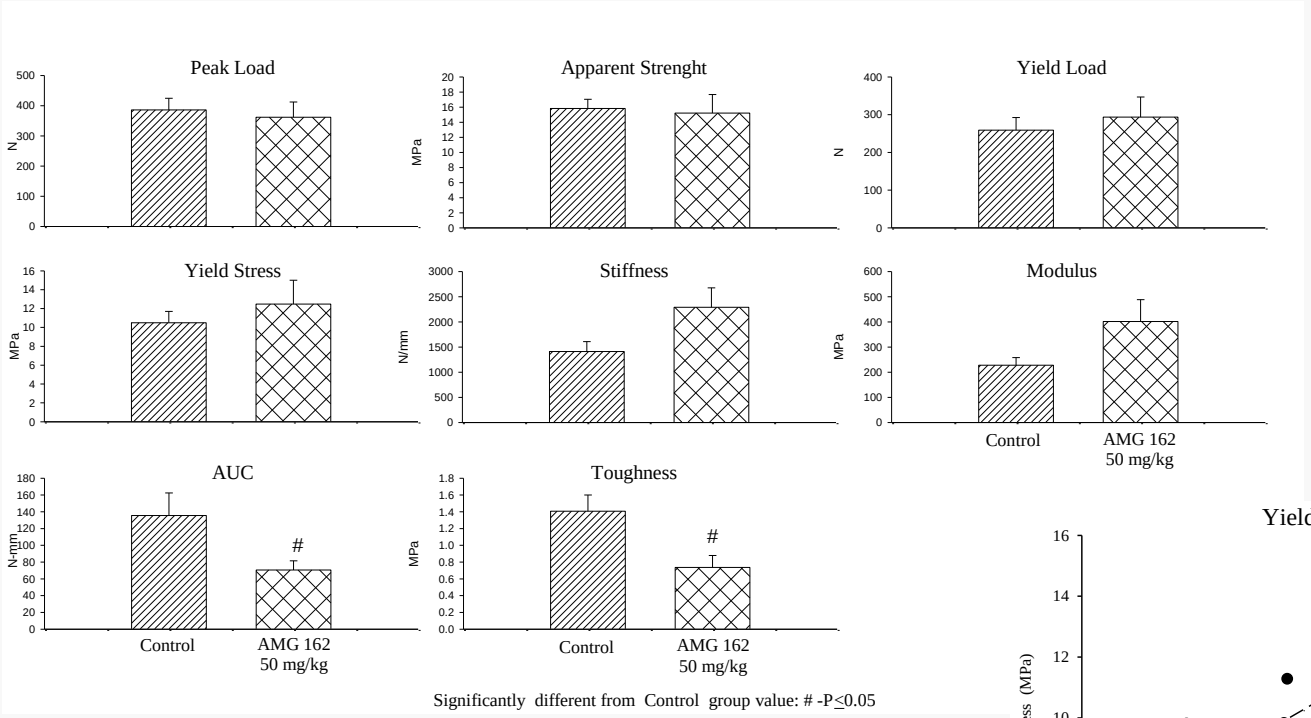
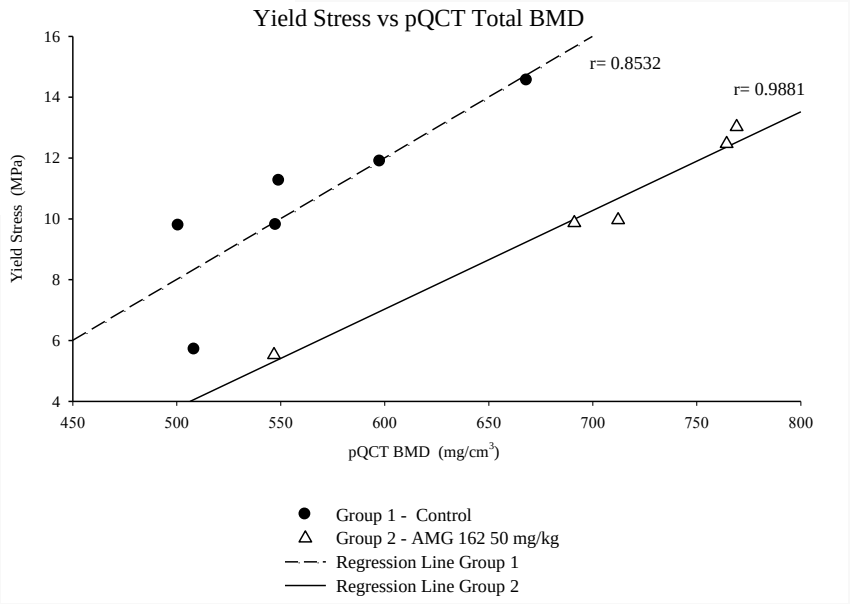
ADVANCED EX VIVO MEASUREMENTS - BIOMECHANICAL TESTING





Modified from Rogely W. Boyce, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014).

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ANABOLIC AGENT: carcinogenesis study

Treatment of skeletally mature ovariectomized rhesus monkeys with PTH(1-84) for 16 months

In vivo Assessments - Radiology

PTH(1-34)

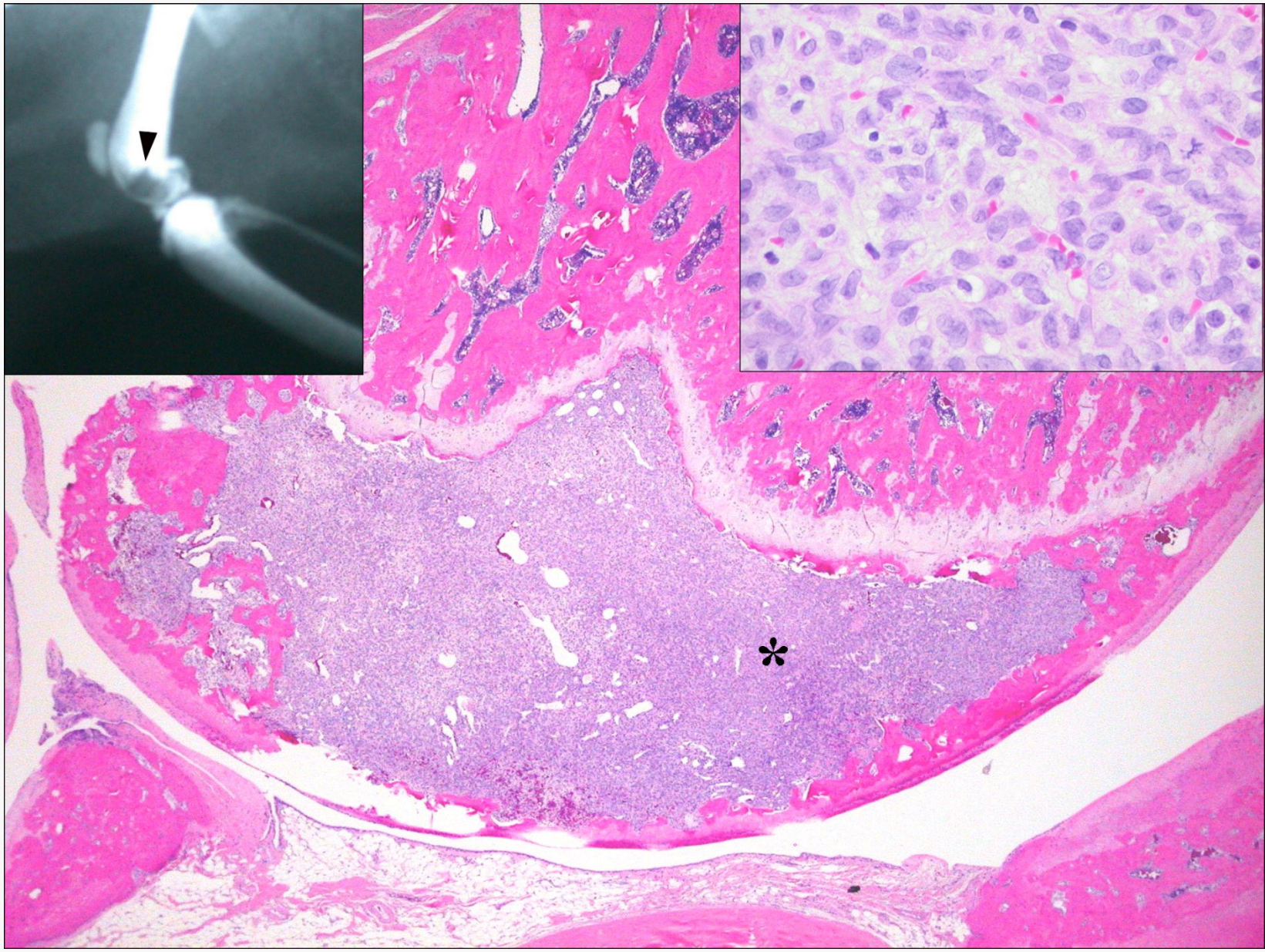
Radiological assessment for non-invasive identification of additional skeletal lesions “occult” lesions from sites not routinely sampled at necropsy or that would otherwise have gone undetected.
Treatement for 13.5 months with recombinant human parathyroid hormone (PTH) 1-84 at 150 mg/kg/ day shows a generalized increase in skeletal radiodensity,
Localized bone loss in the humerus (arrowheads) and tibia (arrow) as indicated by multifocal sites of reduced radiodensity. These lucent areas subsequently were diagnosed as multicentric osteosarcoma.

Modified from Jolette et al. (2006) Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats, Toxicol. Pathol. 34, pp. 929–940

In vivo Assessments - Radiology

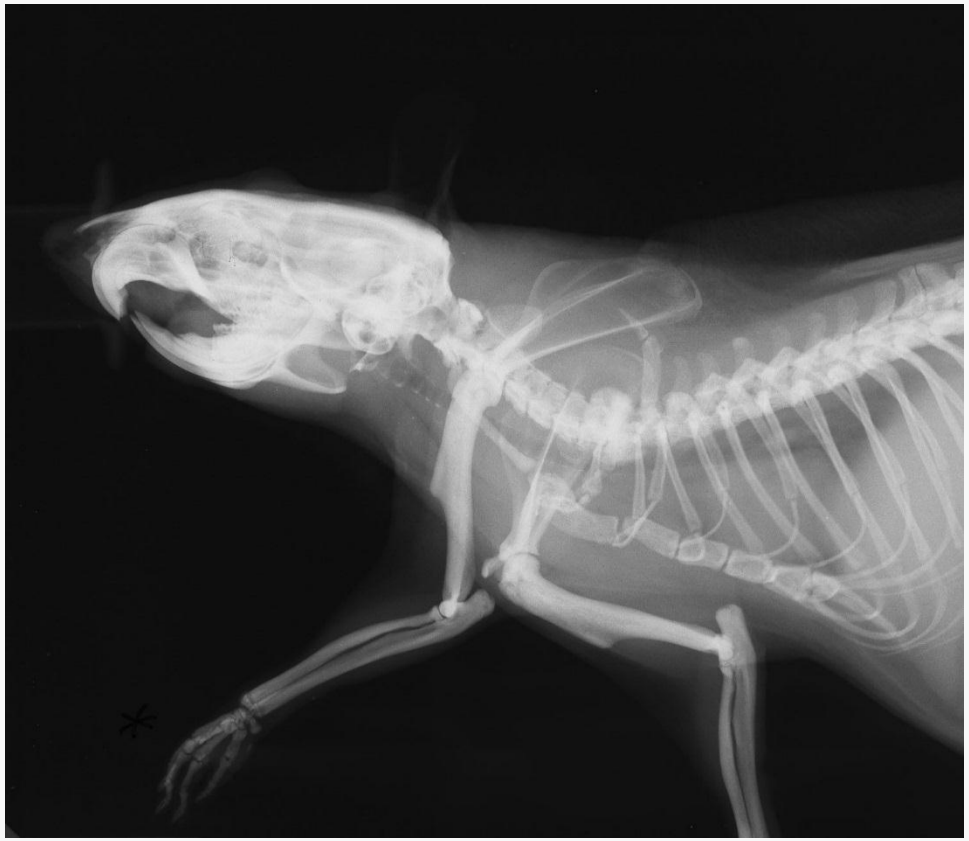
PTH(1-34)

Osteosarcomas in the skeleton that were detected radiologically in a rat treated for an extended period with recombinant human PTH Radiograph showing a lytic area (top left inset) in the distal femur (arrowhead) where the bone was effaced (center, asterisk) by an aggressive population of pleomorphic osteoblasts (high magnification inset at upper right). H&E stain.

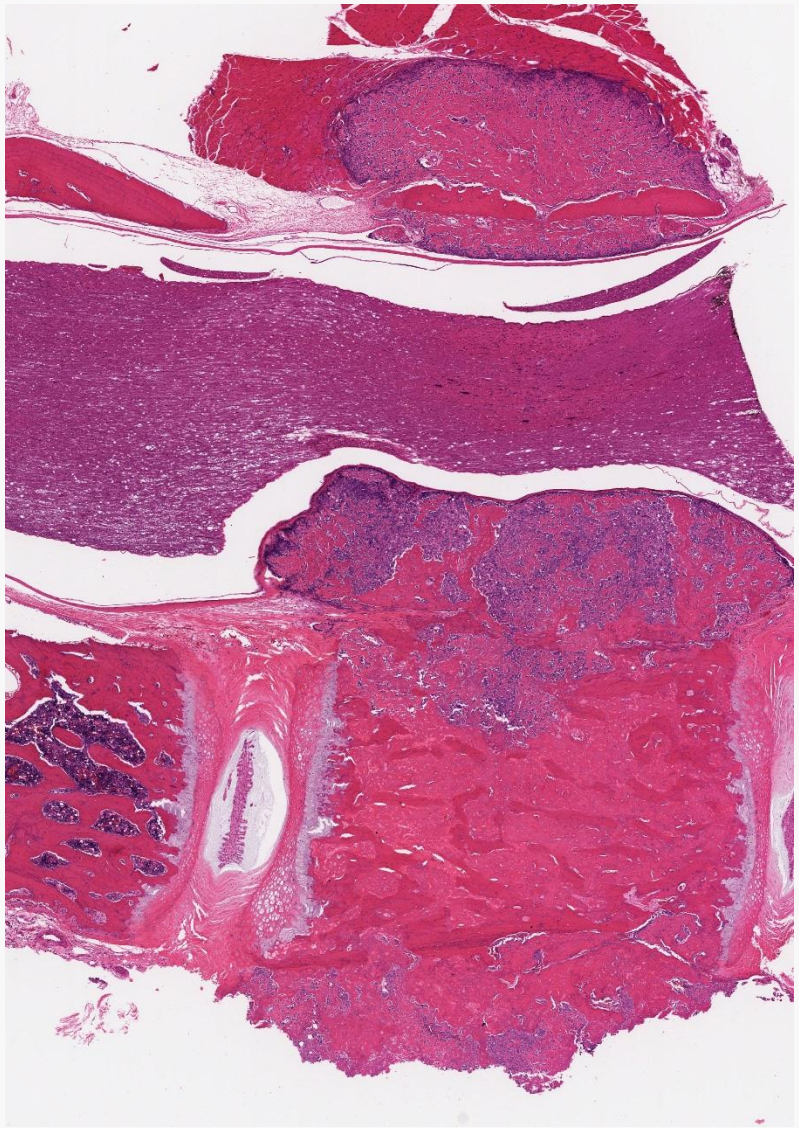


Modified from Jolette et al. (2006) Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats, Toxicol. Pathol. 34, pp. 929–940

IN VIVO ASSESSMENTS - RADIOLOGY



Modified from Jolette et al. (2006) Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats, Toxicol. Pathol. 34, pp. 929–940



Occult osteosarcomas in the axial skeleton that were detected radiologically Comparable radiograph and corresponding microscopic findings of an occult osteosarcoma affecting a vertebral body. H&E stain.

BONE TOXICOLOGY: PPAR γ agonists

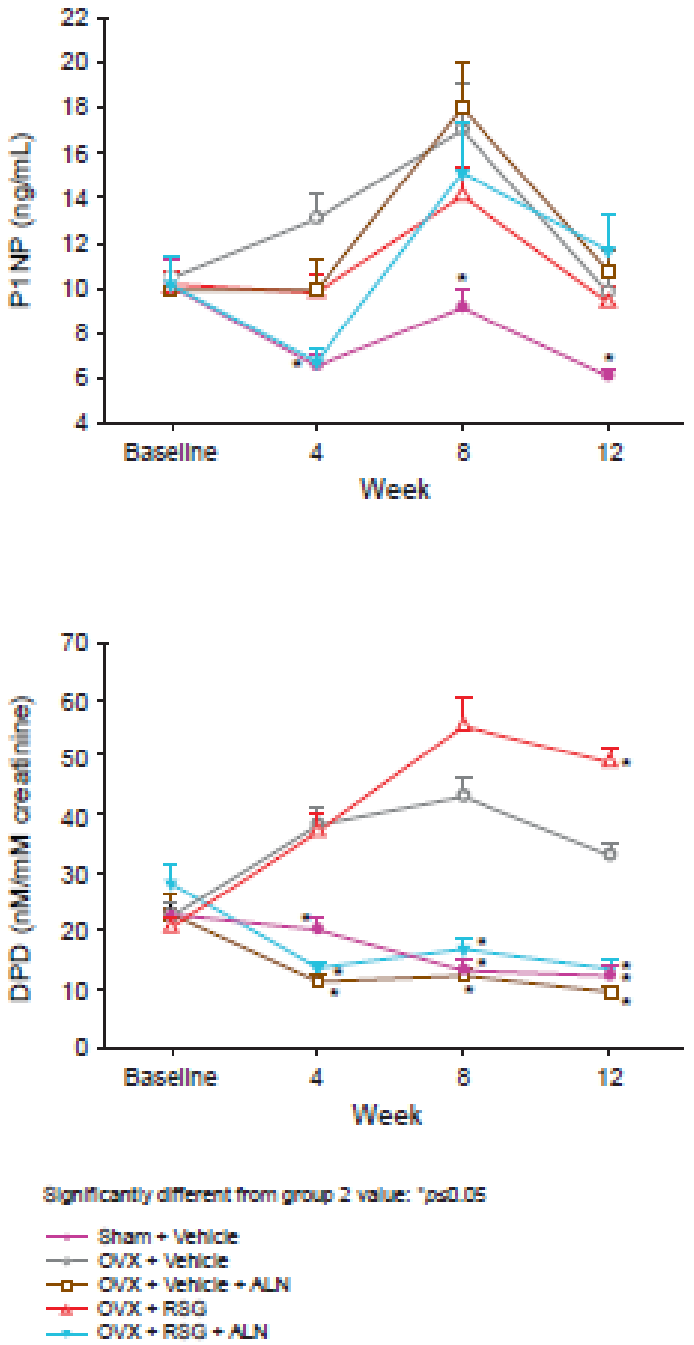
The Effect of Rosiglitazone on Bone Mass and Fragility Is Reversible and Can Be Attenuated With Alendronate

Biochemical Markers of Bone Turnover

PPAR gamma agonist

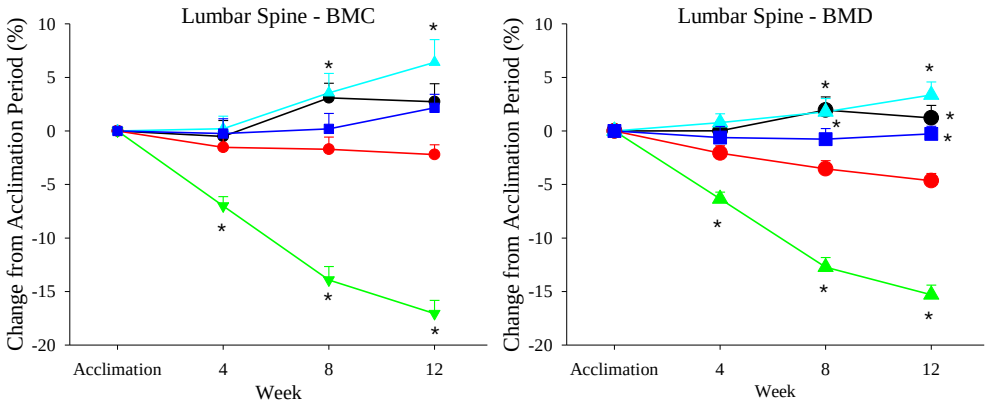
Nine-month-old Sprague-Dawley rats underwent OVX or sham operation and randomized to receive vehicle, RSG, ALN, or RSG plus ALN for 12 weeks. All treatment started the day after ovariectomy. The OVX and RSG groups also underwent an 8-week treatment-free recovery period.
Alendronate, an anticatabolic agent completely prevents PPAR gamma agonist (Rosiglitazone=RSG) induced loss of bone mass in OVX rats.
Changes in **procollagen type 1 N-propeptide (PINP) and deoxypyridinoline (DPD)** over time

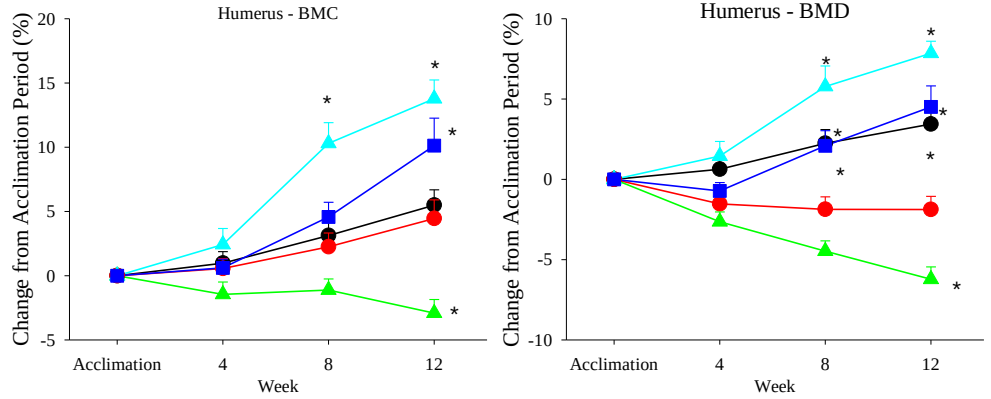
Changes are mean ±SEM
OVX = ovariectomized
RSG = rosiglitazone
ALN = alendronate

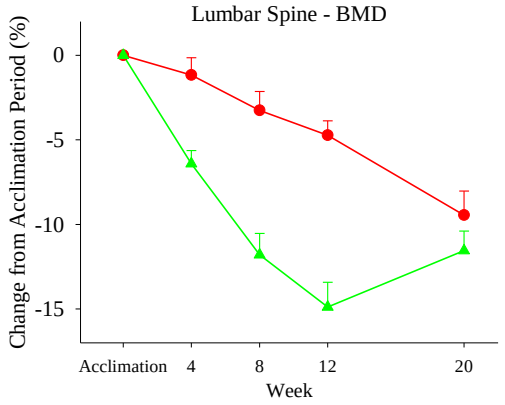


Kumar et al, 2013 JBMR

ADVANCED IN VIVO MEASUREMENTS - DXA







Changes at 12 weeks in BMC and BMD for lumbar spine and humerus

OVX = ovariectomized
RSG = rosiglitazone
ALN = alendronate

Sham Vehicle
OVX Vehicle
OVX RSG 10 mg/kg/day
OVX Alendronate
OVX RSG 10 mg/kg/day+Alendronate

Significantly different from group 2 value: *p≤0.05

Modified from Kumar et al, Journal of Bone and Mineral Research, 28-7, 2013.

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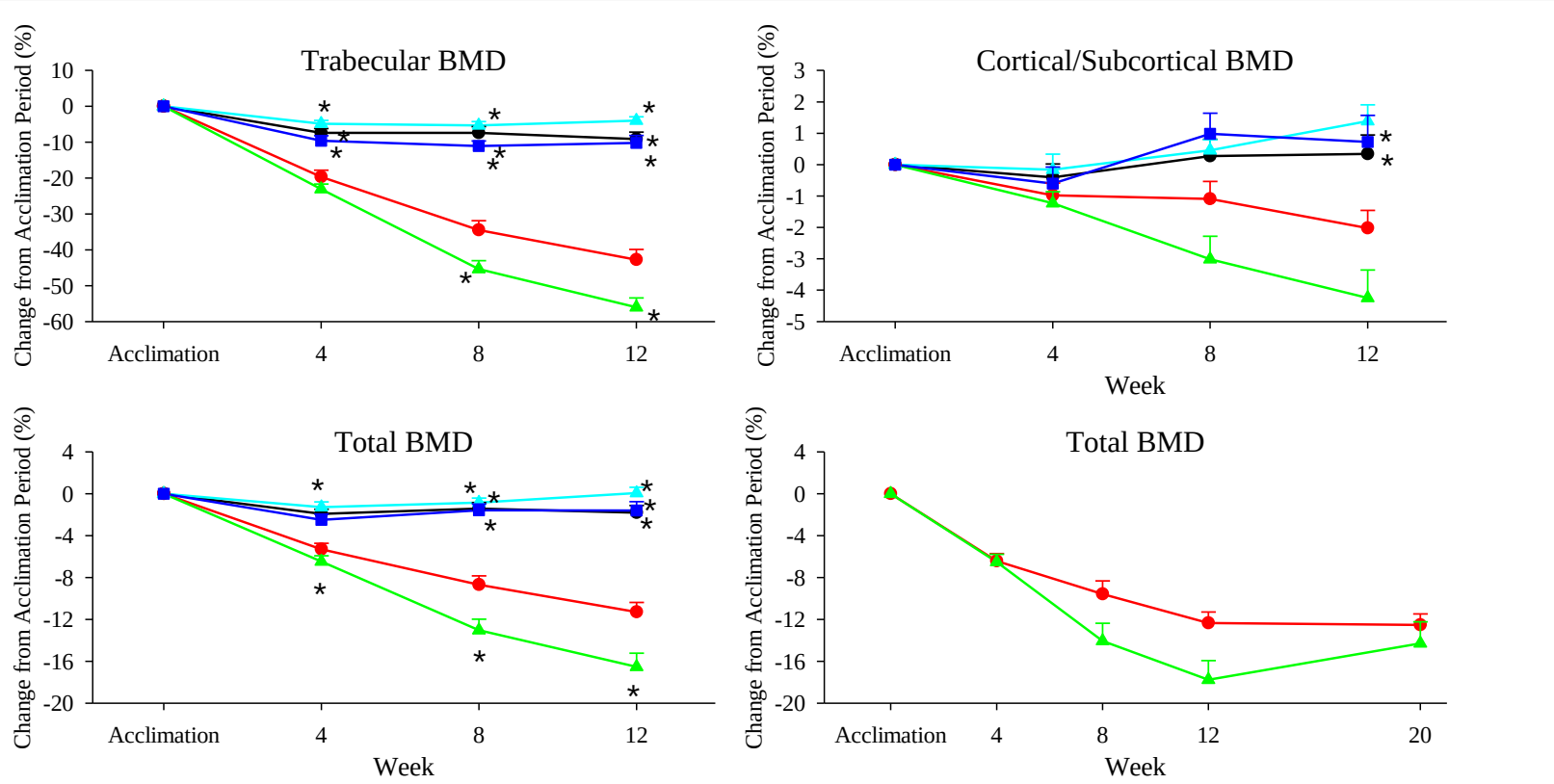
ADVANCED IN VIVO MEASUREMENTS - pQCT



Changes in trabecular, cortical/subcortical and total bone mineral density (BMD) at tibial metaphysis over time as measured by pQCT. Changes are mean ±SEM.
OVX = ovariectomized
RSG = rosiglitazone, ALN = alendronate

Modified from Kumar et al, Journal of Bone and Mineral Research, 28-7, 2013.

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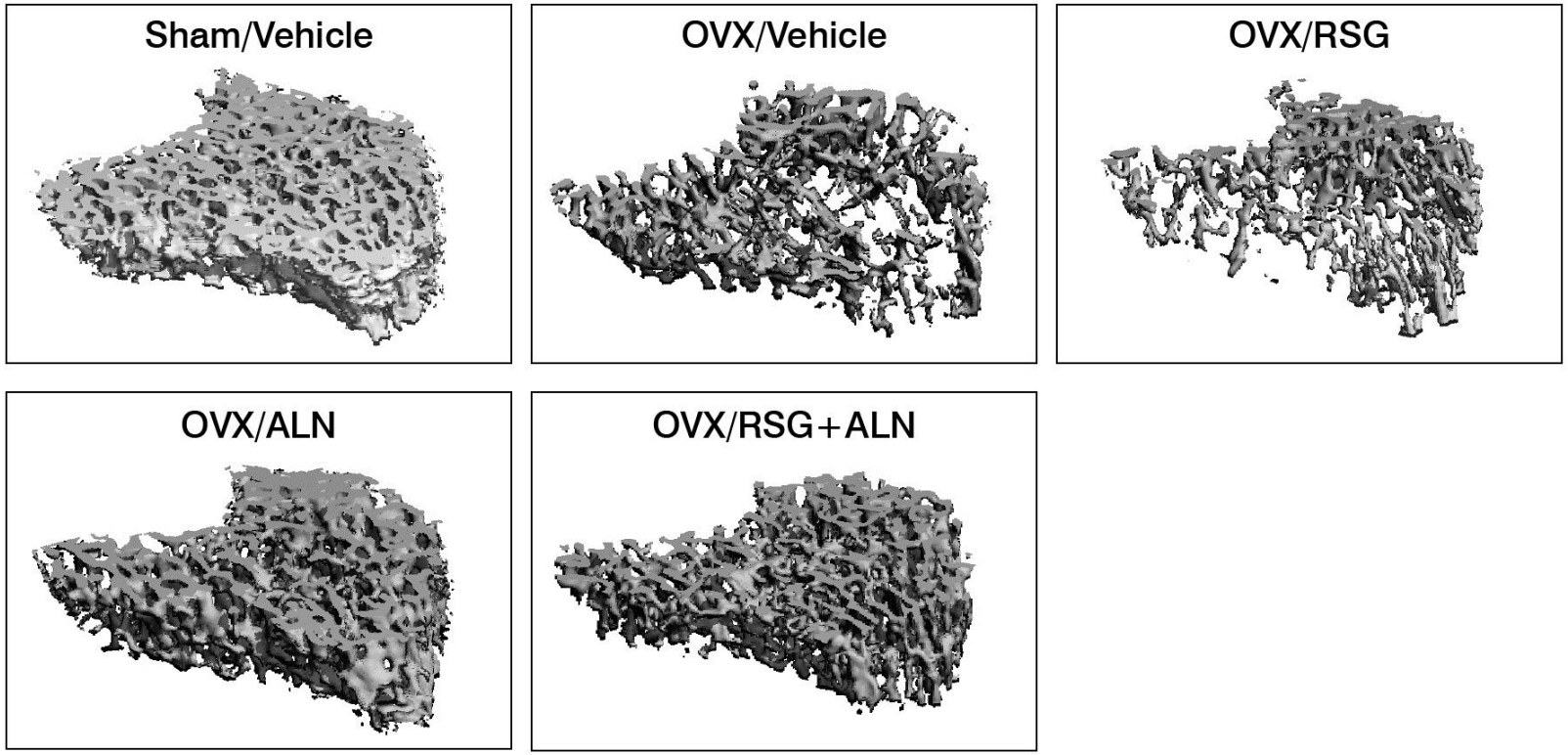
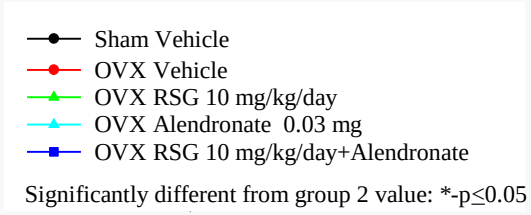
ADVANCED IN VIVO MEASUREMENTS – Micro-CT



Representative ex vivo micro-CT image of proximal tibia at 12 weeks
OVX = ovariectomized, RSG = rosiglitazone, ALN = alendronate



Changes in **trabecular bone mineral density at tibial metaphysis** over time as measured by micro-CT. Changes are mean ±SEM. OVX = ovariectomized RSG = rosiglitazone, ALN = alendronate

Modified from Kumar et al, Journal of Bone and Mineral Research, 28-7, 2013.

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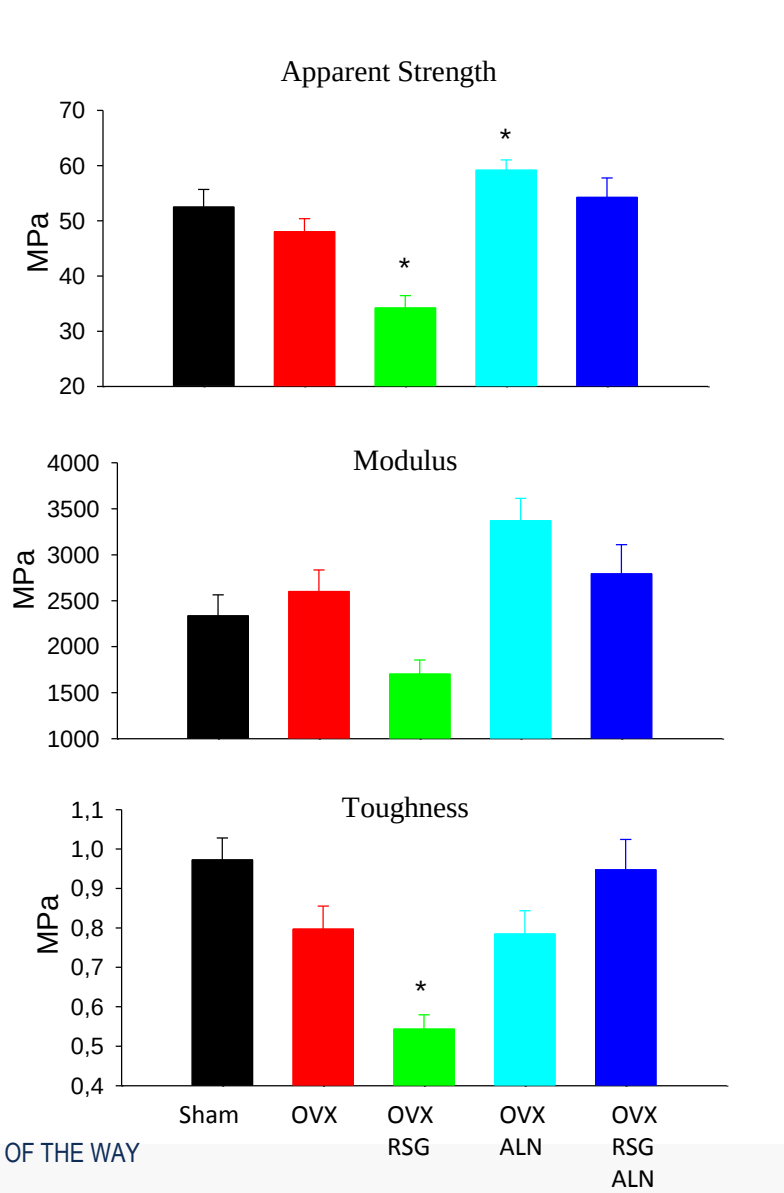
ADVANCED EX VIVO MEASUREMENTS - BIOMECHANICAL TESTING



Bone biomechanical values for the lumbar spine compression

Values are mean ±SEM
Sham
OVX = ovariectomized
RSG = rosiglitazone
ALN = alendronate

Modified from Kumar et al, Journal of Bone and Mineral Research, 28-7, 2013.

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ADVANCED EX VIVO MEASUREMENTS - BONE HISTOMORPHOMETRY

			Main study				
Surgical status **Treatment**			**Sham** **Veh**	**OVX** **Veh**	**OVX** **RSG**	**OVX** **ALN**	**OVX** **RSG+ALN**
Trabecular number	Tb.N	mm^{-1}	**3.98***** 0.13	2.50 0.21	**1.31**** 0.21	**4.28***** 0.13	**4.07***** 0.20
Osteoblast surface	Ob.S/BS	%	**0.55**** 0.11	8.09 1.77	8.04 1.69	**0.18***** 0.04	**0.17***** 0.10
Osteoclast surface	Oc.S/BS	%	1.73 0.36	2.43 0.24	3.14 0.38	**1.07*** 0.14	1.40 0.25
Fat tissue volume	Fa.V/TV	%	**2.38***** 0.49	10.14 1.31	9.02 2.38	**5.08*** 0.82	**2.47***** 0.86
BFR, volume referent	BFR/BV	%/ year	**137.93***** 27.85	376.76 32.49	**576.27*** 52.13	**32.31***** 5.03	**55.68***** 7.62

Effect of treatment on trabecular bone histomorphometry in the tibial proximal metaphysis

Values in bold are significantly different from OVX (control) group: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

ALN=alendronate; BFR=bone formation rate; OVX=ovariectomized; RSG 10=rosiglitazone 10 mg/kg/day; Veh=Vehicle control.

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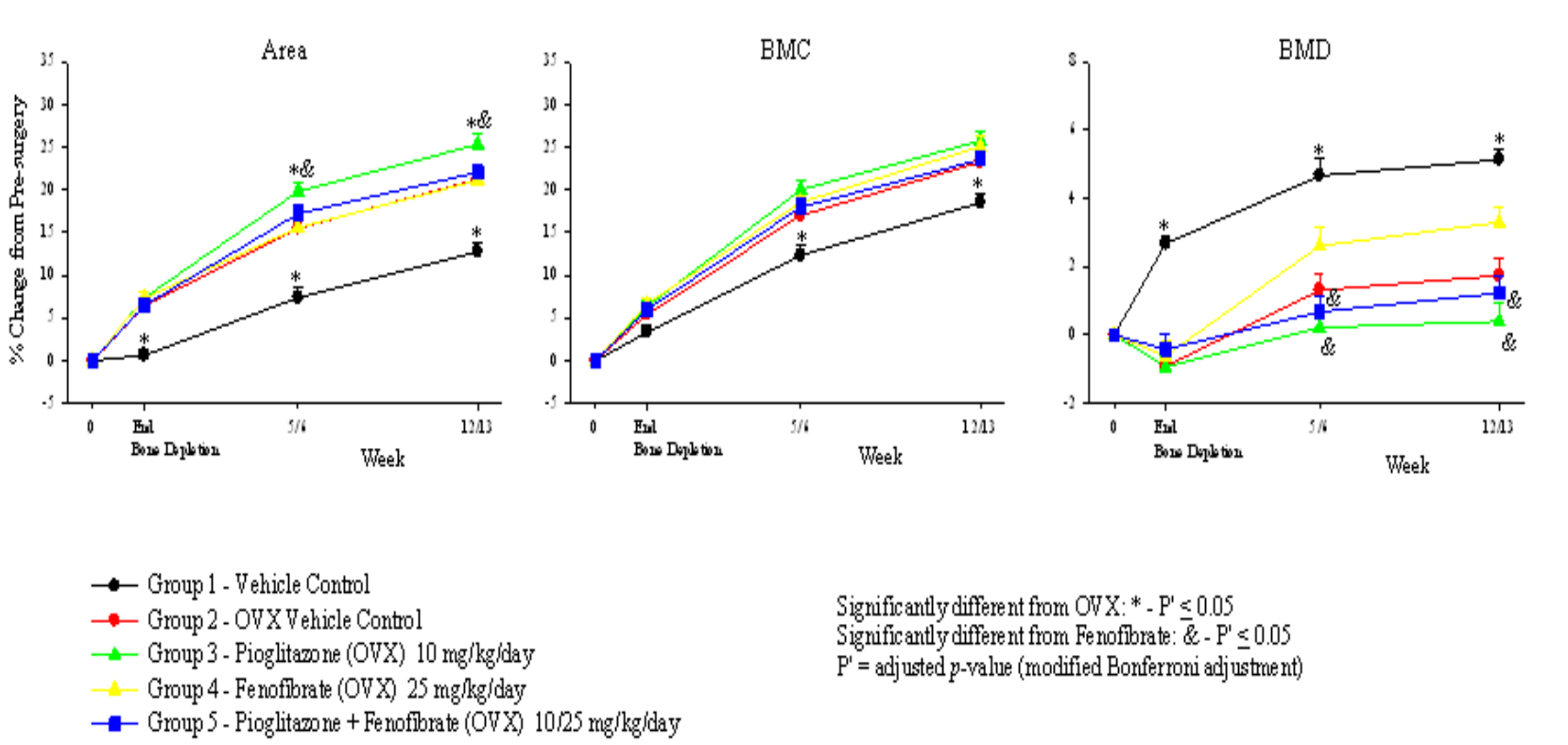
Modified from Kumar et al, Journal of Bone and Mineral Research, 28-7, 2013.

BONE TOXICOLOGY: PPAR γ agonists

Pioglitazone (PIO) and Fenofibrate (FF) on bone when administered to ovariectomized (OVX) Sprague-Dawley (SD) rats for 13 weeks followed by a 6-week treatment-free period

ADVANCED IN VIVO MEASUREMENTS - DXA

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists



Surgery – Sham or OVX
at 6 months of age
N=10
4 weeks bone depletion period
Daily oral gavage for 13 weeks
6 week treatment-free period

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists on **whole body bone densitometry** parameters in OVX rats.

Samadfam et al, 2011 J Endo 212:1-8

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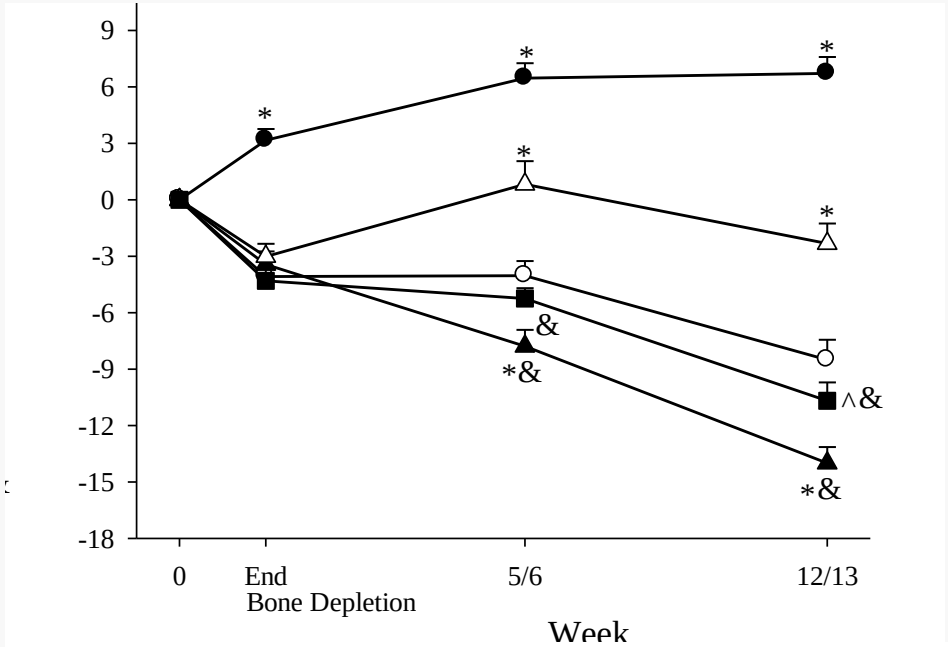
ADVANCED IN VIVO MEASUREMENTS - DXA

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists



Surgery – Sham or OVX
at 6 months of age
N=10
4 weeks bone depletion period
Daily oral gavage for 13 weeks
6 week treatment-free period

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists on **Lumbar Spine BMD** in OVX rats.

Please note: For Treatment-Free Animals Statistical analysis was performed for week 19 only

Significantly different from OVX: * - P' ≤ 0.05
Significantly different from Pioglitazone: ^ - P' < 0.05≤ 0.05
Significantly different from Fenofibrate: & - P' ≤ 0.05
P' = adjusted *p*-value (modified Bonferroni adjustment)

Samadfam et al, 2011 J Endo 212:1-8

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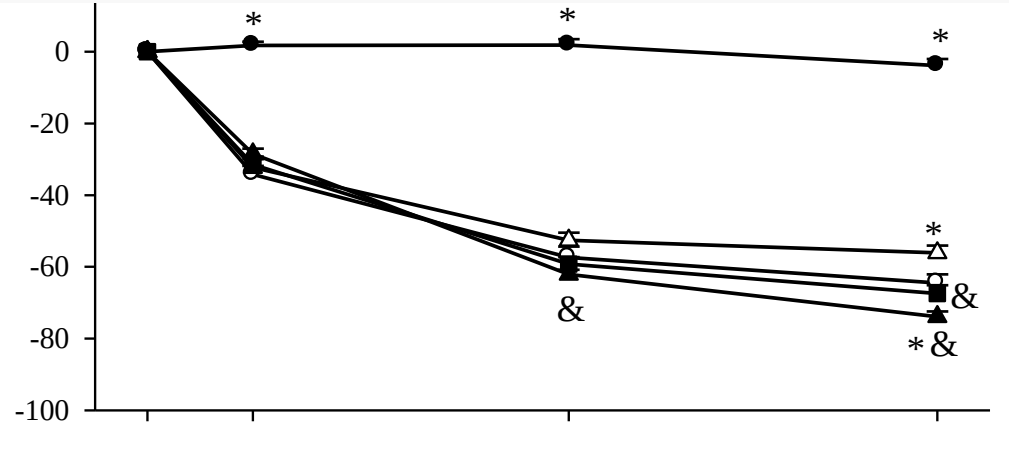
ADVANCED IN VIVO MEASUREMENTS - pQCT

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists



Surgery – Sham or OVX
at 6 months of age
N=10
4 weeks bone depletion period
Daily oral gavage for 13 weeks
6 week treatment-free period

Effects of PPAR gamma (pioglitazone) and alpha (fenofibrate) agonists on **proximal tibia Trabecular BMD pQCT densitometry** parameters in OVX rats.

- Group 1 - Sham Vehicle Control
- Group 2 - OVX Vehicle Control
- Group 3 - Pioglitazone (OVX) 10 mg/kg/day
- Group 4 - Fenofibrate (OVX) 25 mg/kg/day
- Group 5 - Pioglitazone + Fenofibrate (OVX) 10/25 mg/kg/day

Please note: For Treatment-Free Animals Statistical analysis was performed for week 19 only

Significantly different from OVX: * - P' ≤ 0.05
Significantly different from Pioglitazone: ^ - P' < 0.05≤ 0.05
Significantly different from Fenofibrate: & - P' ≤ 0.05
P' = adjusted *p*-value (modified Bonferroni adjustment)

Samadfam et al, 2011 J Endo 212:1-8

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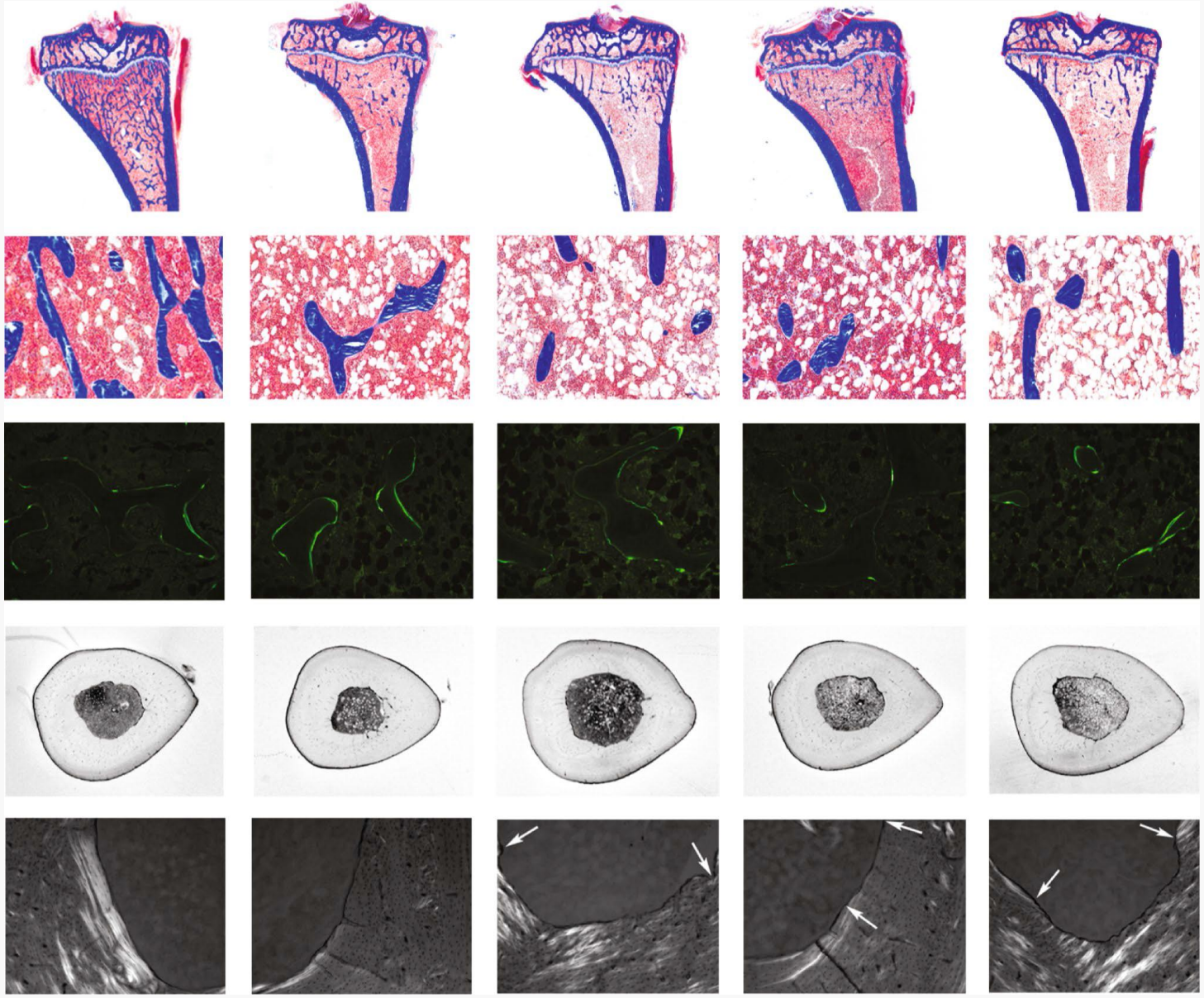
ADVANCED EX VIVO MEASUREMENTS - BONE HISTOMORPHOMETRY



Cancellous bone, bone marrow, bone turnover, cortical bone and bone resporption surface.

Columns (*left* to *right*):
sham vehicle control; OVX vehicle control
pioglitazone (OVX) 10 mg/kg
fenofibrate (OVX) 25 mg/kg
pioglitazone + fenofibrate (OVX) 10/25 mg/kg

Goldner's stain of coronal section of proximal tibia, 1×
Goldner's stain of metaphyseal bone marrow, 20x
Double calcein green labeling of unstained section of metaphyseal region at the proximal tibia, ×20
Unstained cross section at tibia diaphysis, ×2
Unstained cross section at the tibia endocortical surface illustrating smooth and lamellar endocortical surfaces in the control and OVX groups which contrast with the depressed, scalloped, irregular and eroded surfaces (shown by the area between the arrows) in the pioglitazone, fenofibrate and pioglitazone + fenofibrate groups, ×20. Note that no arrows are included in control and OVX groups as there were no areas of measureable erosion

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CONCLUSION

- Techniques and study designs are available to evaluate concerns for skeletal safety and development
- Simple, non-invasive techniques can be used to measure in vivo skeletal growth and skeleton in rodents and non-rodents using standard group sizes typical in toxicology studies
- Ex vivo bone measurements can be readily added to toxicology studies
- Imaging, Biomechanics and Histomorphometry provide complementary and comprehensive assessment of bone in preclinical studies
- Tier approach

Questions

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REFERENCES

- Smith SY, Recker RR, Hannan M, Muller R, Bauss F. Intermittent intravenous administration of the bisphosphonate ibandronate prevents bone loss and maintains bone strength and quality in ovariectomized cynomolgus monkeys. Bone. 2003 32:45-55.
- Fox J, Miller MA, Newman MK, Turner CH, Recker RR, Smith SY. Treatment of skeletally mature ovariectomized rhesus monkeys with PTH(1-84) for 16 months increases bone formation and density and improves trabecular architecture and biomechanical properties at the lumbar spine. J Bone Miner Res. 2007 Feb:22(2) 260-73.
- Fox J, Miller MA, Newman MK, Recker RR, Turner CH, Smith SY. Effects of daily treatment with parathyroid hormone 1-84 for 16 months on density, architecture and biomechanical properties of cortical bone in adult ovariectomized rhesus monkeys. Bone. 2007 Sep;41(3):321-30.
- Fox J., Newman M.K., Turner C.H., Guldberg R.E., Varela A., Smith S.Y. Effects of Treatment with Parathyroid Hormone 1–84 on Quantity and Biomechanical Properties of Thoracic Vertebral Trabecular Bone in Ovariectomized Rhesus Monkeys. Calcif Tissue Int. 2008, 82:212-220.
- Boyce RW, Aurore Varela, Luc Chouinard, Jeanine L. Bussiere, Gary J. Chellman, Michael S. Ominsky, Ian T. Pyrah. Infant cynomolgus monkeys exposed to denosumab in utero exhibit an osteoclast-poor osteopetrotic like skeletal phenotype at birth, Bone (2014), http://dx.doi.org/10.1016/j.bone.2014.04.002
- L Pouliot, M Schneider, M DeCristofaro, R Samadfam, SY. Smith, and DA Beckman. Assessment of a nonsteroidal aromatase inhibitor, letrozole, in juvenile rats. Birth Birth Defects Research (Part B) 98:374–390 (2013)

REFERENCES

- Jolette, J., Wilker, C.E., Smith, S.Y., Doyle, N., Hardisty, J.F., Metcalfe, A.J., Marriott, T.B., Fox, J., Wells, D.S., 2006. Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats. Toxicol. Pathol. 34, 929–940.
- Luc Chouinard, Melanie Felx, Nacera Mellal, Aurora Varela a, Peter Mann, Jacquelin Jolette a, Rana Samadfam a, Susan Y. Smith a, Kathrin Locher c, Sabina Buntich, Michael S. Ominsky c, Ian Pyrah c, Rogely Waite Boyce. Carcinogenicity risk assessment of romosozumab: A review of scientific weight-of-evidence and findings in a rat lifetime pharmacology study. Regulatory Toxicology and Pharmacology 81 (2016) 212-222
- Kumar S.,Sandra J Hoffman, Rana Samadfam, Peter Mansell, Jacquelin Jolette, Susan Y Smith, Robert E Guldberg, and Lorraine A Fitzpatrick. The Effect of Rosiglitazone on Bone Mass and Fragility Is Reversible and Can Be Attenuated With Alendronate. Journal of Bone and Mineral Research, Vol. 28, No. 7, July 2013, pp 1653–1665
- Samadfam R, Awori M, Benardeau A, Bauss F, Sebokova E, Wright MB, Smith SY (2012) Combination treatment with pioglitazone and fenofibrate attenuates pioglitazone-mediated acceleration of bone loss in ovariectomized rats. J Endocrinol 212:179–186
- Susan Y. Smith, Rana Samadfam, Luc Chouinard, Malaika Awori, Agnes Benardeau, Frieder Bauss, Robert E. Guldberg, Elena Sebokova, Matthew B. Wright: Effects of pioglitazone and fenofibrate co-administration on bone biomechanics and histomorphometry in ovariectomized rats. J Bone Miner Metab. 2015 Nov;33(6):625-41.