



Animal Models for Osteoporosis and Rheumatoid Arthritis

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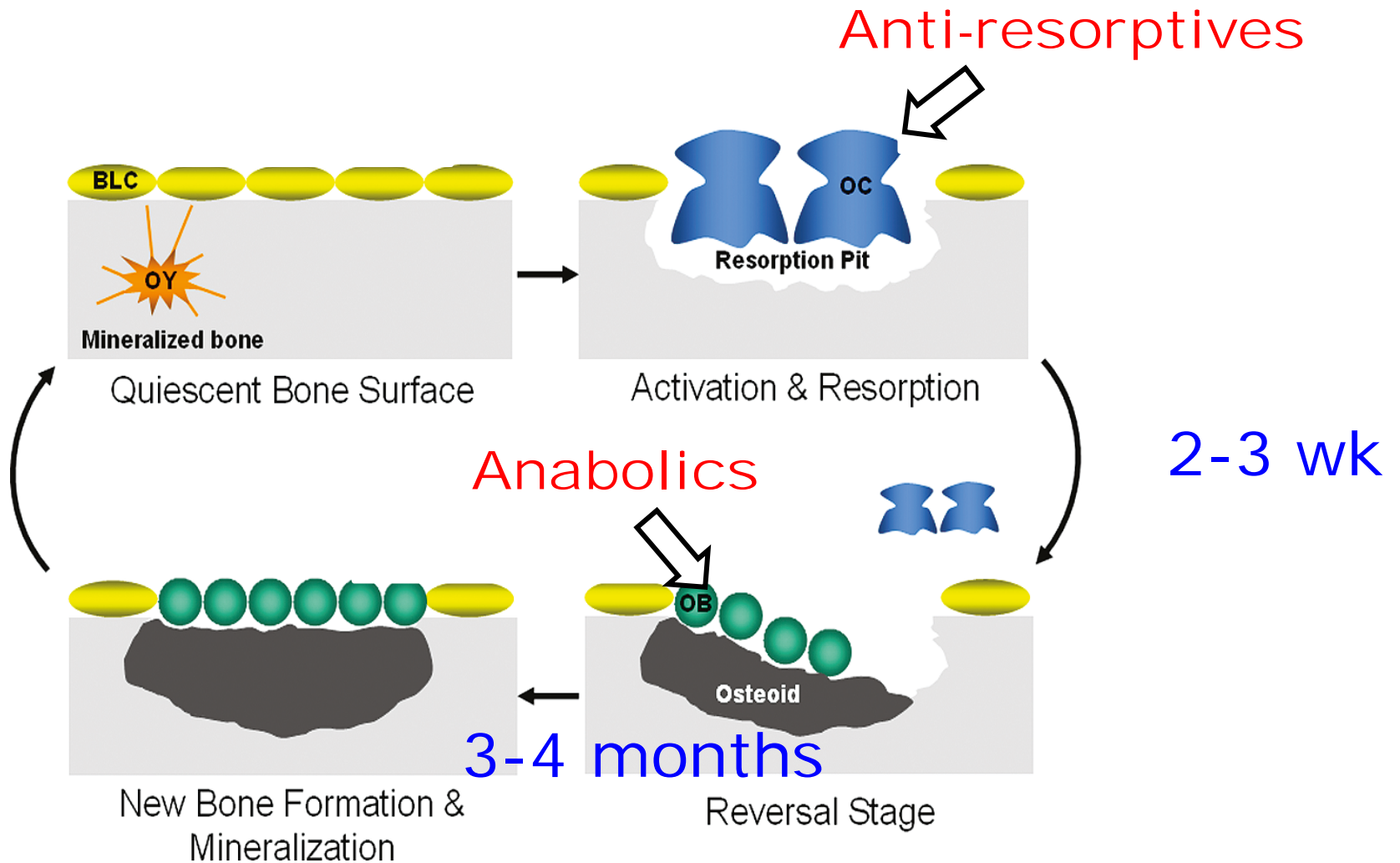


***Col: Alkem Lab, GSK, Glenmarck, Enem-Nostrum**

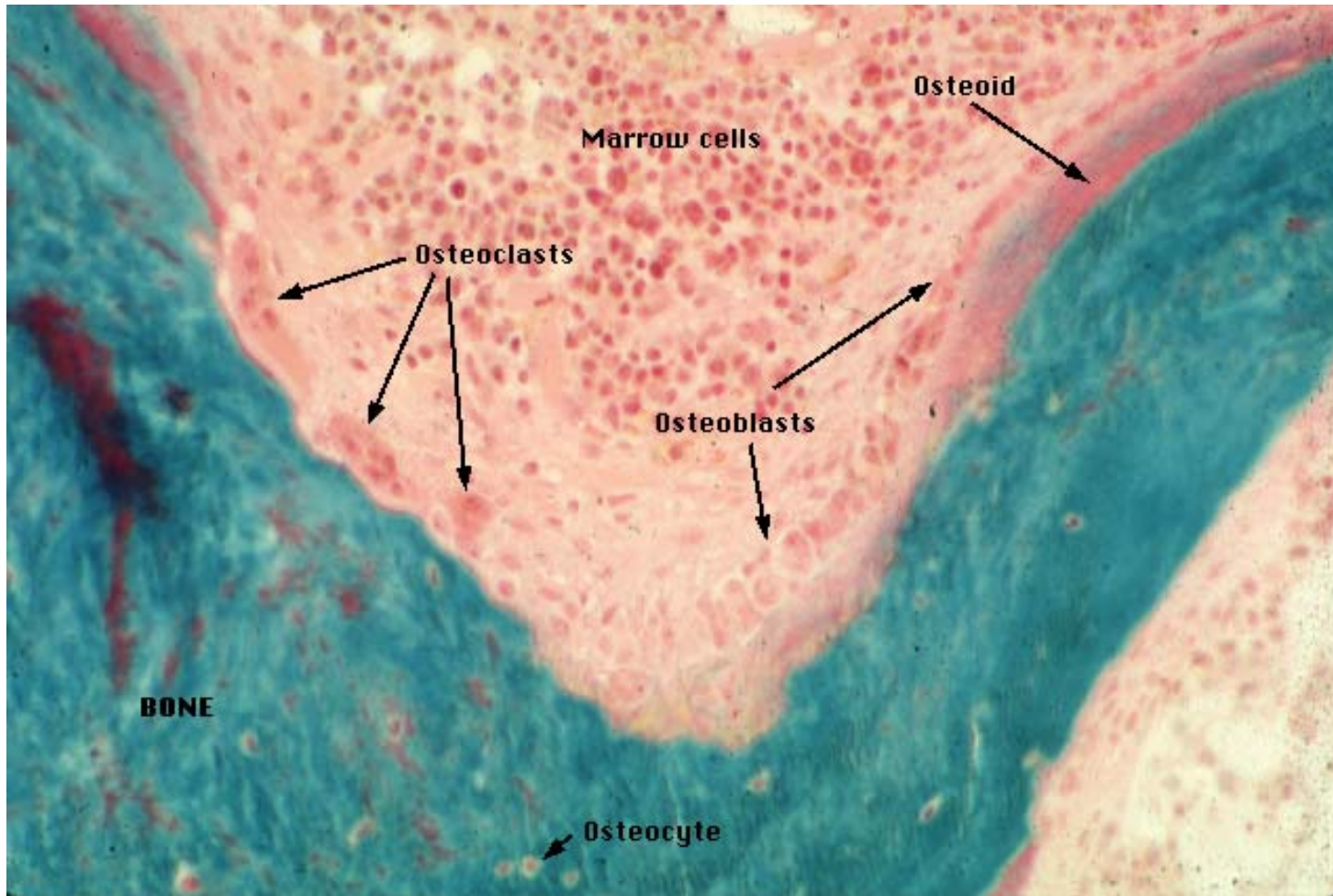
AREAS TO BE COVERED

- **Bone remodeling and modeling**
- **Bone envelopes in humans**
- **FDA guideline on PMO**
- **Preclinical models of PMO**
- **Corticosteroid-induced osteoporosis**
- **Diabetes-induced bone loss**
- **Senescence-accelerated bone loss model**
- **Rheumatoid arthritis and osteoporosis**
- **FDA guideline on RA**
- **CIA-induced RA model**
- **Other RA models**

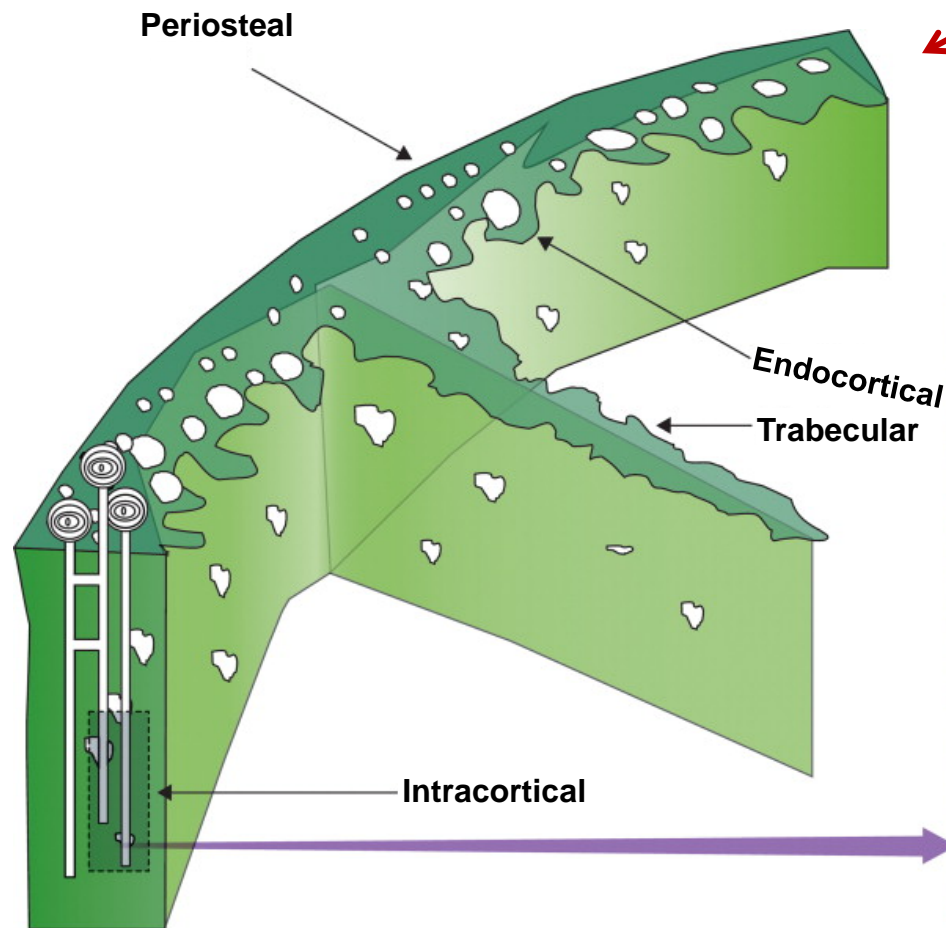
BONE REMODELING CYCLE



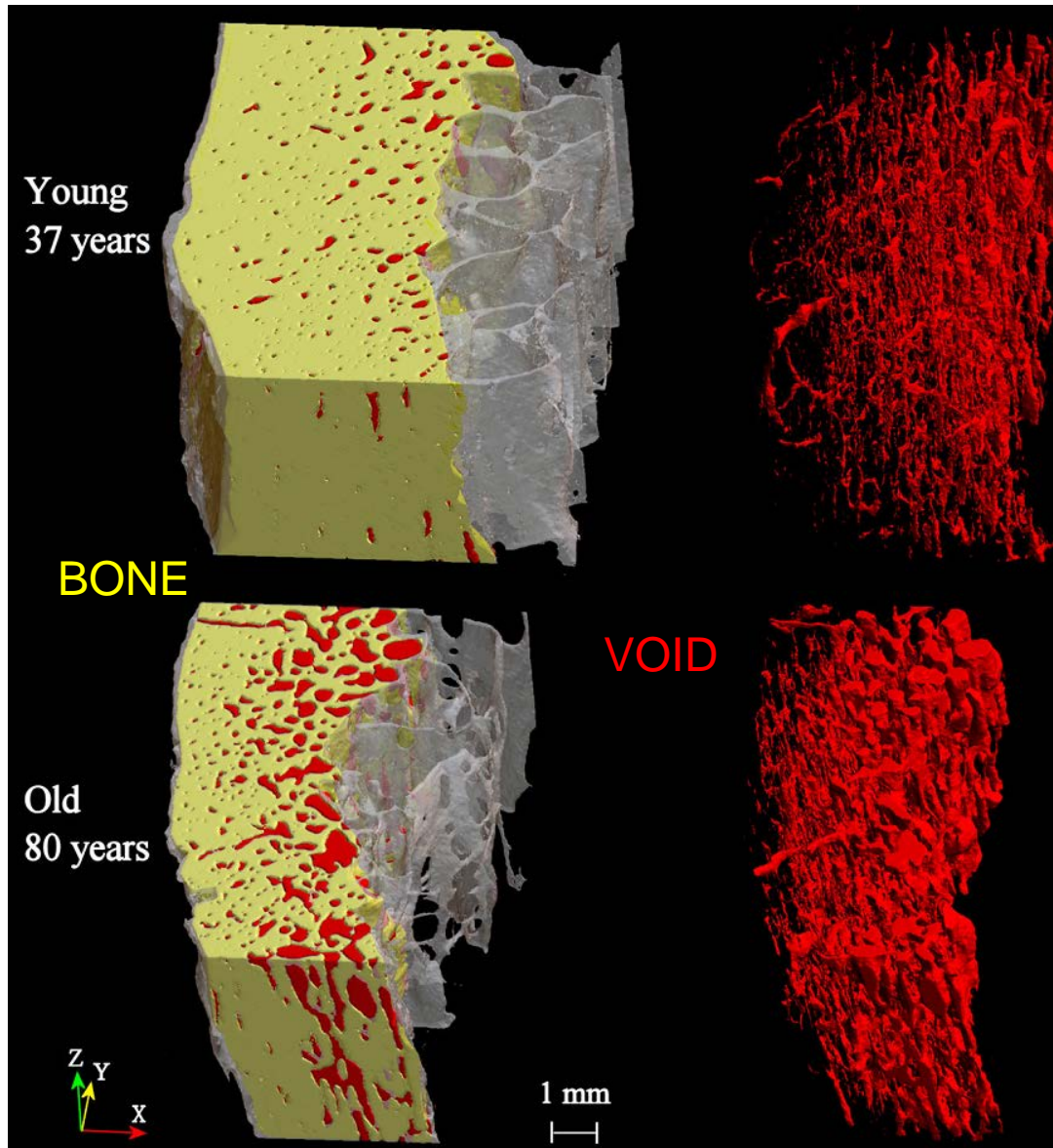
BASIC MULTICELLULAR UNITS



BONE ENVELOPES IN HUMANS



INTRACORTICAL CANALS IN HUMAN FEMUR



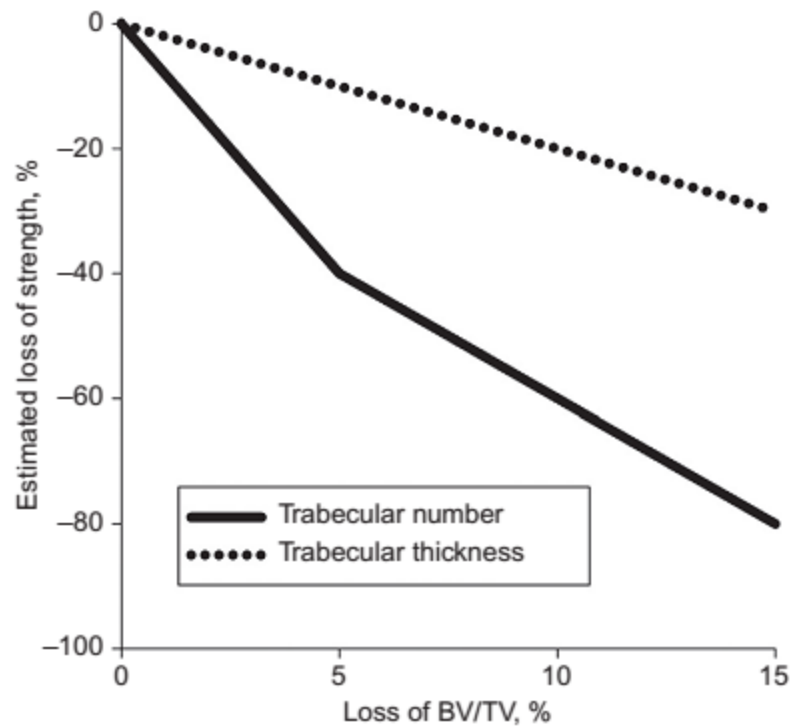
Vo.V/TV – 3%

Vo.V/TV % - 11%

Perilli et al 2015

HUMANS ARE UNIQUE!

- Bipedal primate
- Live much long after menopause
- A disproportionate loss of strength



FDA GUIDELINES ON PRECLINICAL PMO (1994)



OVX **Rodent (rats)** and a non-rodent

(Modeling) (intracortical remodeling)

(Screening) (Validation)

(12 months) (24 months)

Parameters

- Bone mass (DEXA, QCT, ash-measurement)
- Tb Static histomorphometry (structural analyses)
- Bone volume, cross-sectional area, cortical thickness, MI
- Dynamic histomorphometry
- Biomechanical analyses

FEMALE REPRODUCTIVE CYCLE AND ESTROGEN IN VARIOUS MODELS



Species	Cycle (E/M)	E2 (Basal) pg/ml	E2 (Peak) pg/ml
RAT	4-5 d (E)	7-17	50-88
DOG	1-2/year (E)	5-10	50-100
PIG	21 d (E)	5-10	80-90
RHESUS MONKEY	28-30 d (M)	10-30	150-300
HUMAN	28 d (M)	10-50	120-375

Miller et al. 1995

RAT OVX (ADVANTAGES & DISADVANTAGES)

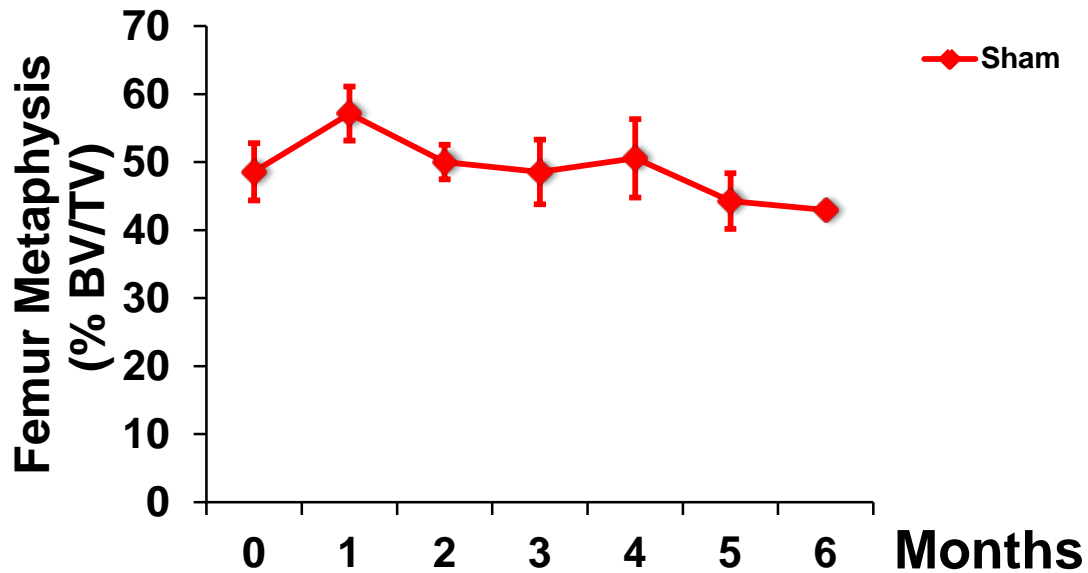
ADVANTAGES

- Lamellar bone
- Cancellous bone remodeling
- Mechanical tests under standardized condition
- No periosteal cover on femoral neck (proximal)
- OVX-induced cancellous changes = human PM

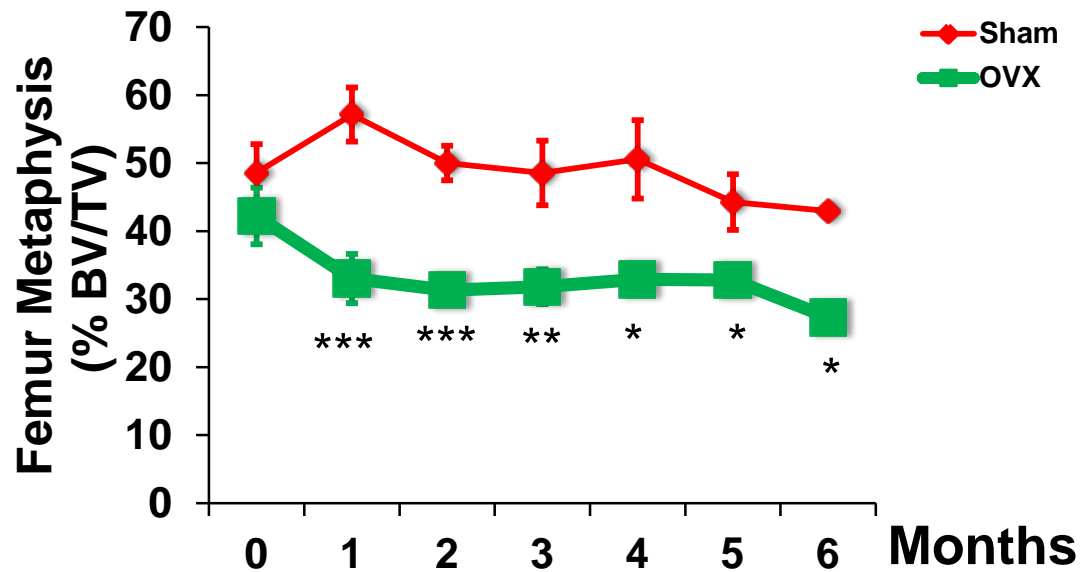
DISADVANTAGES

- Loading pattern different
- Open epiphysis (12-24 months)
- Very little intracortical bone remodeling
- Pronounced bone modeling throughout life
- Hematopoietic marrow at most sites throughout life

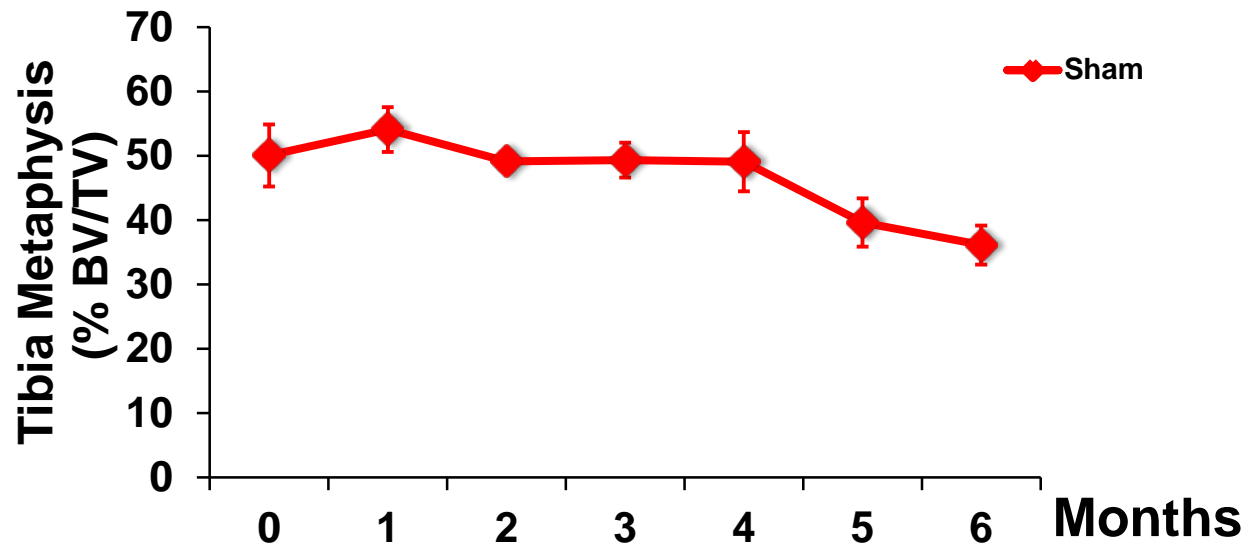
OVX (LONGITUDINAL ASSESSMENT)



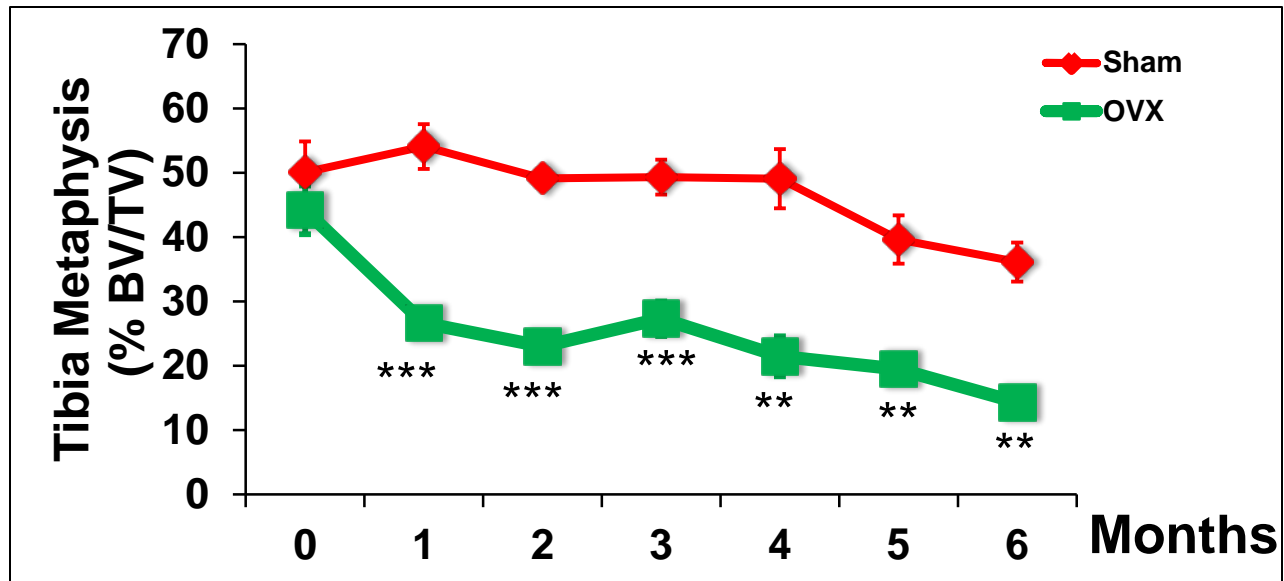
OVX (LONGITUDINAL ASSESSMENT)



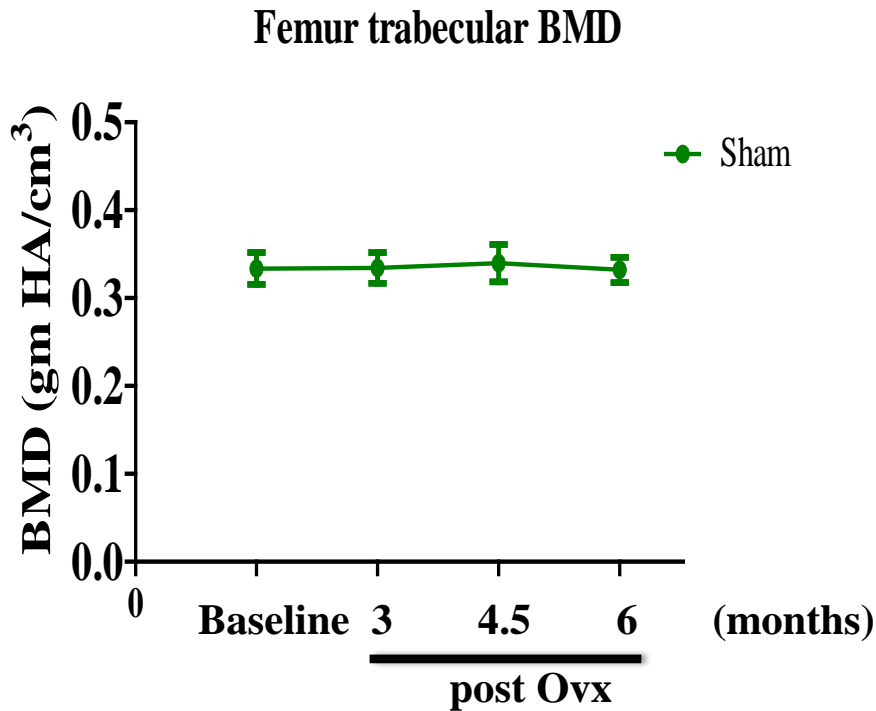
OVX (LONGITUDINAL ASSESSMENT)



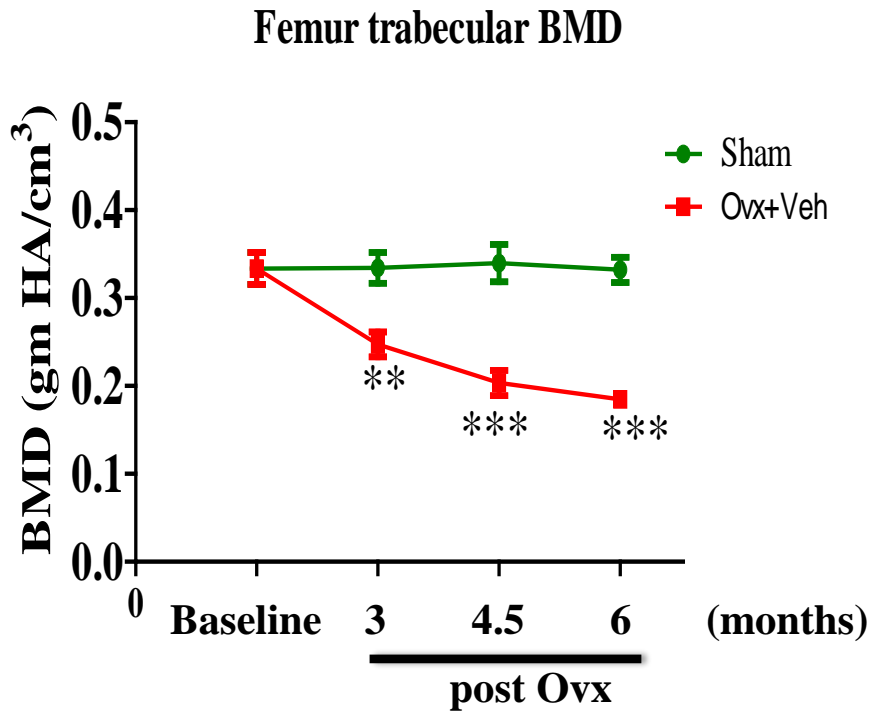
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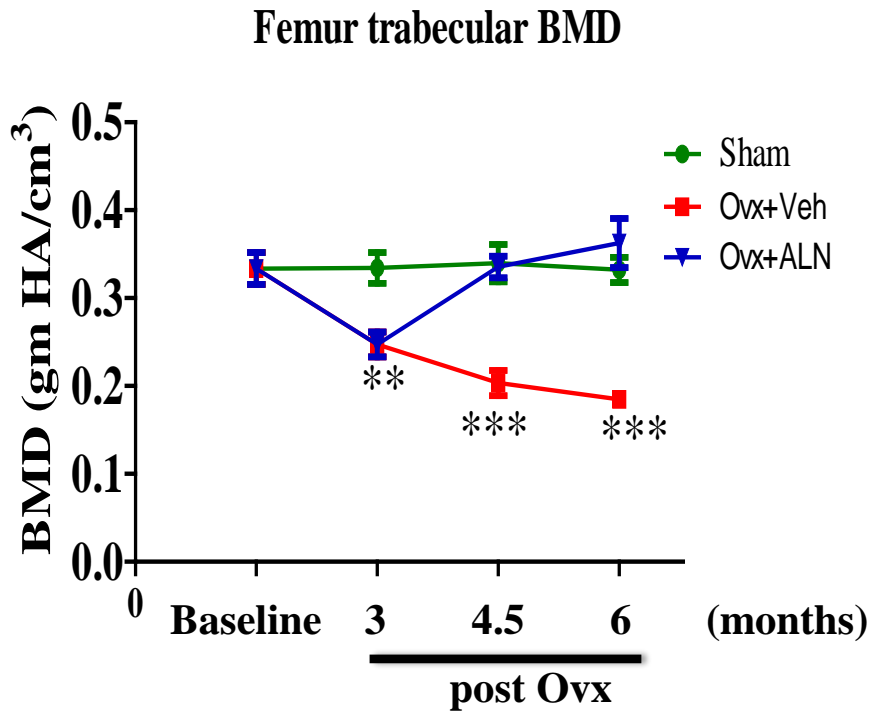
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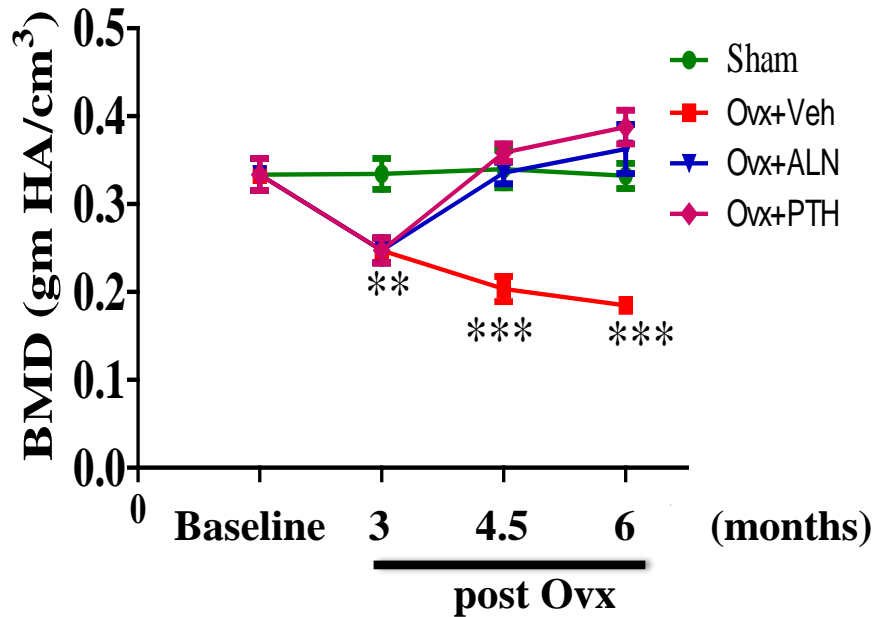


OVX (LONGITUDINAL ASSESSMENT)



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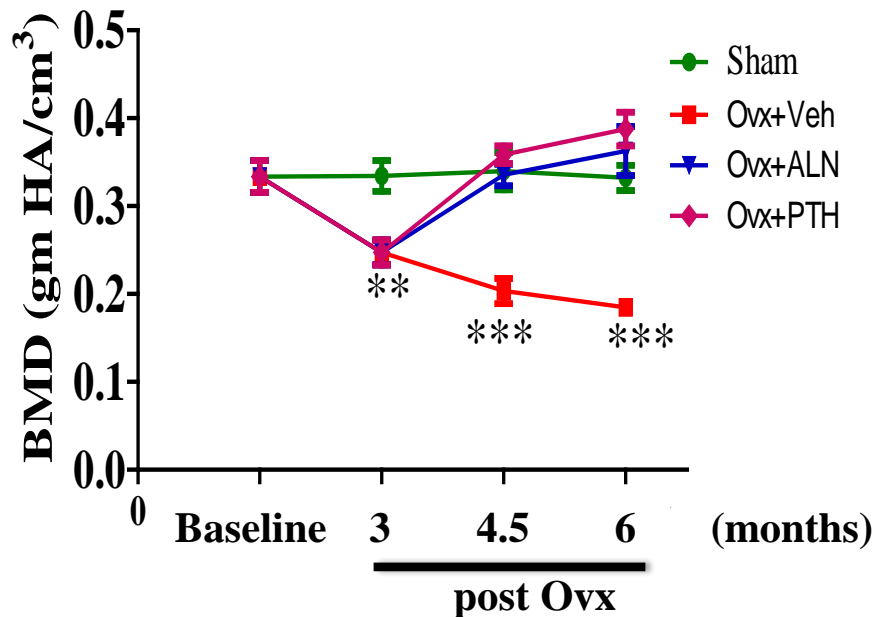
Femur trabecular BMD



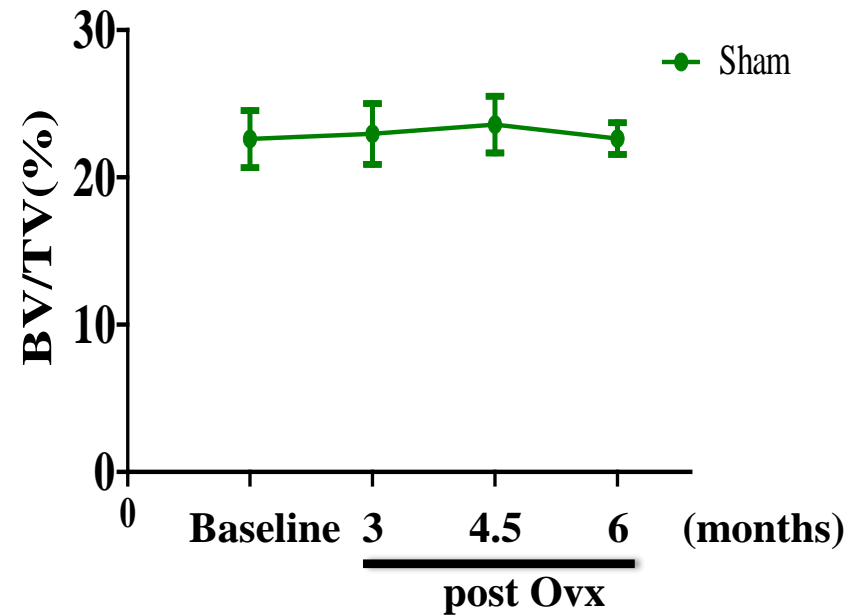
OVX (LONGITUDINAL ASSESSMENT)



Femur trabecular BMD

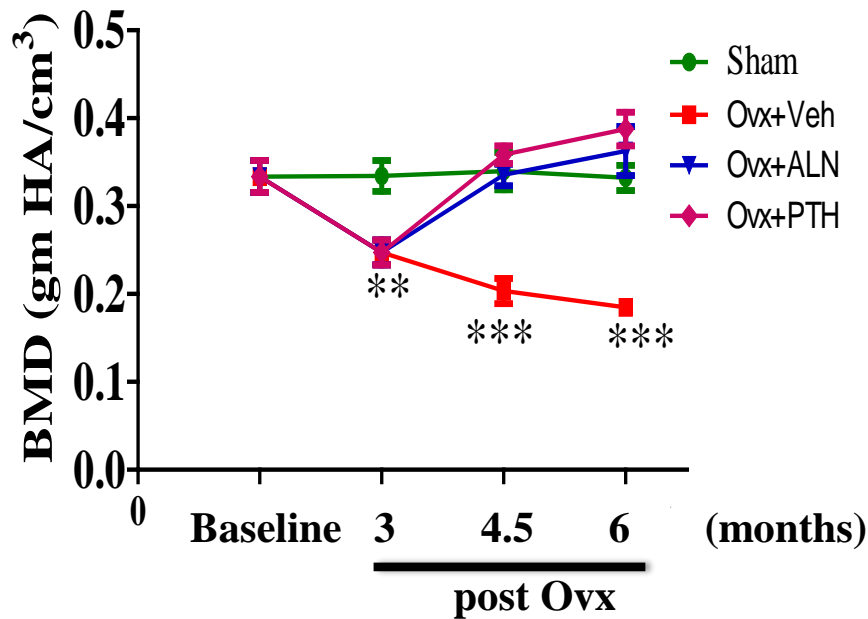


Femur trabecular BV/TV

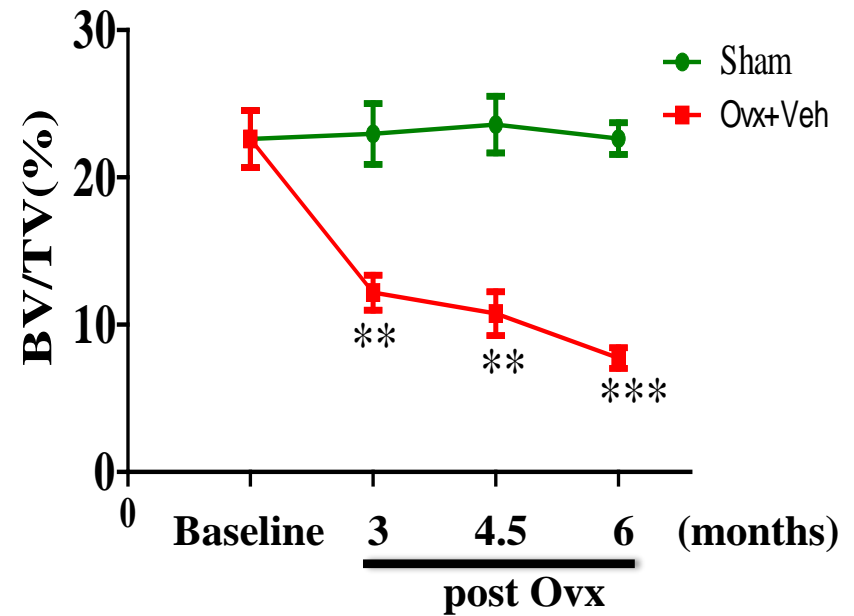


OVX (LONGITUDINAL ASSESSMENT)

Femur trabecular BMD

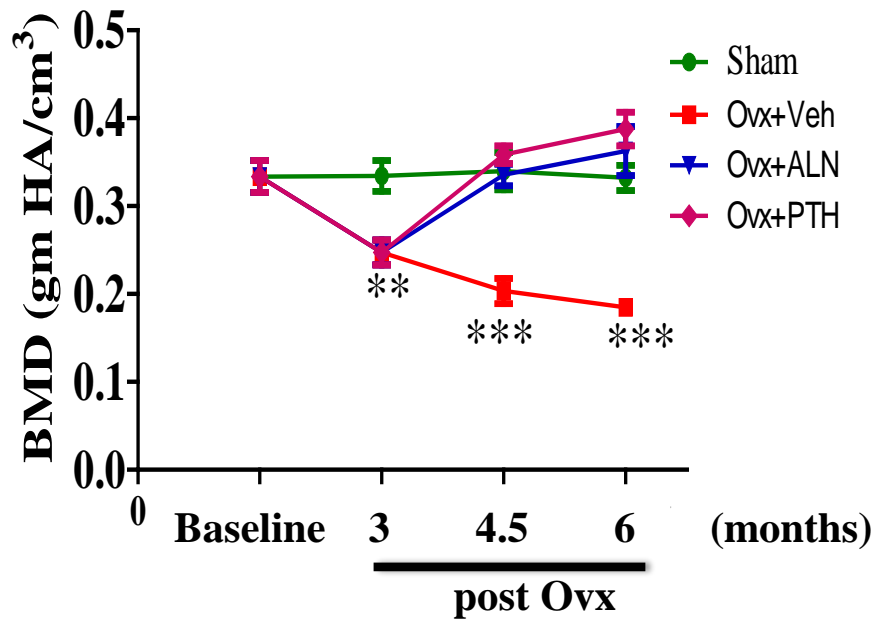


Femur trabecular BV/TV

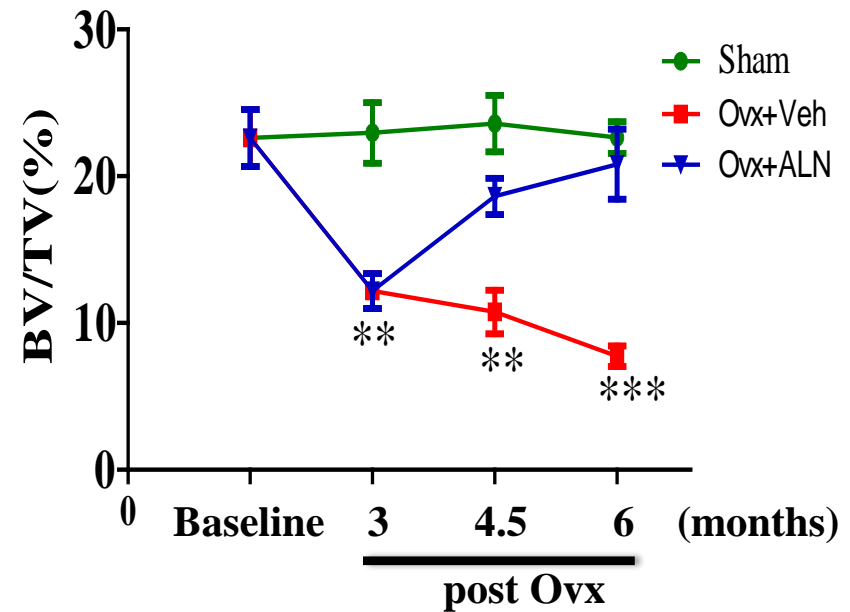


OVX (LONGITUDINAL ASSESSMENT)

Femur trabecular BMD

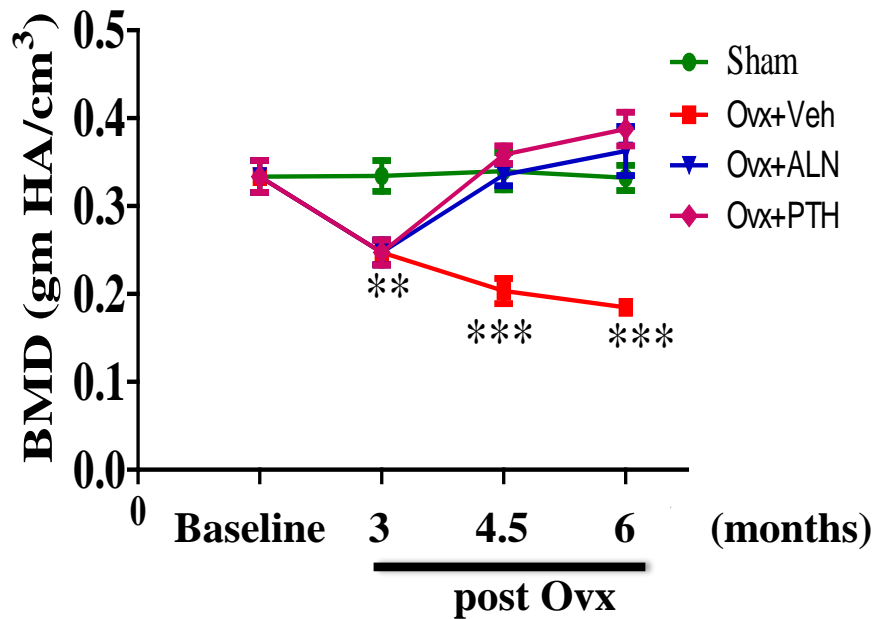


Femur trabecular BV/TV

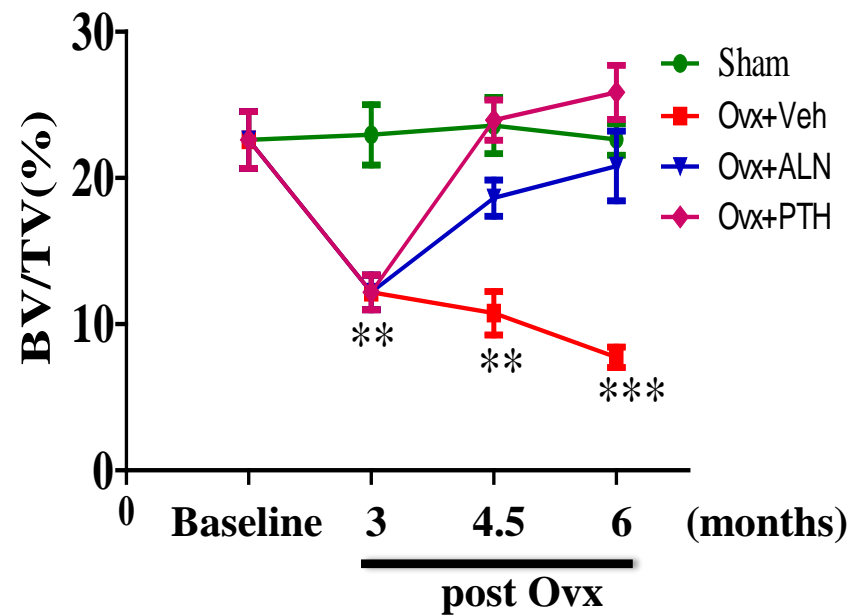


OVX (LONGITUDINAL ASSESSMENT)

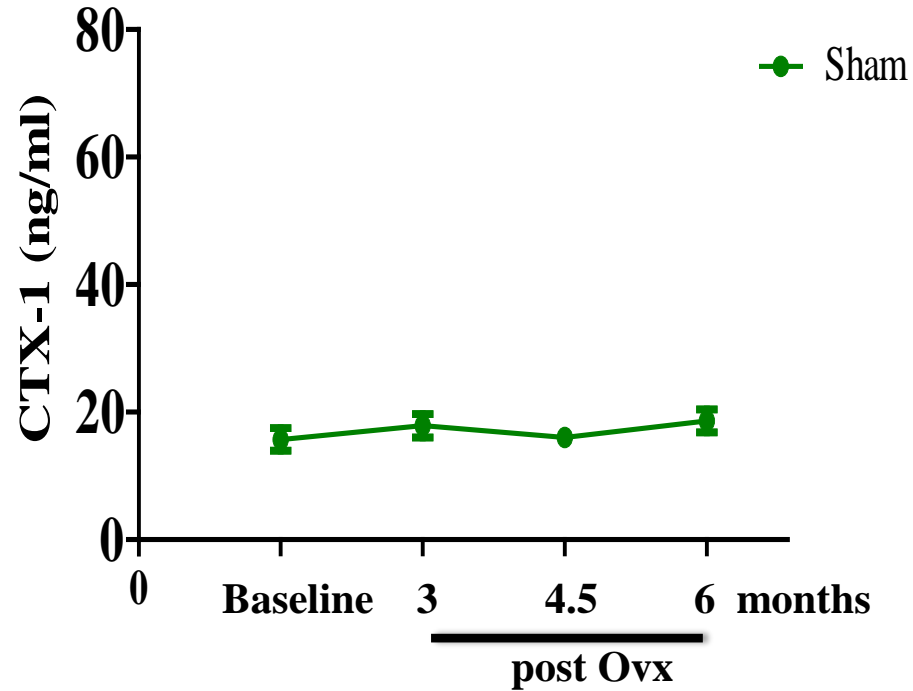
Femur trabecular BMD



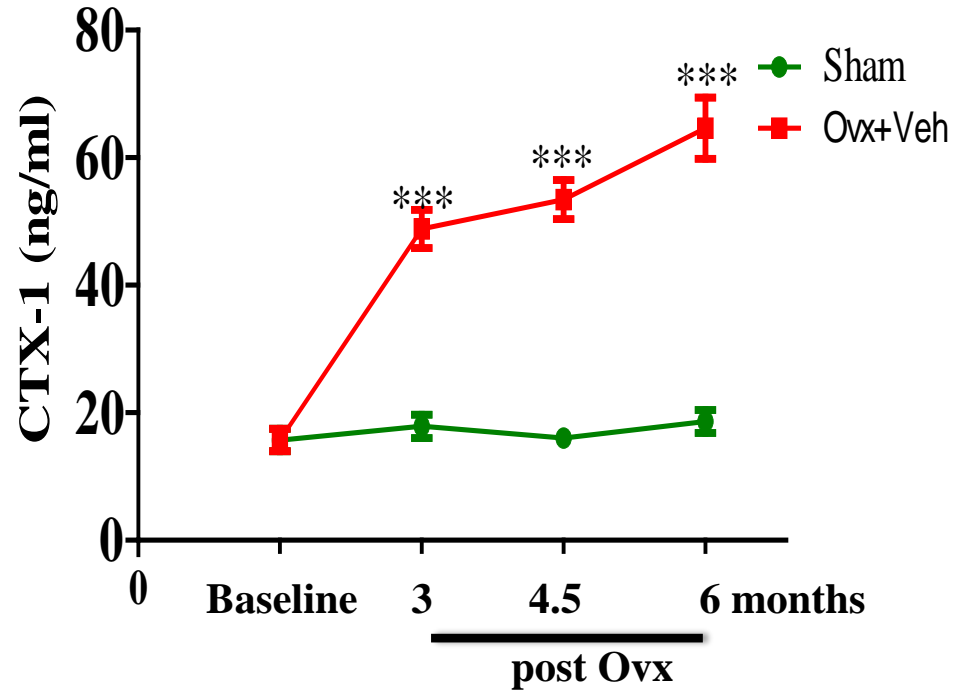
Femur trabecular BV/TV



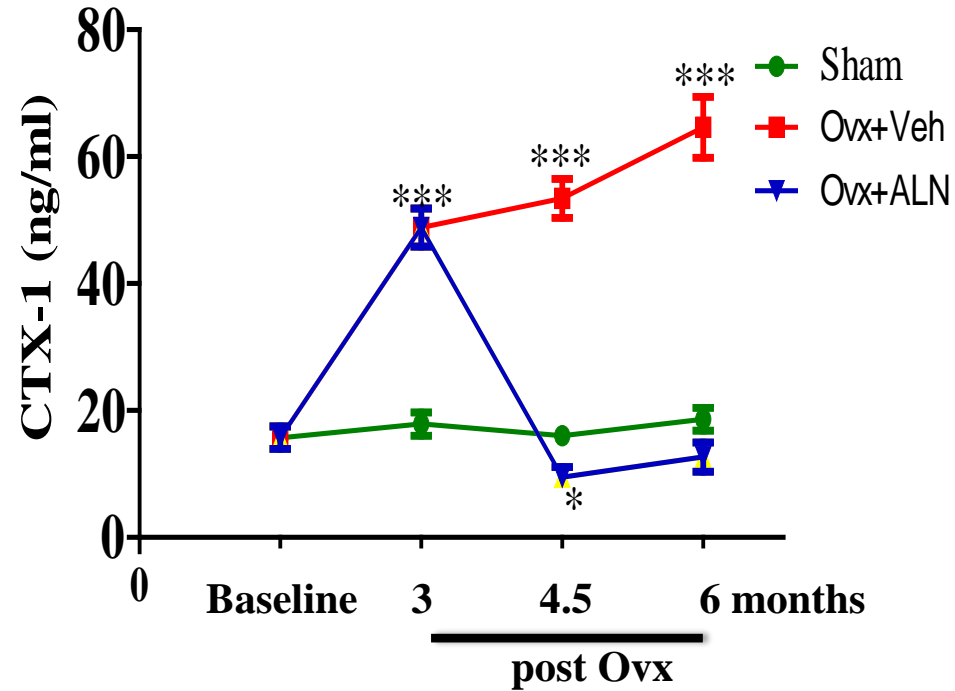
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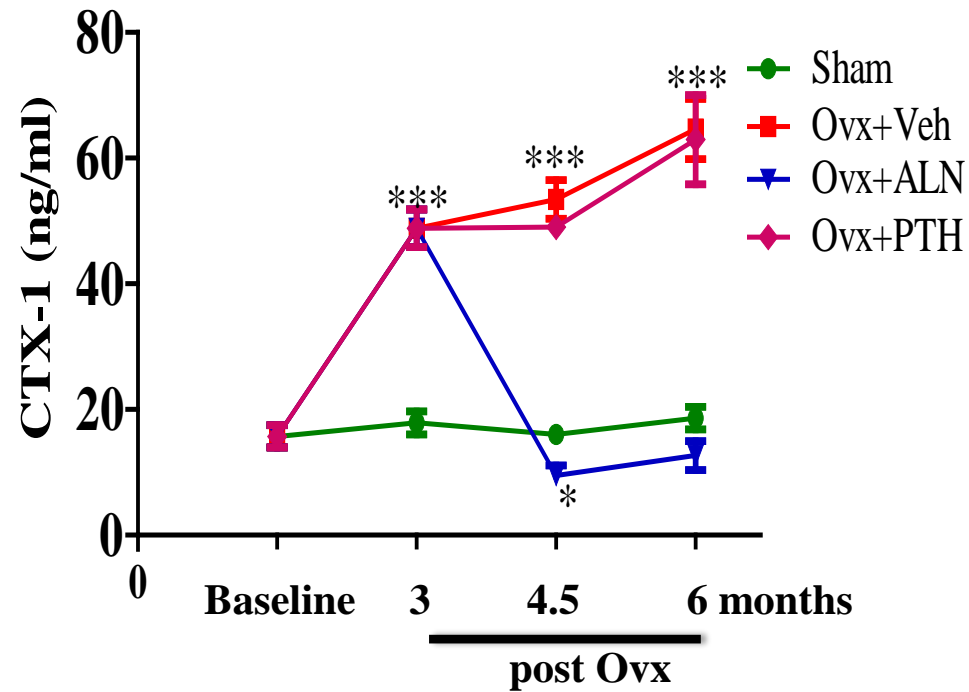
OVX (LONGITUDINAL ASSESSMENT)



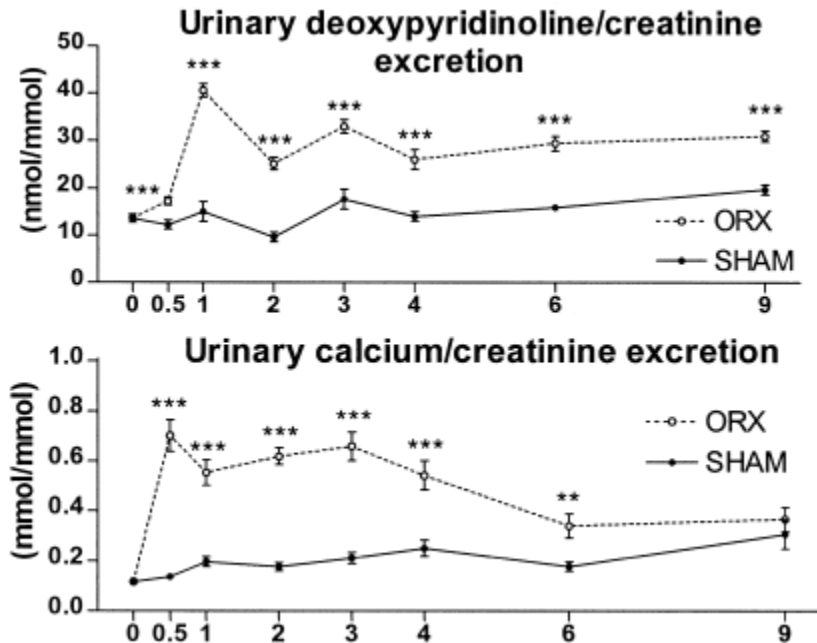
OVX (LONGITUDINAL ASSESSMENT)



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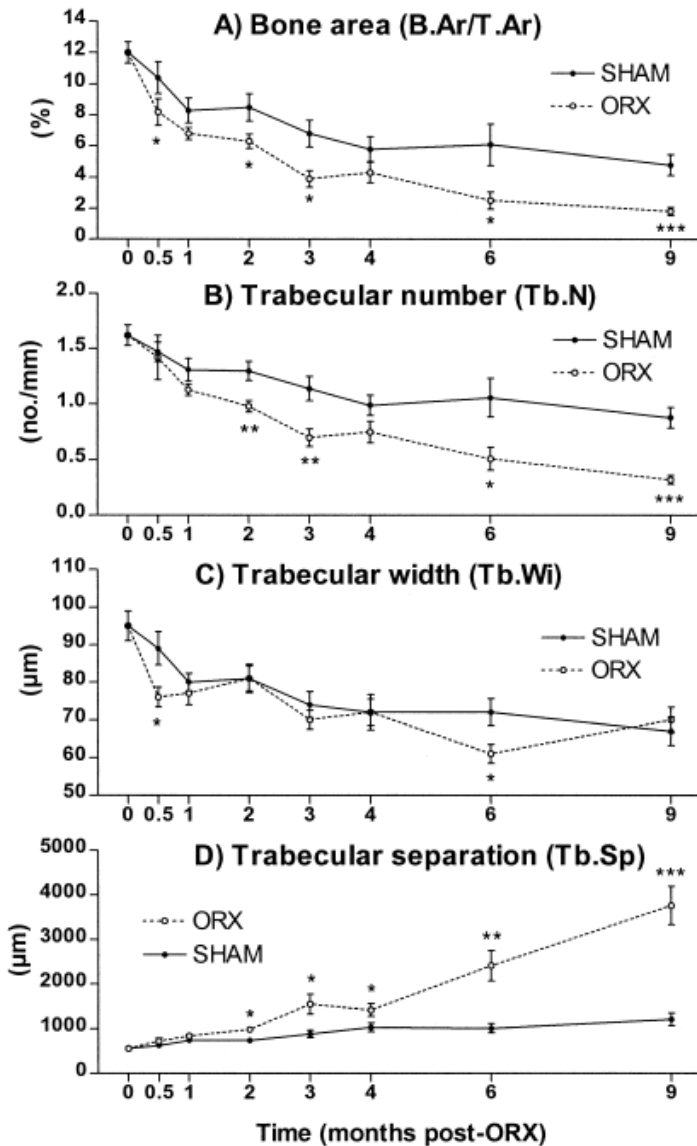
ORX IN AGED RATS



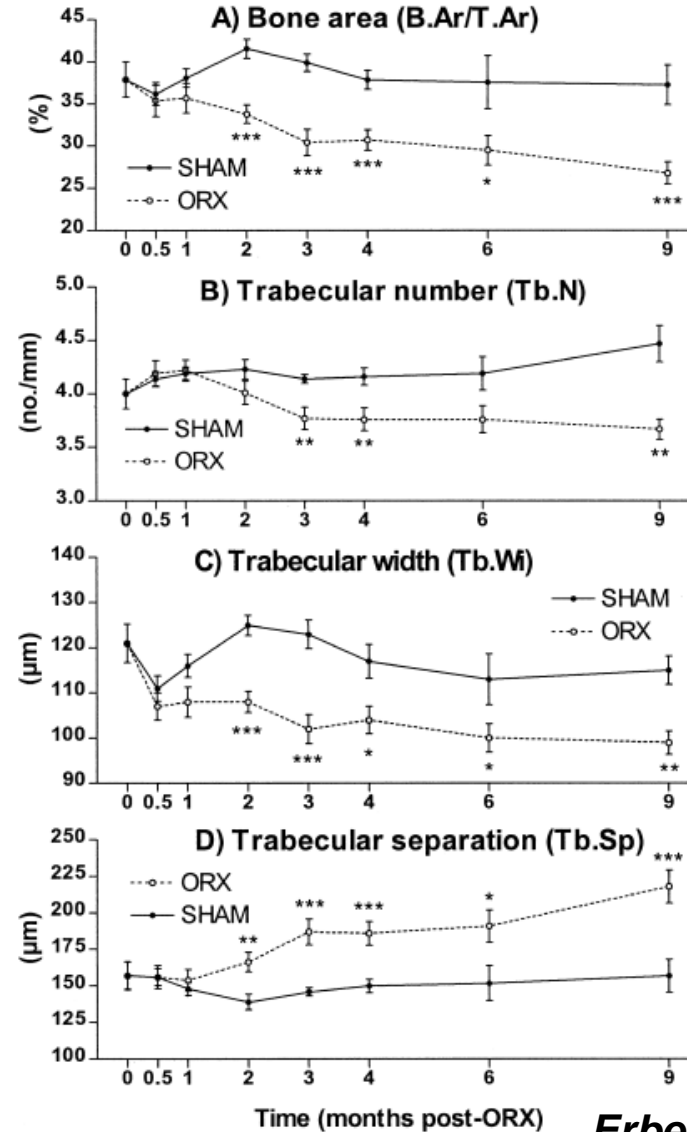
	Sham	ORX	ORX + T
Total T (ng/ml)	1.09±0.33*	0.09±0.01	2.39±0.16*
Free T (pg/ml)	1.6±0.72*	--	4.93±0.59*
5α-DHT (pg/ml)	53.5±6.1	30.8±1.4	98±12.1*
E2 (pg/ml)	1.06±0.23*	0.2±0.09	1.23±0.14*

ORX IN AGED RATS

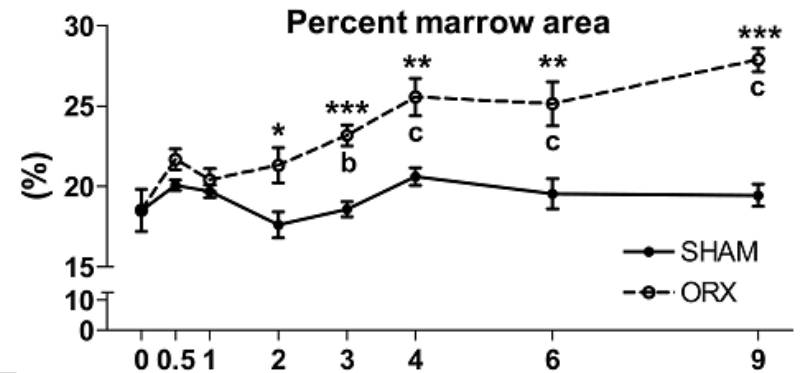
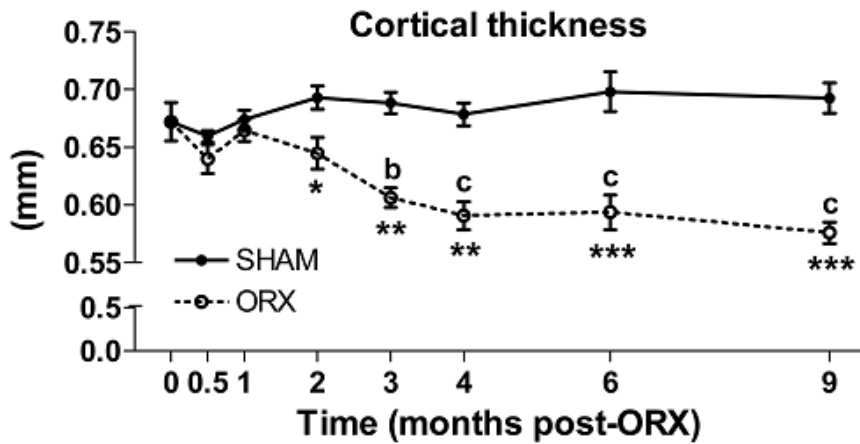
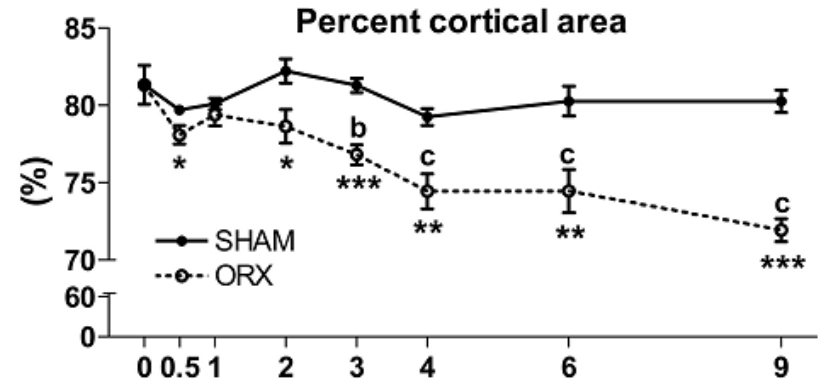
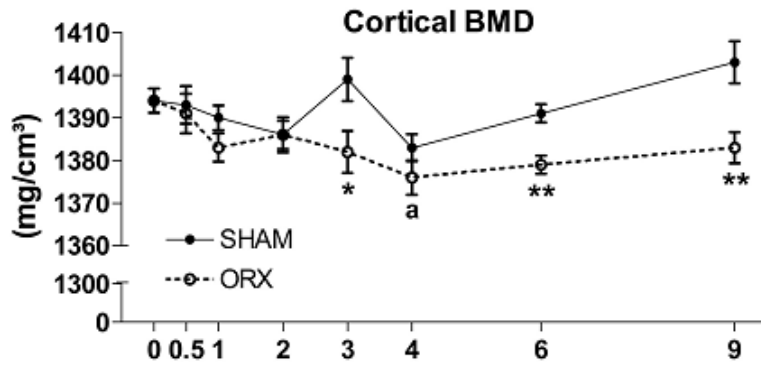
Tibia



Lumbar spine



ORX IN AGED RATS



LARGE ANIMAL MODELS

- A standardized large animal with proven intracortical bone remodeling
 - Primate, minipig, dog, sheep and ferret
- Proven osteopenic response to mechanical, hormonal or nutritional manipulation
 - Immobilization
 - Aging
 - Ca-restriction
 - OVX
 - Lactation

HUMAN VERSUS RHESUS

SIMILARITIES

- Spine loading similar when sitting
- “Menstrual cycle” and “menopause”
- Progressive loss of bone mass with aging
- Loss of cortical bone with aging in females

DIFFERENCES

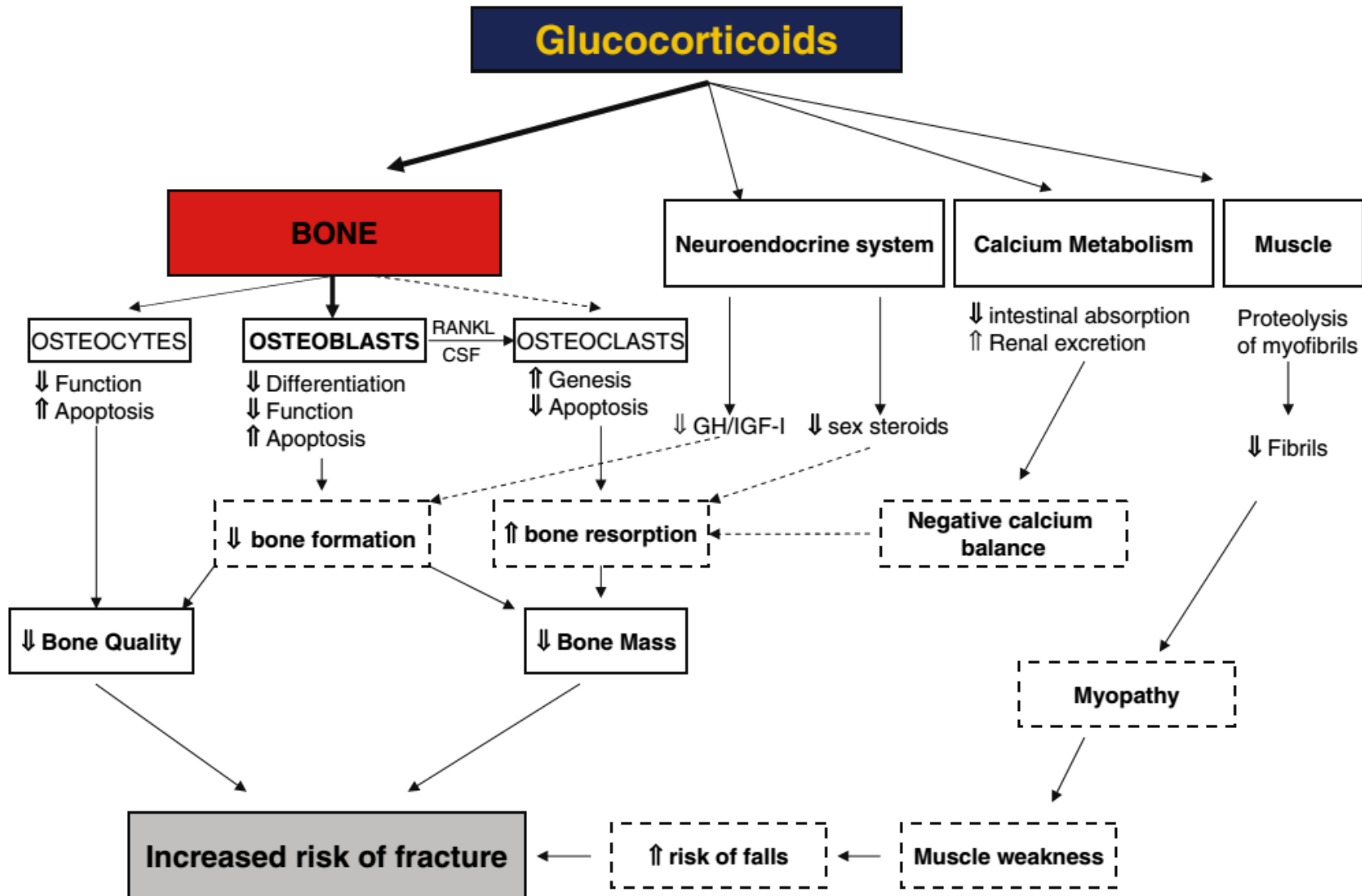
- A thick cortical shell in the vertebral bodies
- Cancellous network much denser in NHP

OSTEOPENIA IN RHESUS MONKEY

Lumbar spine	Sham	OVX (16 months)
vBMD (mg.cm ³)	506±10	460±10 (<0.05)
BMC (mg/mm)	81.2±3.3	69.5±2.8 (<0.05)
vTbBMD (mg.cm ³)	346±9	301±10
Yield load (N)	4054±175	3523±198
Yield stress (MPa)	25.4±1.1	23.3±1.1
Stiffness (N/mm)	15,908±1050	16512±828
Modulus (MPa)	960±60	884±61

Femur metaphysis	Sham	OVX (16 months)
vBMD (mg.cm ³)	699±21	685 15
BMC (mg/mm)	129±5	125±5
vTbBMD (mg.cm ³)	186±8	181±9

CORTICOSTEROID-INDUCED OSTEOPOROSIS



ANIMAL MODELS OF CS ADMINISTRATION



	Model description	Observations
Mouse	BALB/C (Dex daily, i.p.); 2-3 wk	↓ BFR, Tb.Th, serum OCN
Mouse	BALB/C (Dex daily p.o.) >4wk	ON lesions in long bones
Rat	Wistar; MP daily s.c., 4wk	↓ BFR, ↑ adipocyte in BM
Rat	S.D.; Prednisolone daily p.o. 12 wk	Tb osteopenia, ↑ adipocyte in BM
Rat	SHRSP (17 wk); MP once IM; after 2 wk	Severe early ON
Rabbit	NZ White; cortisone, daily IM, 9 wk	Osteopenia (5 wk), no ON
Rabbit	NZ White; MP, IM depot prep for 8 wk	Osteopenia (6 wk), some ON

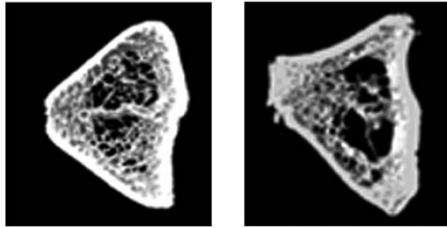
DEX VERSUS MP

	Control	Dex (0.2 mpk)
Initial wt (g)	177.9±1.9	173.3±2.4
Final wt (g)	207.7±3.9 (16%)	158.3±4.7*** (-9%, -24%)
Survival (%)	100	46.7

	Control	MP (3 mpk)
Initial wt (g)	192.6±5.2	193.9±3.5
Final wt (g)	221.3±5.9 (15%)	171.8±4.5*** (-12%, -22%)
Survival (%)	100	100
Na ⁺ (mmol/L)	110±7.2	132±0.4**
K ⁺ (mmol/L)	5.83±1.1	3.75±0.6**
Ca ²⁺ (mmol/L)	1.425±0.0 8	1.03±0.04*
Osteocalcin (ng/ml)	386±21.2	286.4±4.2***

MP-INDUCED OSTEOPENIA

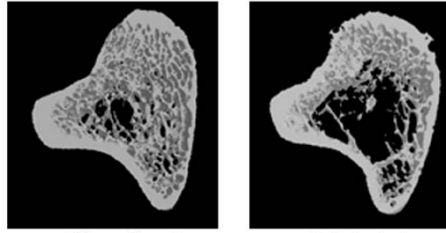
Tb. Femur



Control

MP+V

Tb. Tibia



Control

MP+V

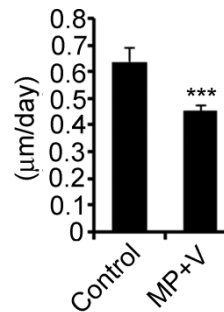
Ct. Femur



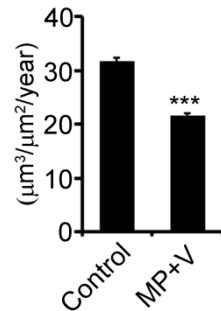
Control

MP+V

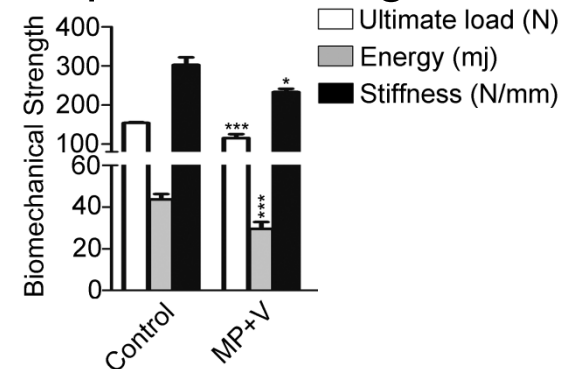
pMAR



pBFR/BS



3-point bending

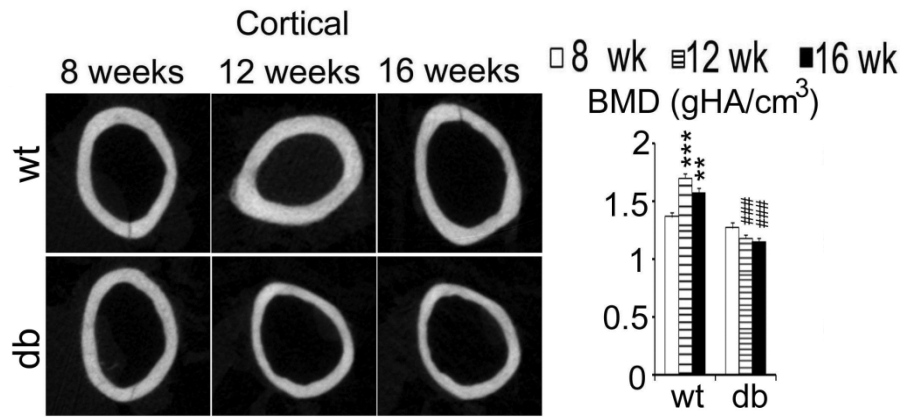
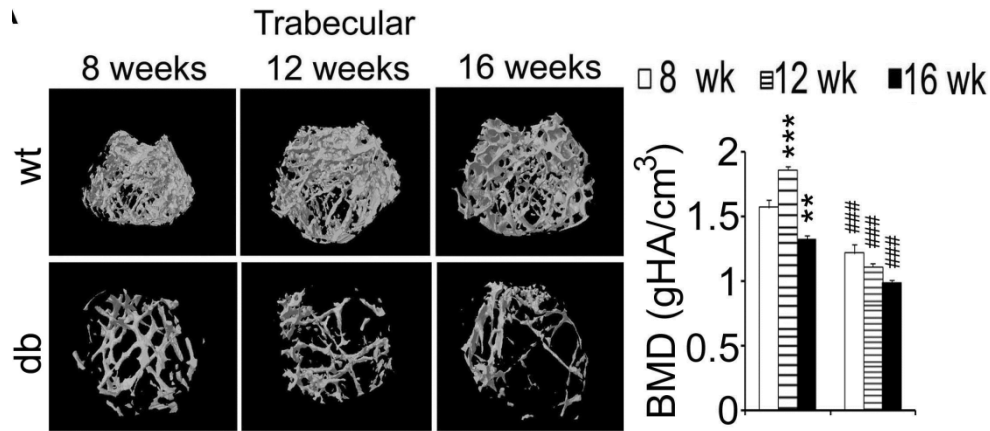


DIABETES-INDUCED BONE LOSS

- Frax at normal/high BMD levels
- AGE alters collagen fibril diameter and inhibits OB

Body composition	Age of onset	Species	BMD	Biomechanical strength
Obese	Prematurity	<i>ob/ob</i> mouse	↓	↓
Obese	Prematurity	<i>db/db</i> mice	↓	↓
Obese	Prematurity	Zucker diabetic rats	↓	↓

DIABETES-INDUCED BONE LOSS



ANIMAL MODEL FOR SENILE OSTEOPOROSIS

- **Senescence-accelerated mouse (SAMP3 & SAMP6)**

- ❖ Low peak bone mass
- ❖ Severe osteopenia after 5 months
- ❖ Spontaneous fracture in long bones
- ❖ Reduced OB and OC functions

- **Klotho mouse**

- ❖ Multi-organ defect
- ❖ Low bone turnover

RA AND OSTEOPOROSIS (TWO FACES OF THE SAME COIN)

- Focal marginal articular erosion
- Subchondral bone loss
- Periarticular bone loss
- Systemic osteoporosis

FDA GUIDELINES ON PRECLINICAL RA (1999)



- Collagen-induced arthritis (CIA)
- Naturally occurring arthritis
- Rat carrageenin-induced acute model of inflammation
- Adjuvant-induced arthritis in rats
- Streptococcal cell wall-induced arthritis
- Transgenic animal models

CIA INDUCTION

Native chicken sternal collagen type II (CII)

+

CFA containing 4mg/ml *M. tuberculosis*, strain H37Ra
(1:1)



Intradermal injection at the base of tail of 8-10 wk
DBA/1J mice (100 μ l containing 200 μ g CII)



At day 21, booster with 200 μ g CII in IFA



Assessment of clinical parameters

CIA SCORING

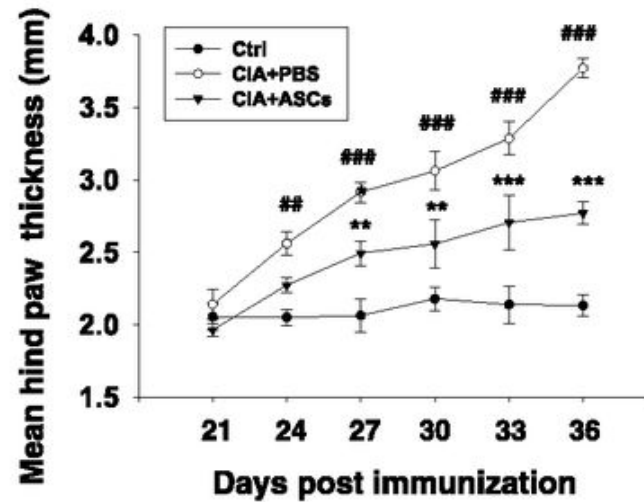
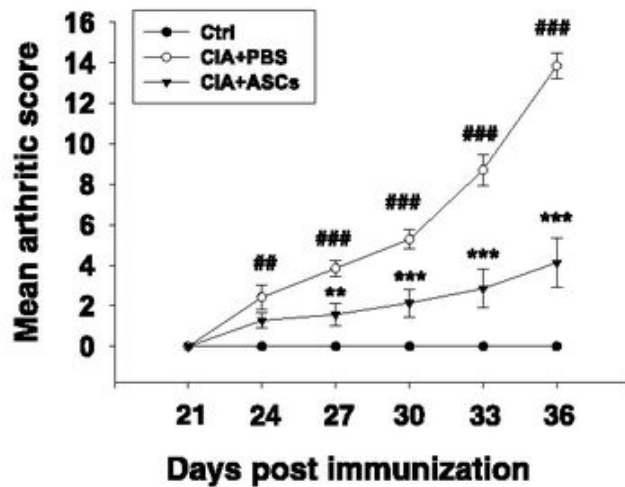
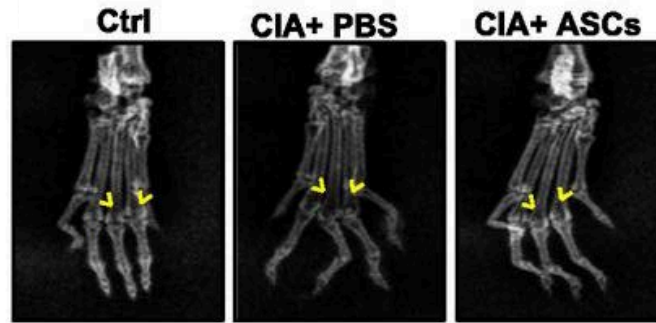
Score	Observations
0	No erythema and swelling
1	Erythema and mild swelling in tarsal or ankle joint
2	Erythema and mild swelling extending from tarsal to ankle joint
3	Erythema and moderate swelling extending from tarsal to ankle joint
4	Erythema and severe swelling surrounding ankle, foot and digits

Each limb scored individually; max score/mouse 16

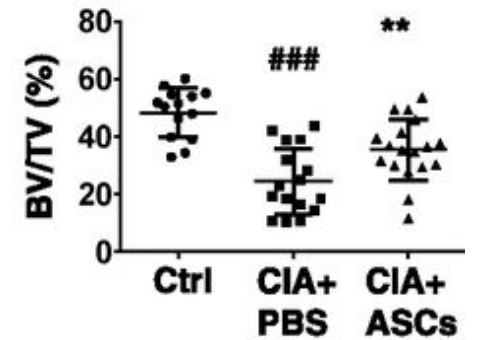
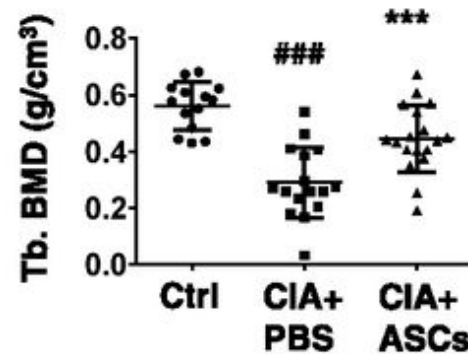
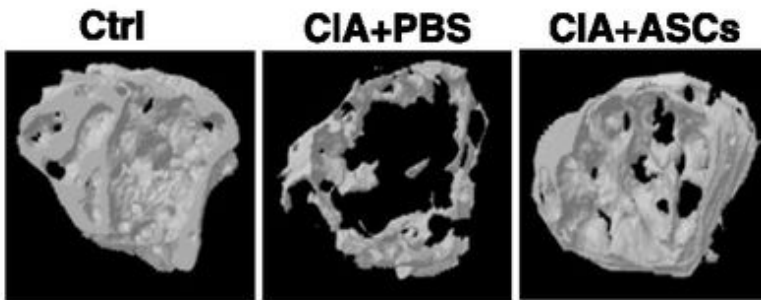
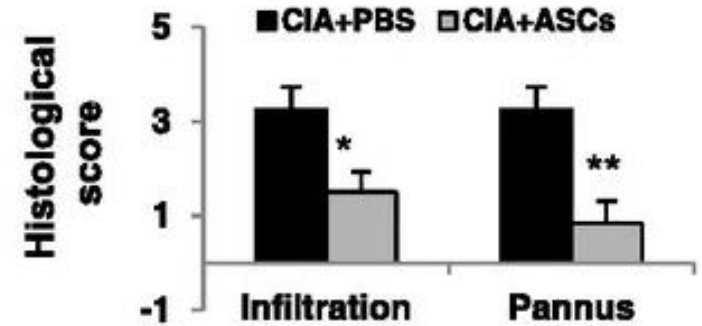
Hind paw thickness by caliper

Hind limbs – soft X-ray radiography (Siemens)

ASSESSMENT OF CIA



ASSESSMENT OF CIA



COLLAGEN-INDUCED ARTHRITIS

Similarities to RA

- Susceptibility linked to MHC immunologically mediated
- Erosions of cartilage & bone
- Autoimmunity to collagen seen in some RA patients
- Arthritic rather than multisystem

Differences to RA

- Disease is induced
- The model “resolves”
- New bone formation more marked
- No synovial vasculitis
- No cycles of relapse & remission
- No RF
- No sex predilection

OTHER RA MODELS



Model	Species	Characteristics
CAIA	Mouse	<ul style="list-style-type: none">▪ Self limiting polyarthritis in 100% animals▪ onset within 48 hours▪ innate but not acquired▪ inducible in most strains
Zymosan-induced arthritis	Mouse	<ul style="list-style-type: none">▪ Monoarthritis (rapid onset and tapering off)▪ relapse after day 25▪ inducible in multiple strains
Spontaneous transgenic model of arthritis	Mouse	Spontaneous chronic and progressive polyarthritis (KBxN, SKG and DNAs II-/- IFN-IR-/- and human TNF α transgenic mice)



Department of Science and Technology

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