骨と歯の疾患の
生命科学的研究

分子薬理学へようこそ

09. 6. 10

Unloading Causes Rapid Bone Loss

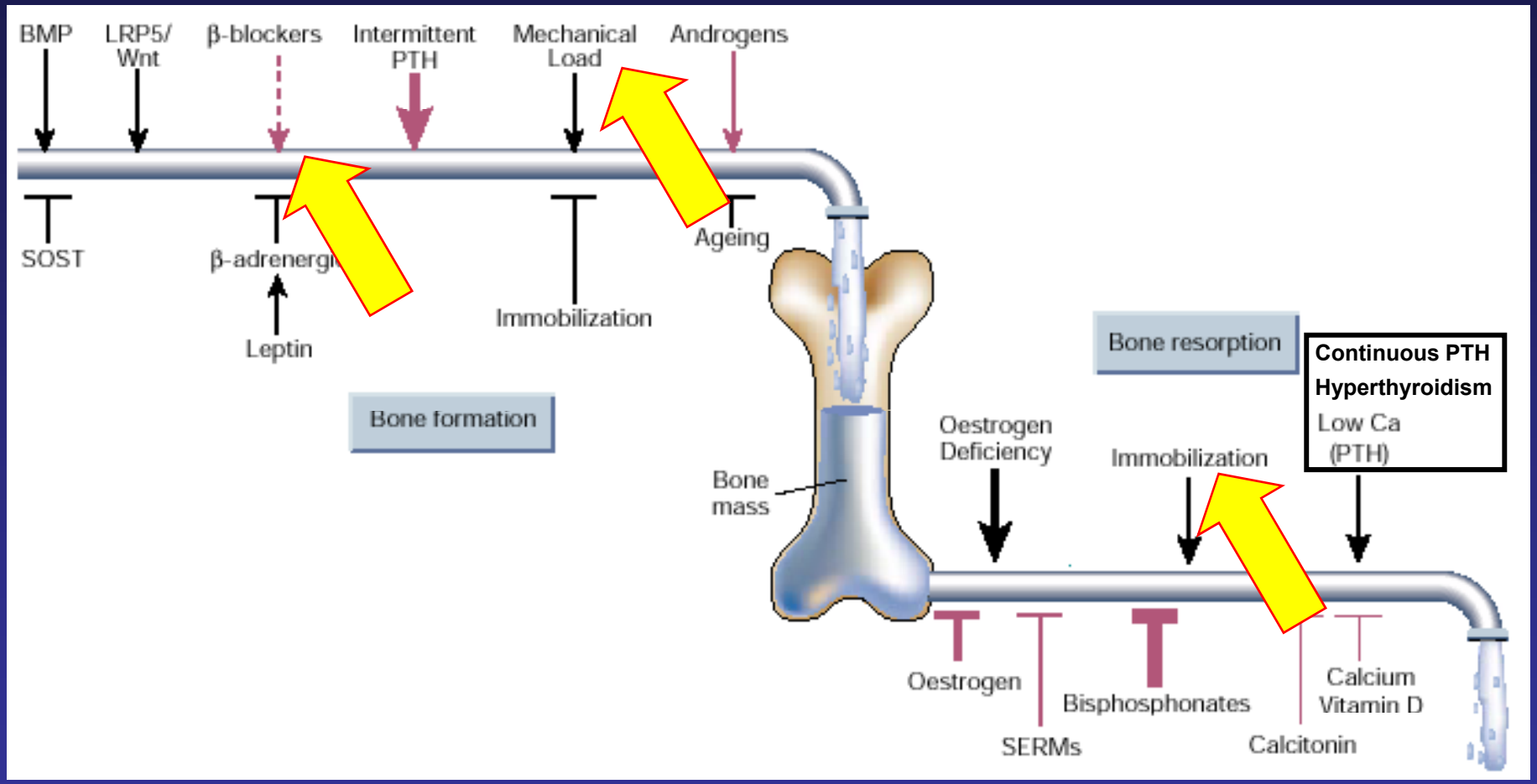
Bed-Ridden Patients

Astronauts

↓ Mechanism?

Osteoporosis

骨形成と骨吸収

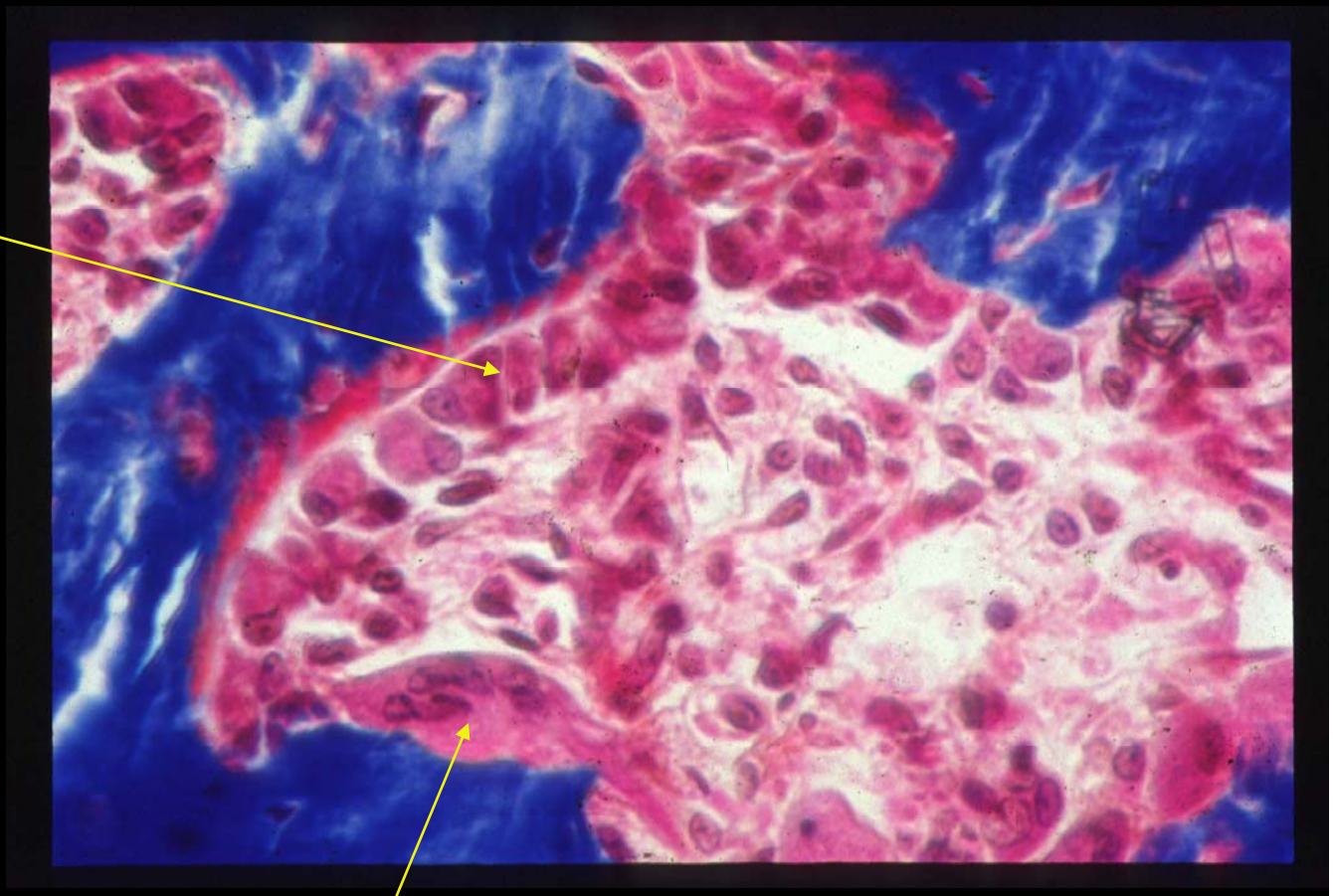


Physiological (black) and pharmacological (pink) stimulators and inhibitors of bone formation and resorption

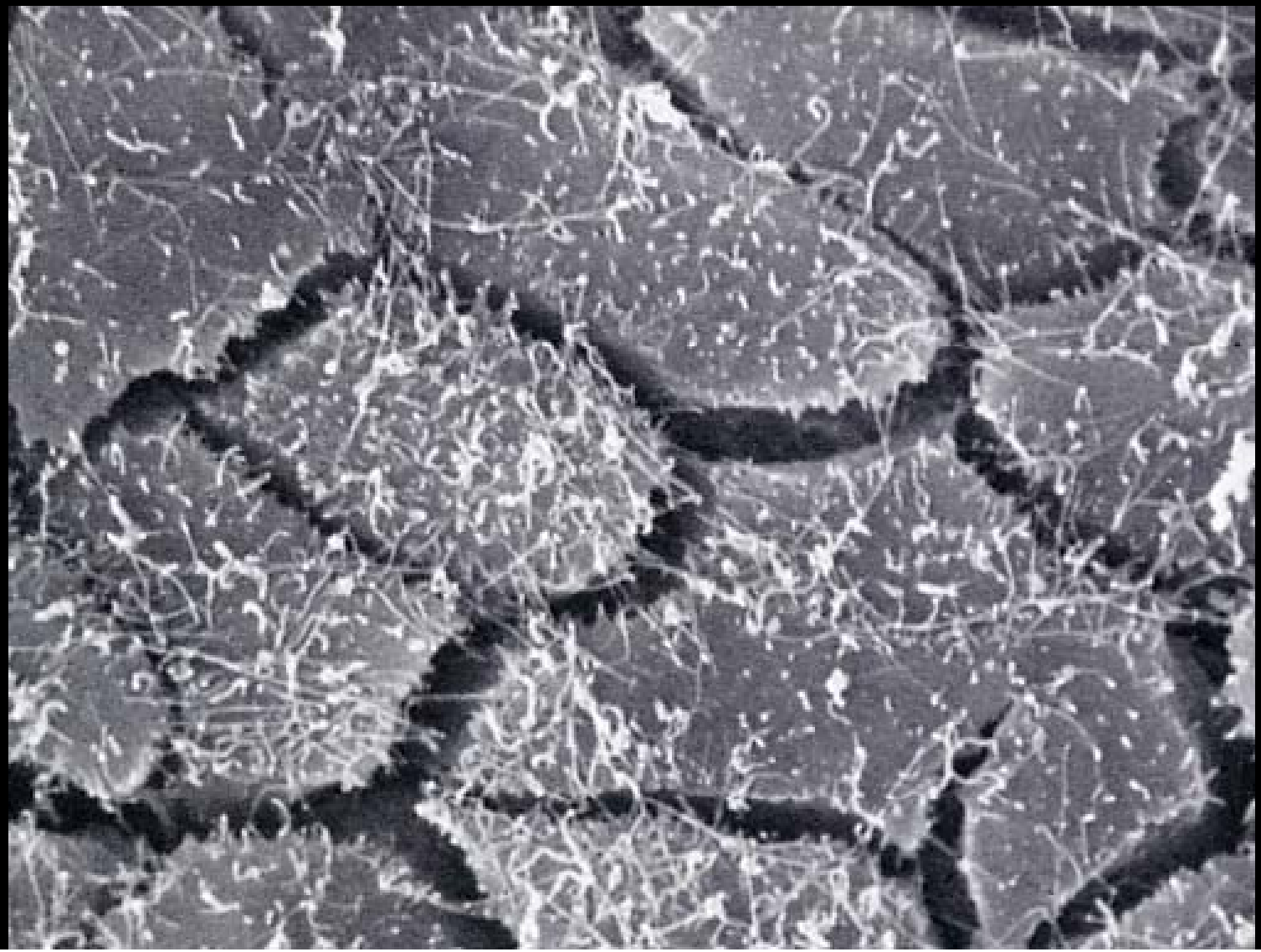
Determinants of skeletal homeostasis and bone mass

(Harada & Rodan, Nature 2003)

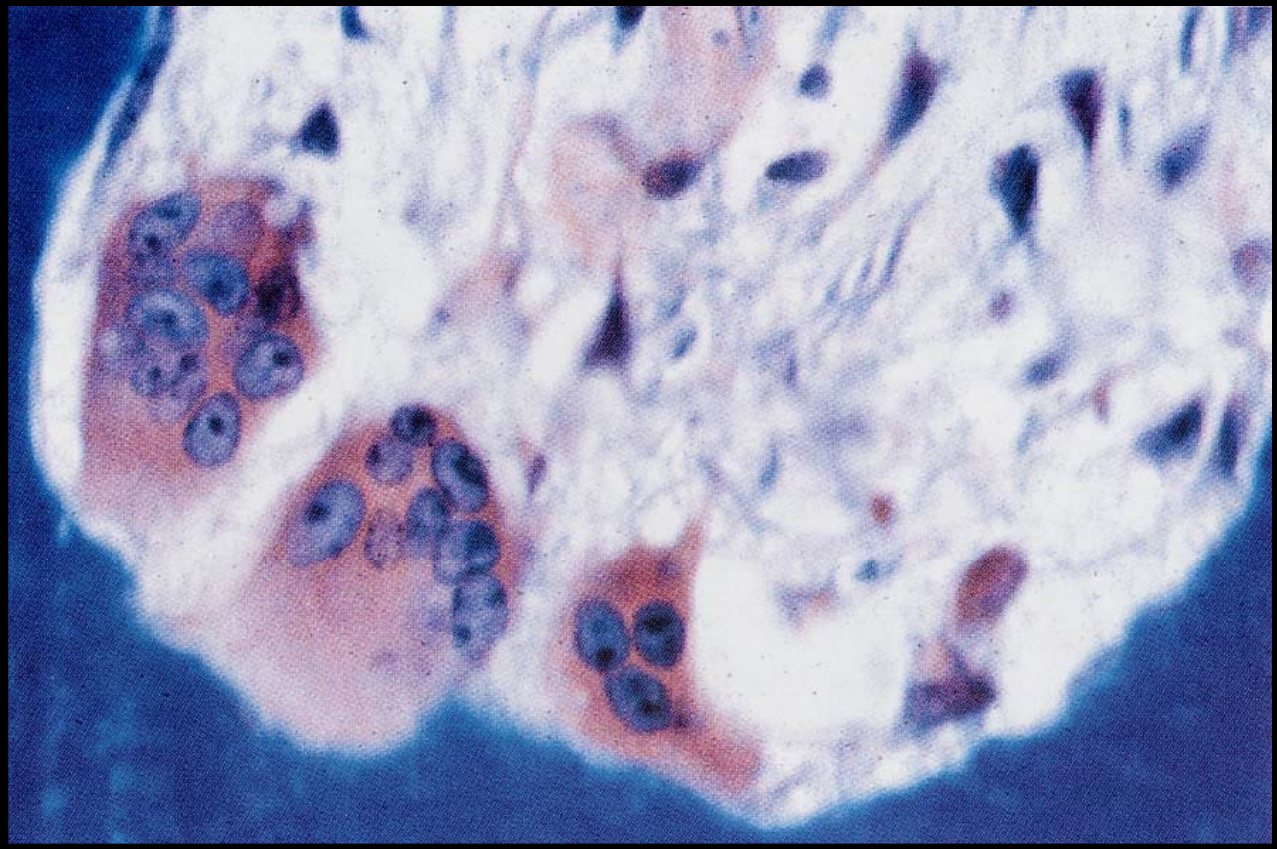
骨芽細胞と破骨細胞の共同作業の場



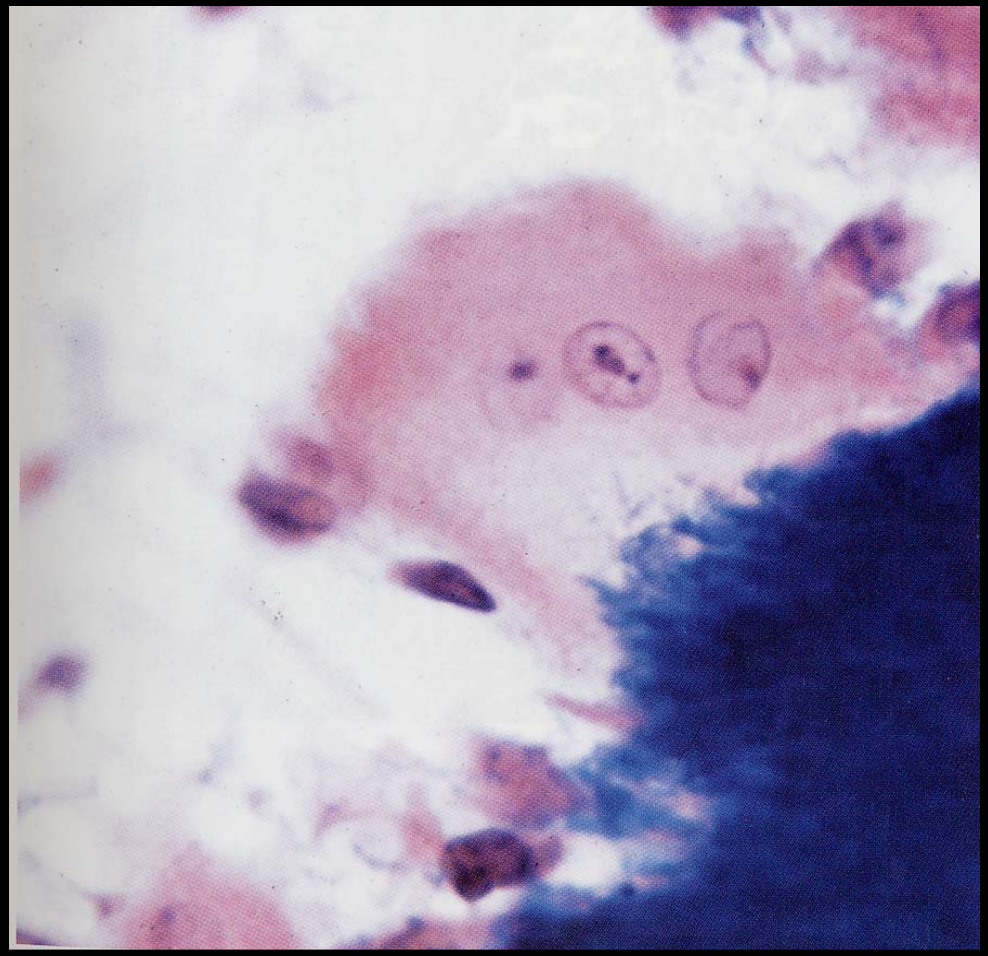
骨芽細胞



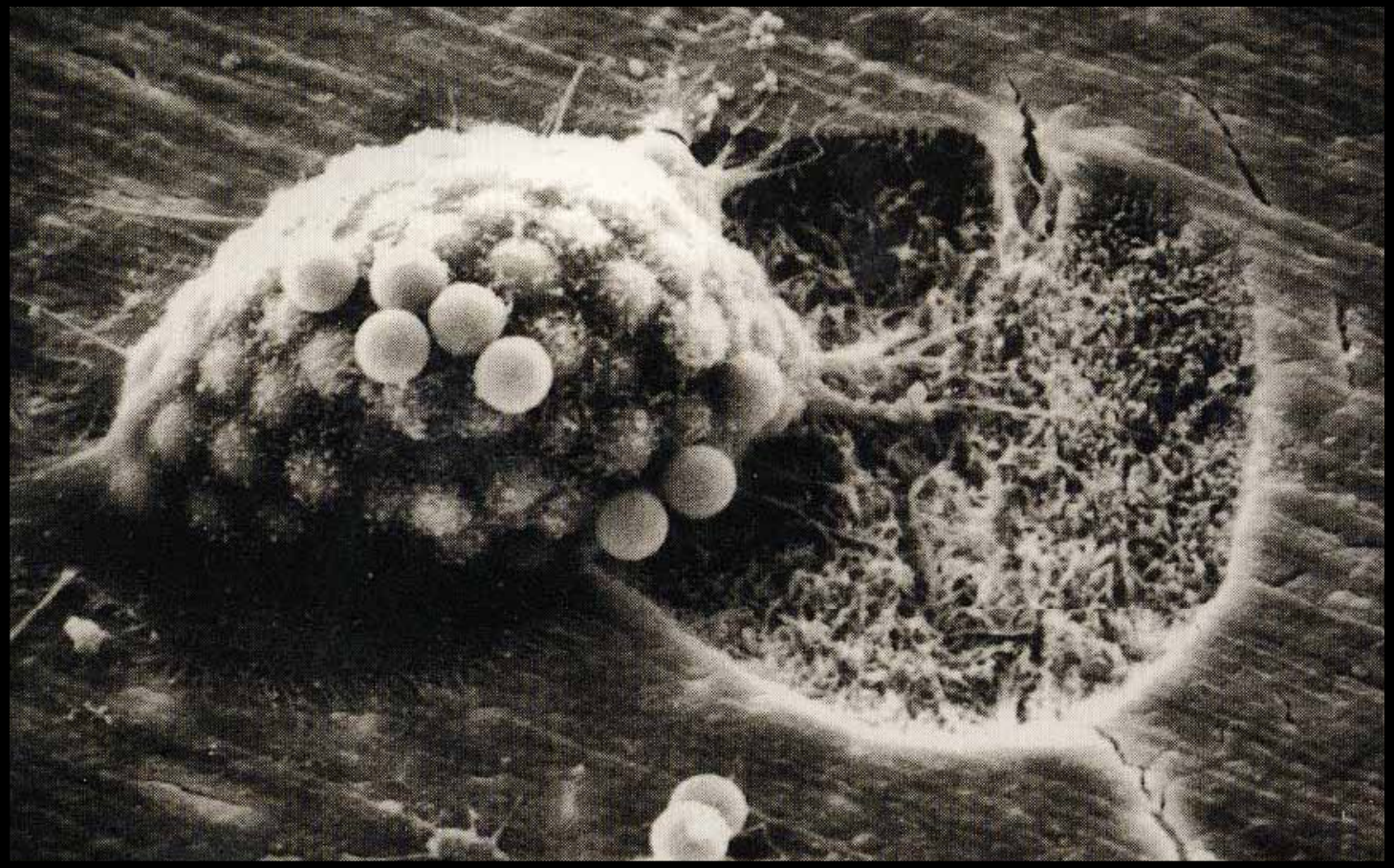
破骨細胞



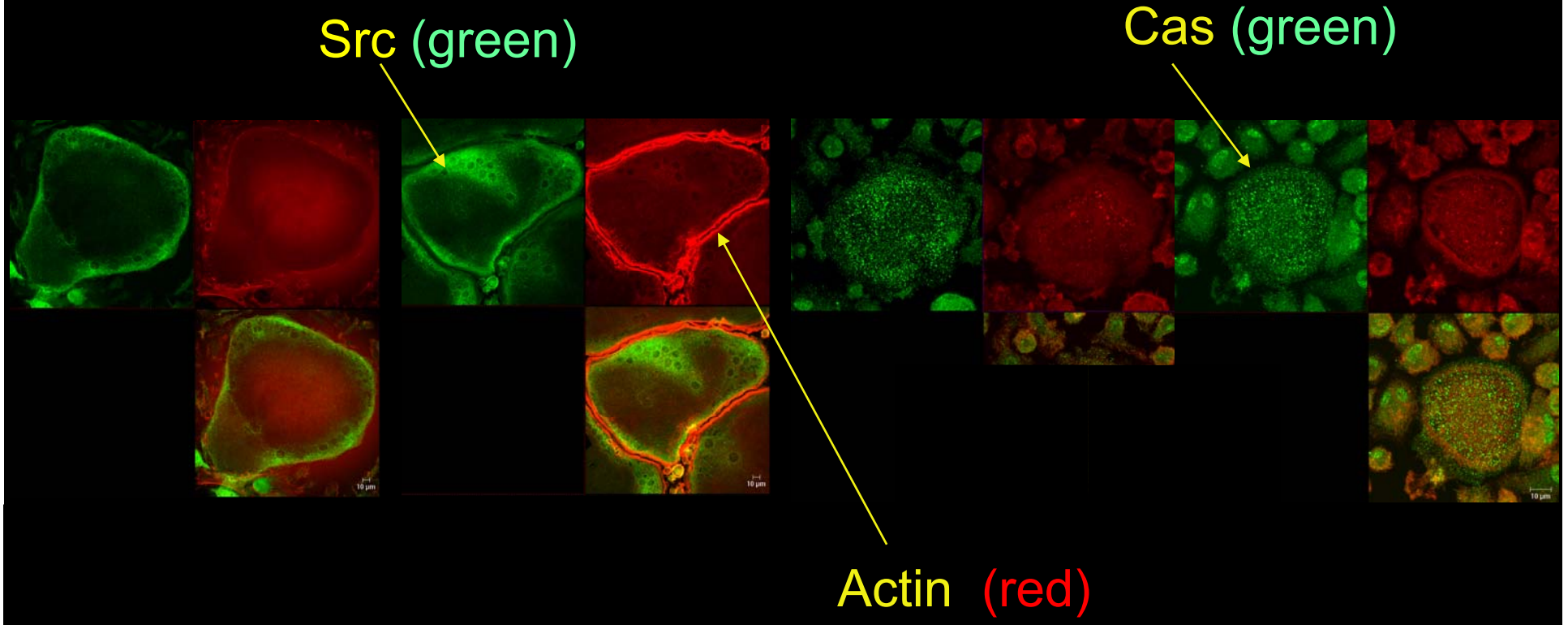
破骨細胞

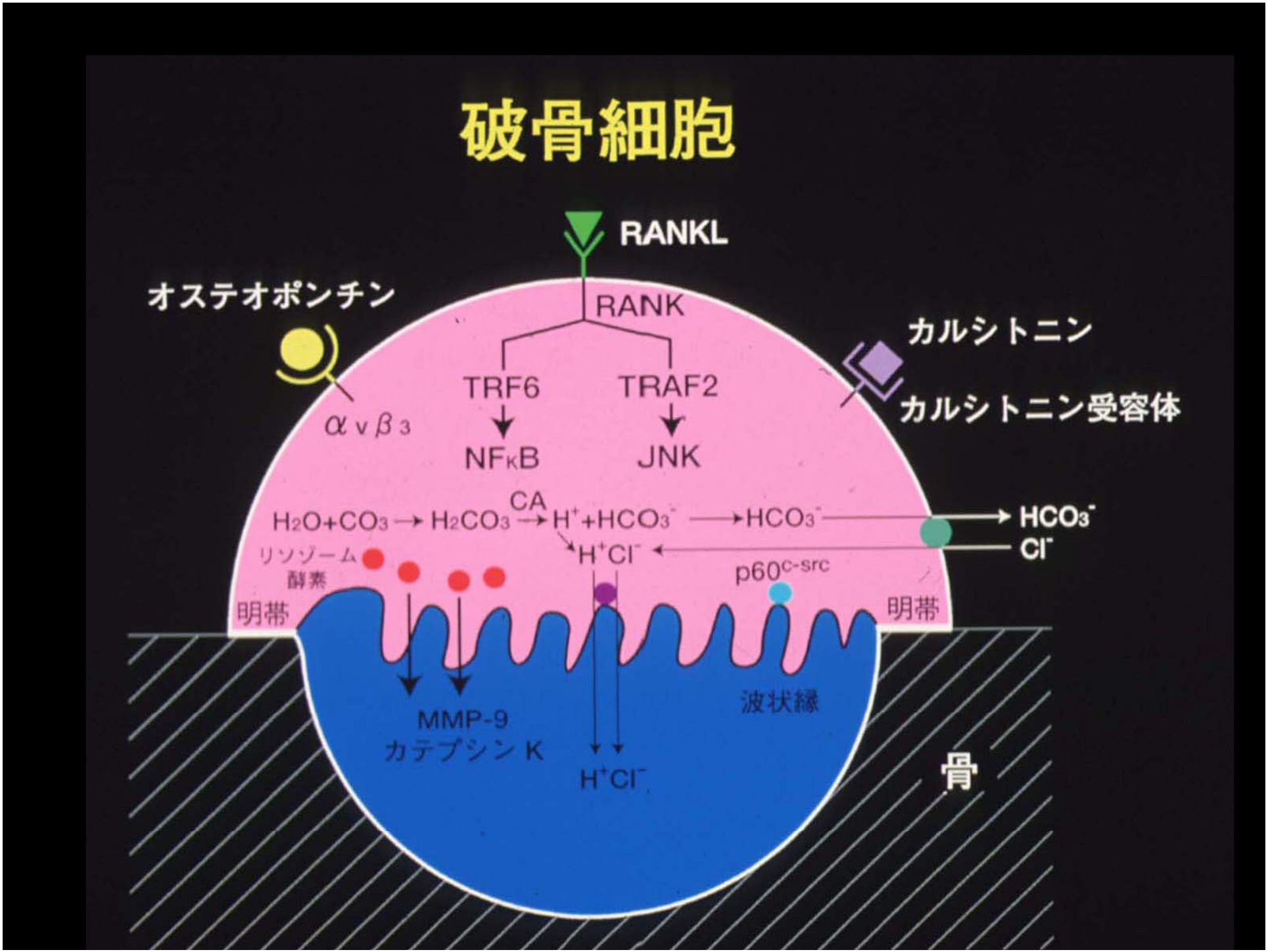


破骨細胞の骨吸収

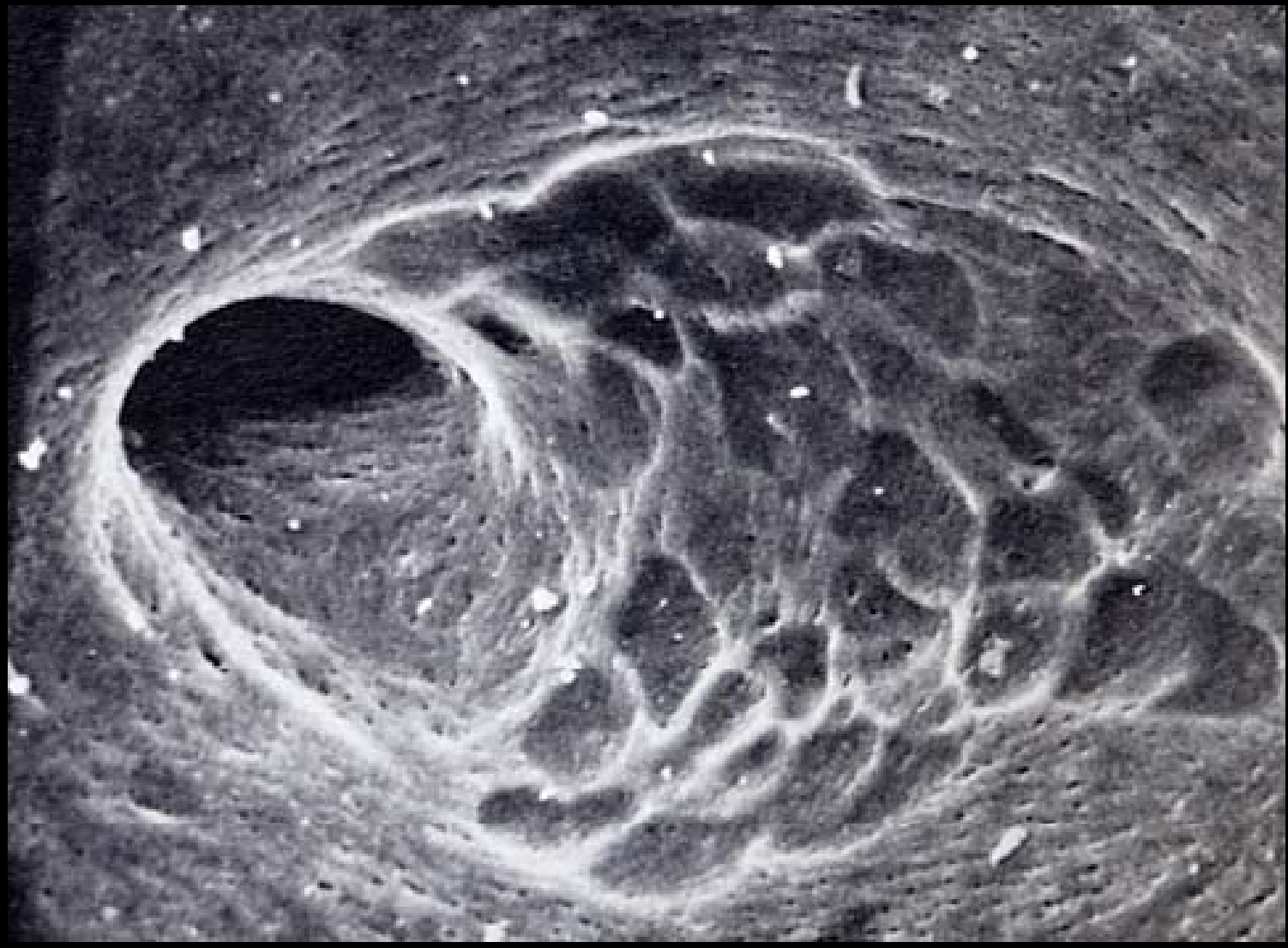


破骨細胞は細胞骨格により閉鎖腔を構成する

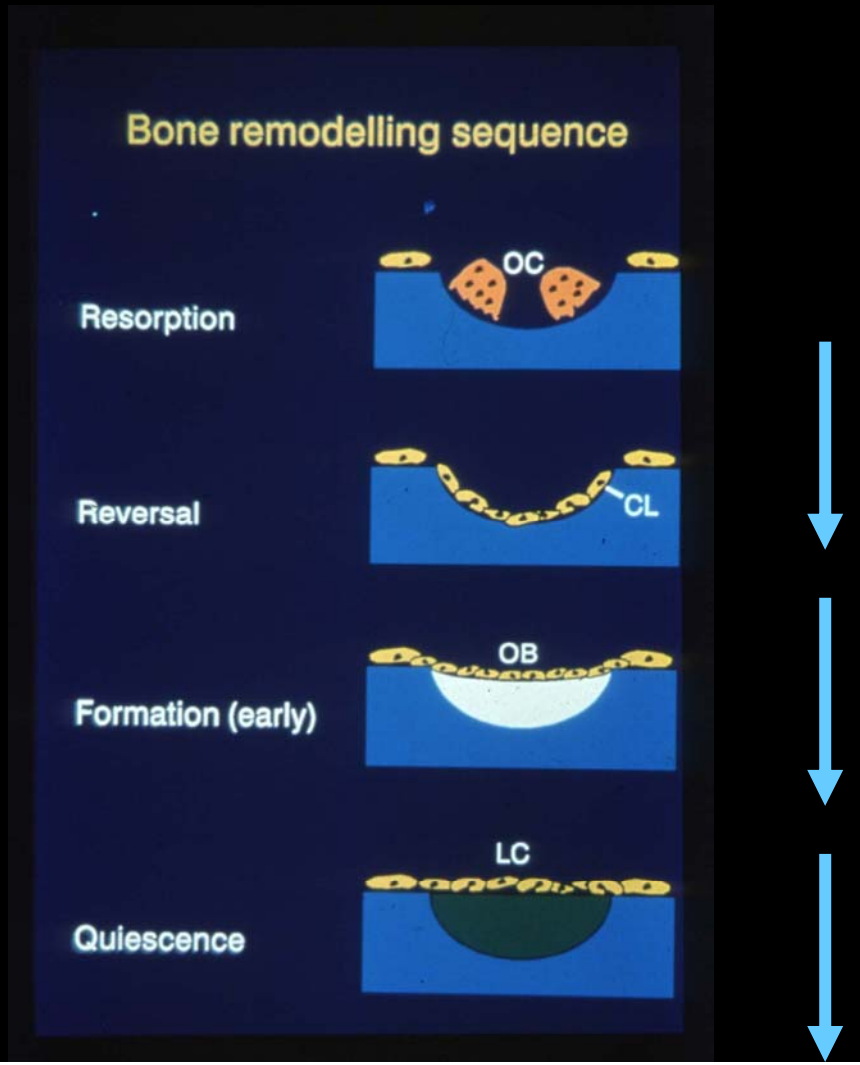


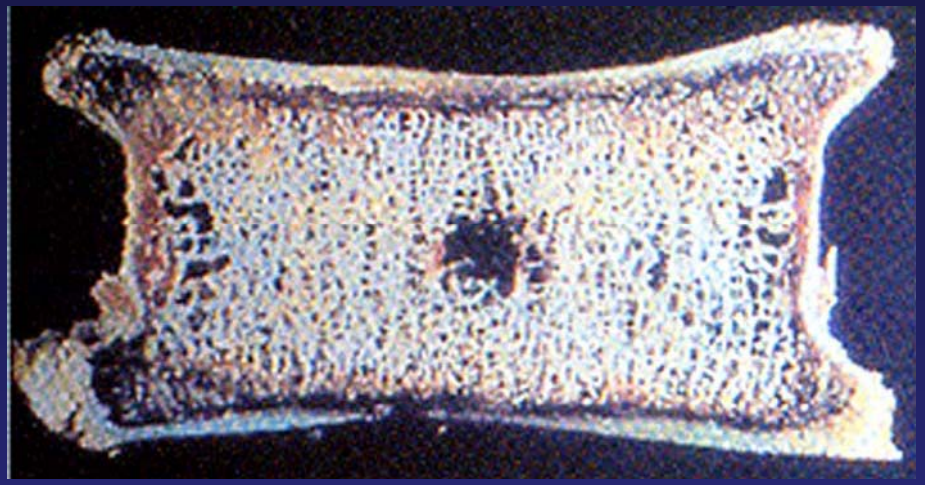

破骨細胞
RANKL
RANK
オステオポンチン
カルシトニン
カルシトニン受容体
TRF6
TRAF2
αvβ3
NFκB
JNK
CA
H2O+CO3 → H2CO3 → H+HCO3- → HCO3-
HCO3-
Cl-
H+Cl-
リソゾーム酵素
p60c-src
明帯
明帯
MMP-9
カテプシンK
H+Cl-
波状縁
骨

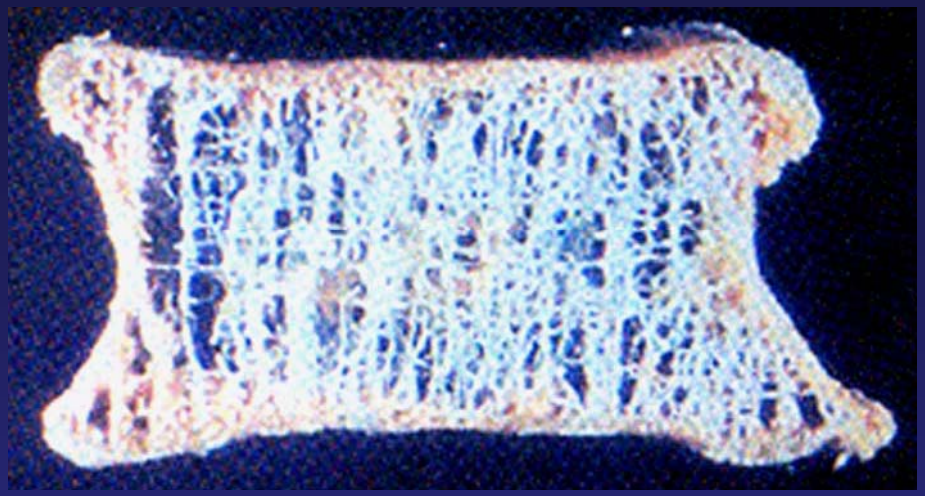
破骨細胞により形成された骨吸収窩



骨芽細胞･破骨細胞による骨のリモデリングの進行

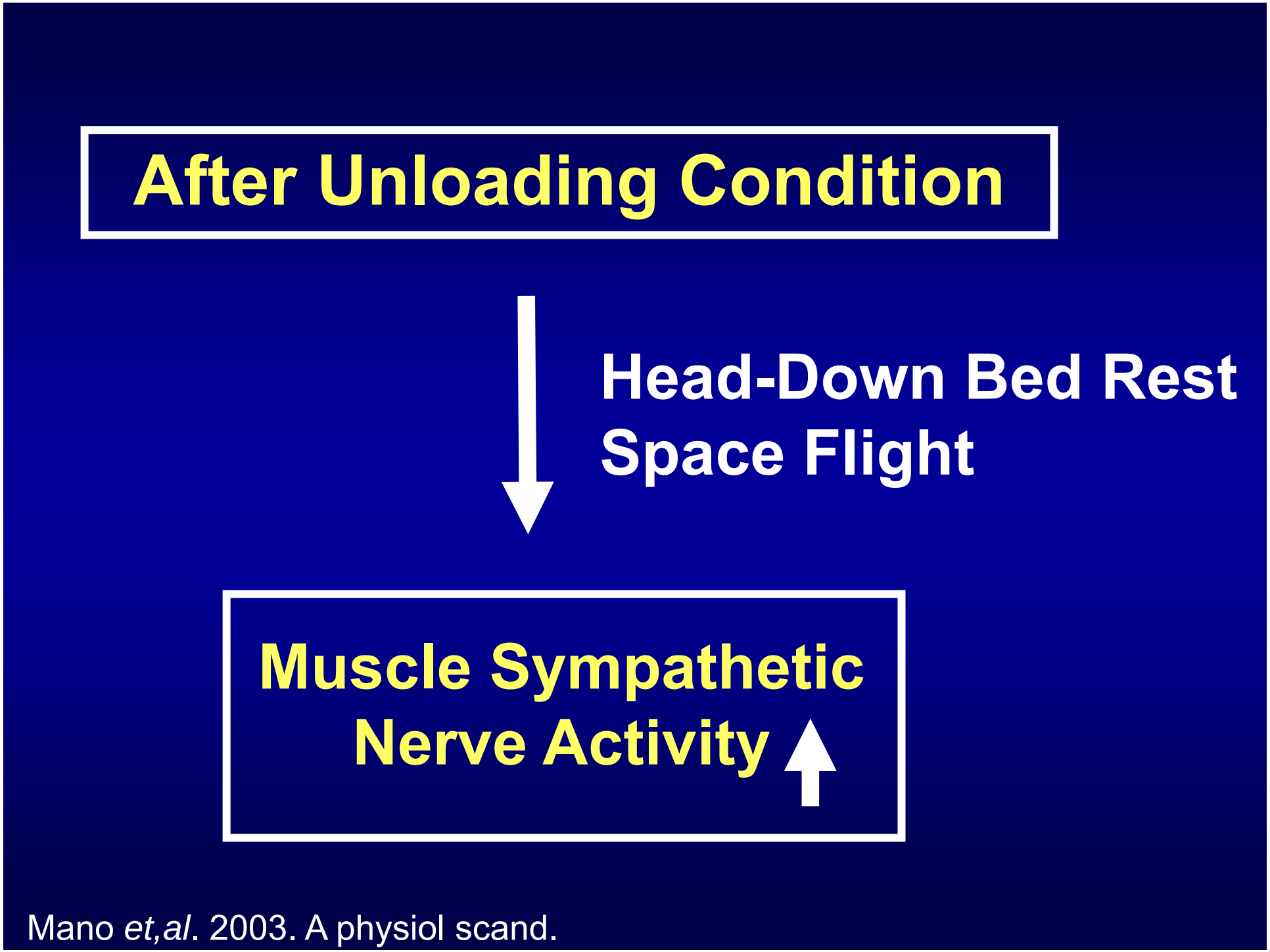




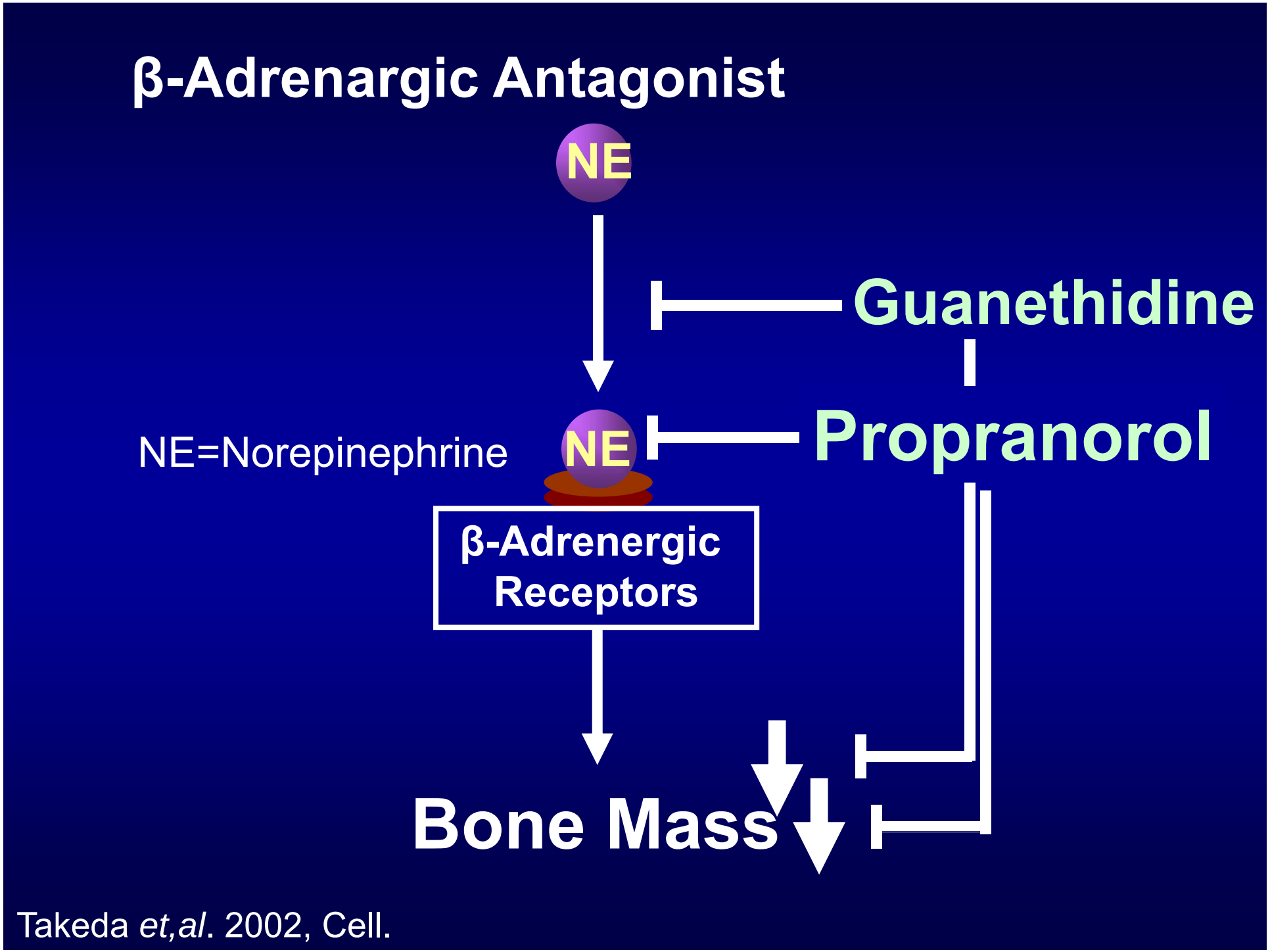


Normal Osteopo

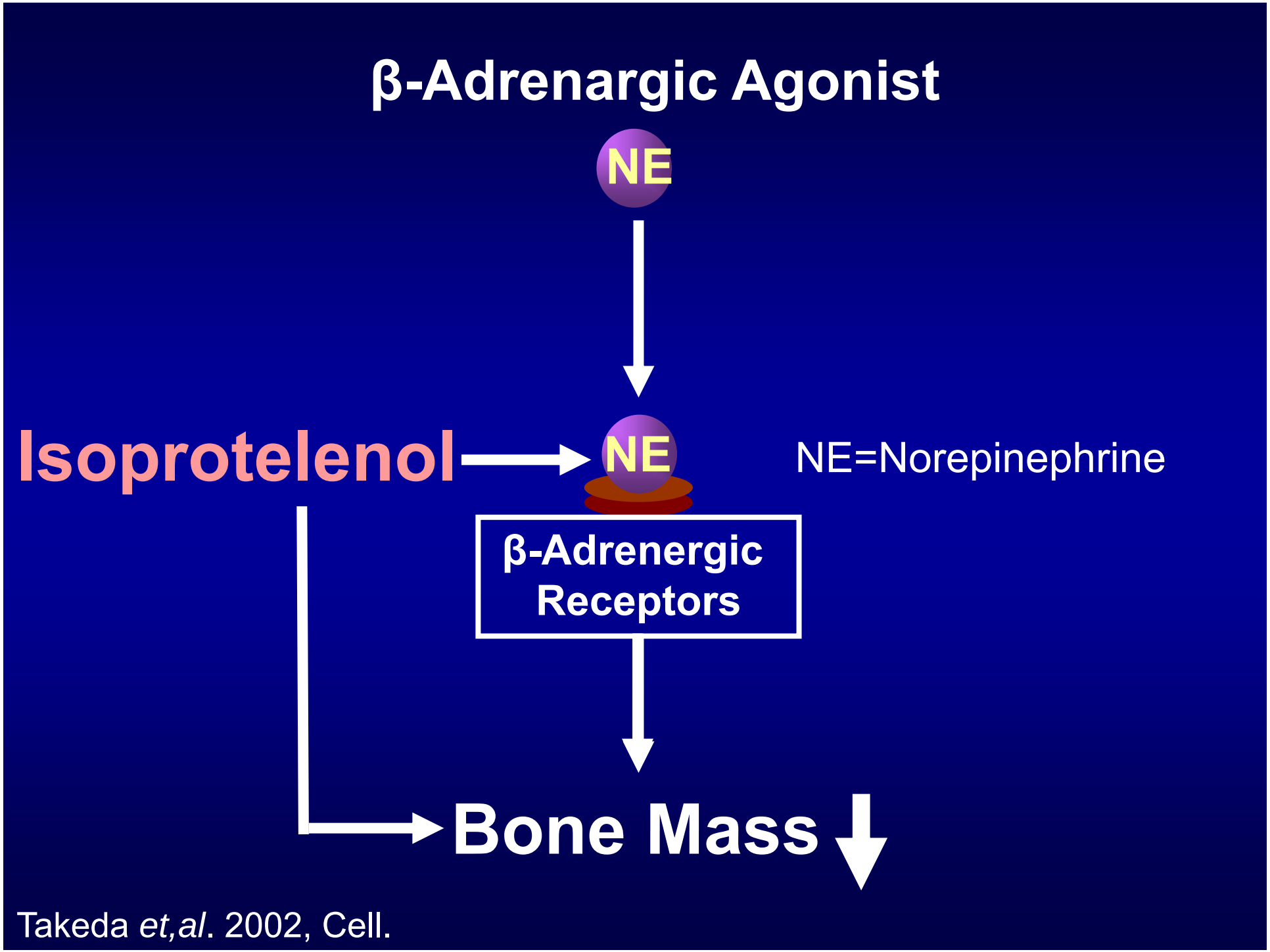
Remodeling / Mechanical Stress



Mano *et,al*. 2003. A physiol scand.

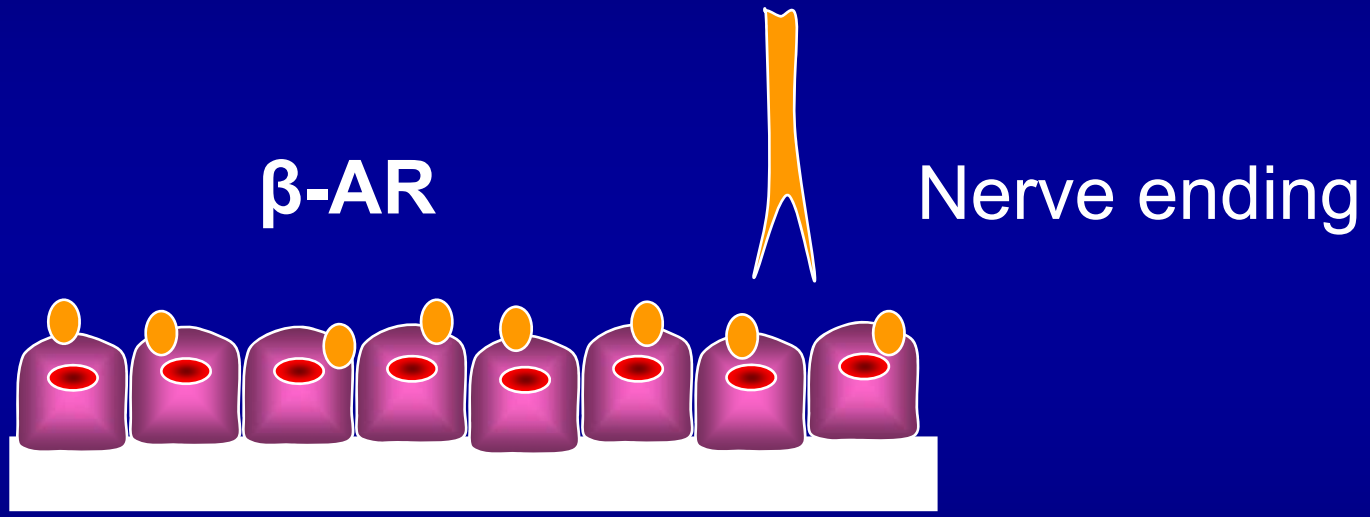


Takeda *et,al*. 2002, Cell.



Takeda *et,al*. 2002, Cell.

Osteoblasts Express β-Adrenergic Receptors



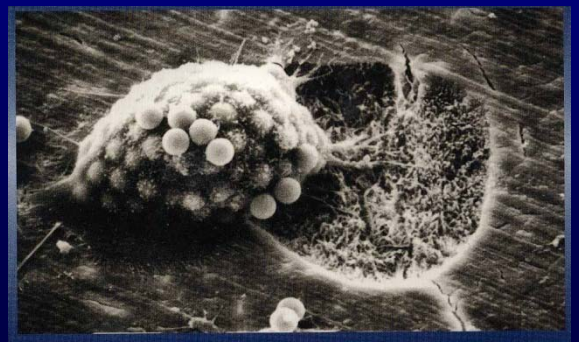
Takeda *et,al*. 2002, Cell.

Togari *et,al*.1997, Neuroscience Letters.

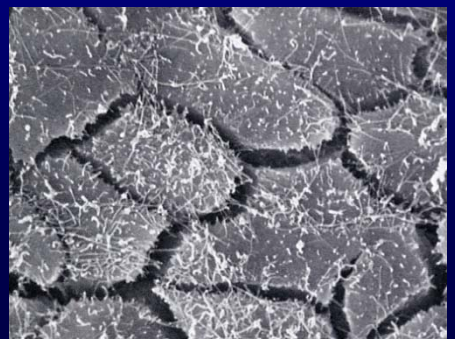
Hind Limb Unloading

Resorption by Osteoclasts

Formation by Osteoblasts



Bone Mass

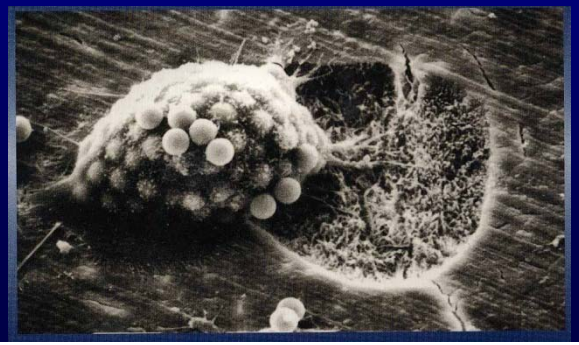


Hind Limb Unloading

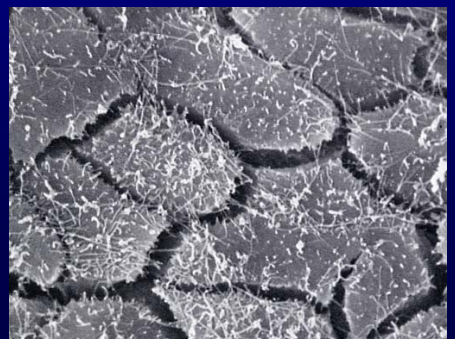
Sympathetic Nervous System ?

Resorption by Osteoclasts

Formation by Osteoblasts



Bone Mass



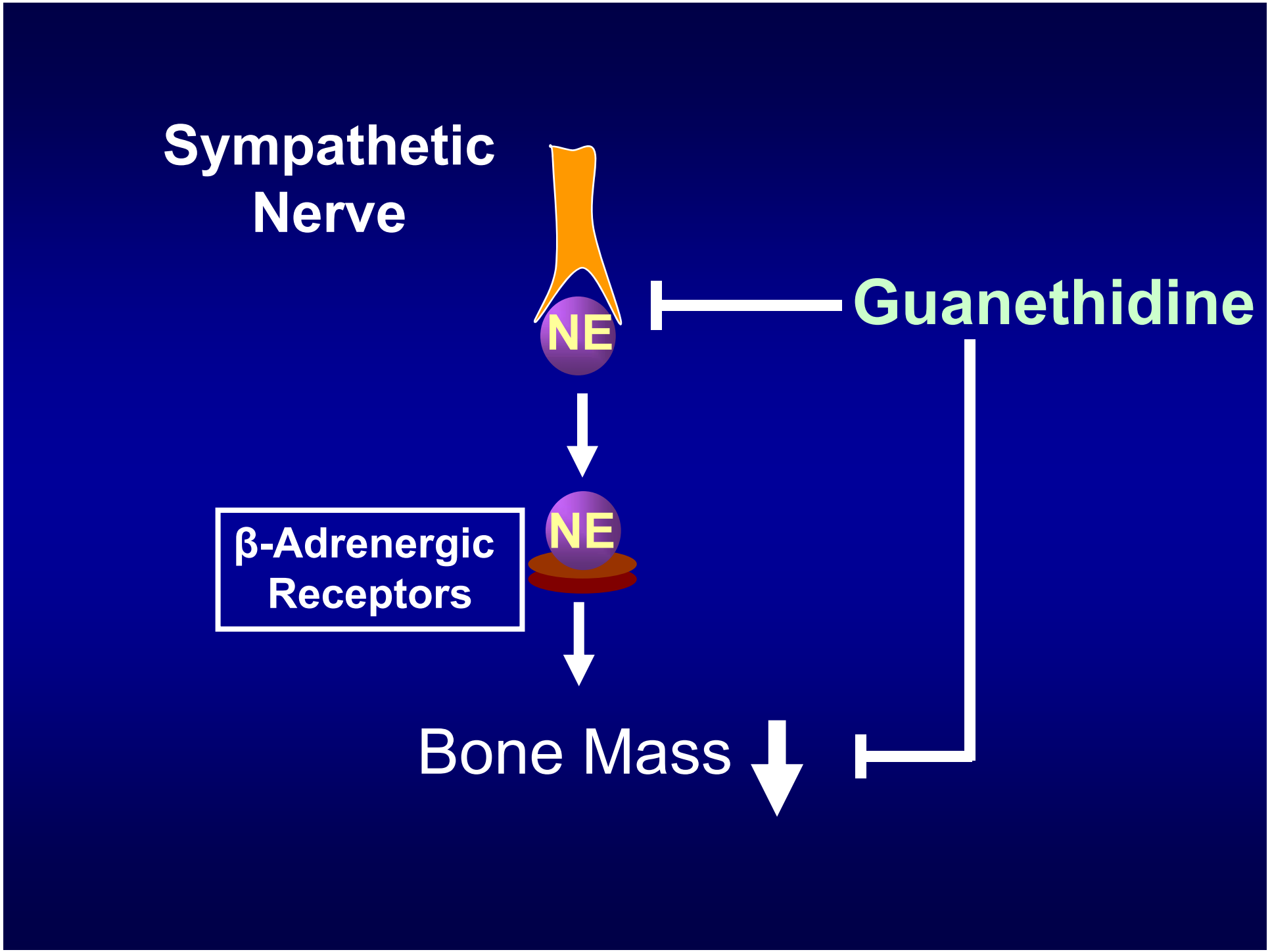
To address whether the sympathetic nervous system is involved in unloading-induced bone loss by using pharmacological modulators.

Animals: 129 , C57BL/6 10-15 Week-Old Male Mice
Unloading:Hind Limb (tail) Unloading Model
Bone Volume: 2 Dimensional μCT
(2DCT)
Bone Density: Dual Energy X-ray Absorptiometry
(DEXA)
Bone Resorption: Deoxypyridinoline in Urine
Bone Formation: Ex Vivo Nodule Formation
Assay in Bone Marrow Cells

Tail suspension model

Adult mice

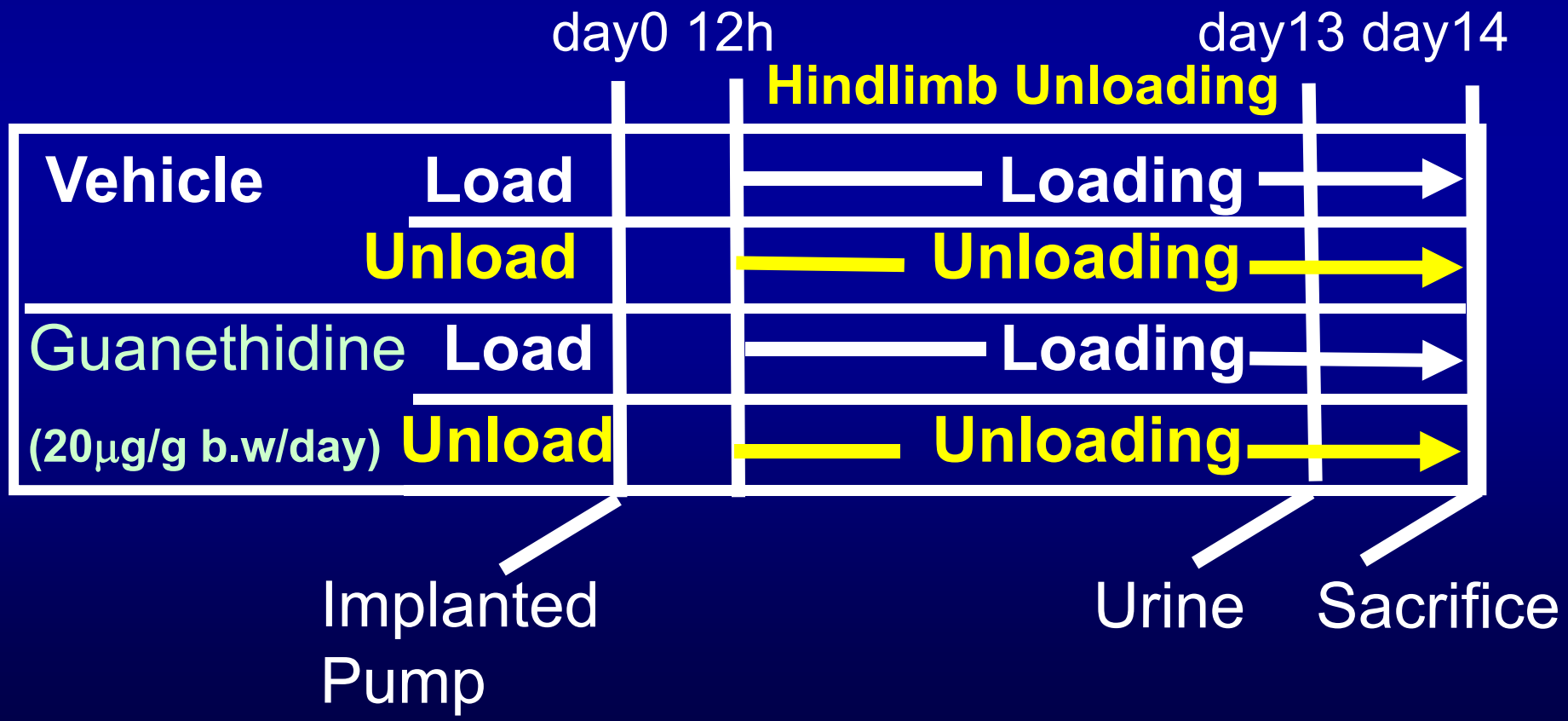
2 weeks


Sympathetic Nerve
NE
Guanethidine
NE
β-Adrenergic Receptors
Bone Mass

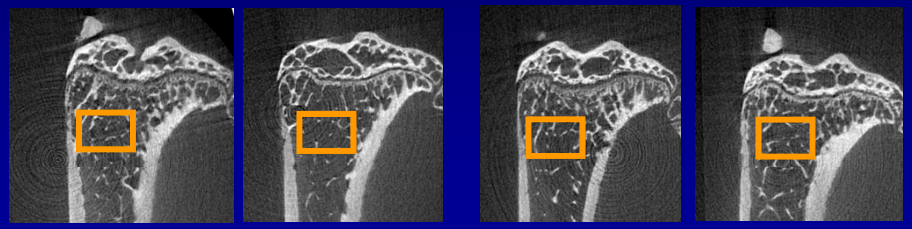
β-Antagonist Guanethidine Treatment

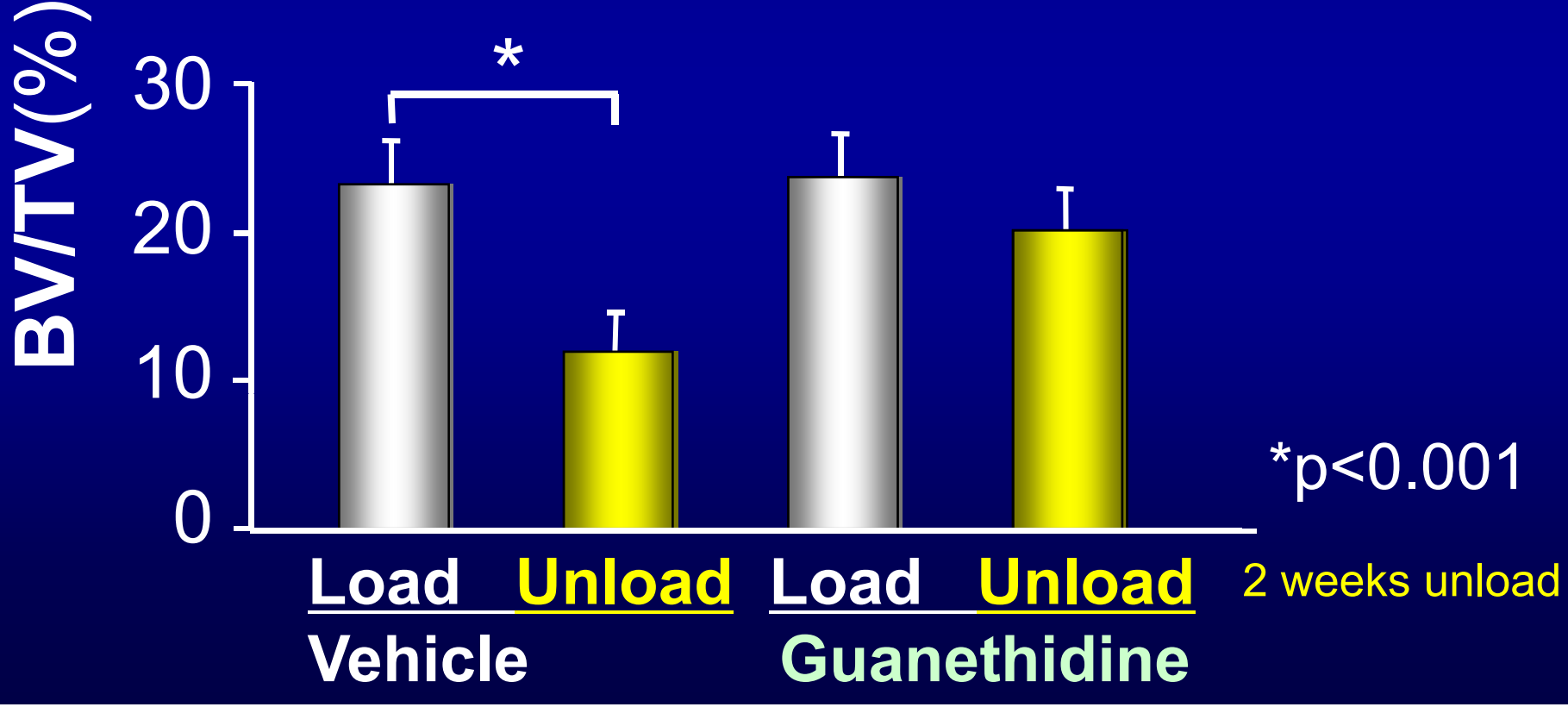
Animals: 15 week-old male

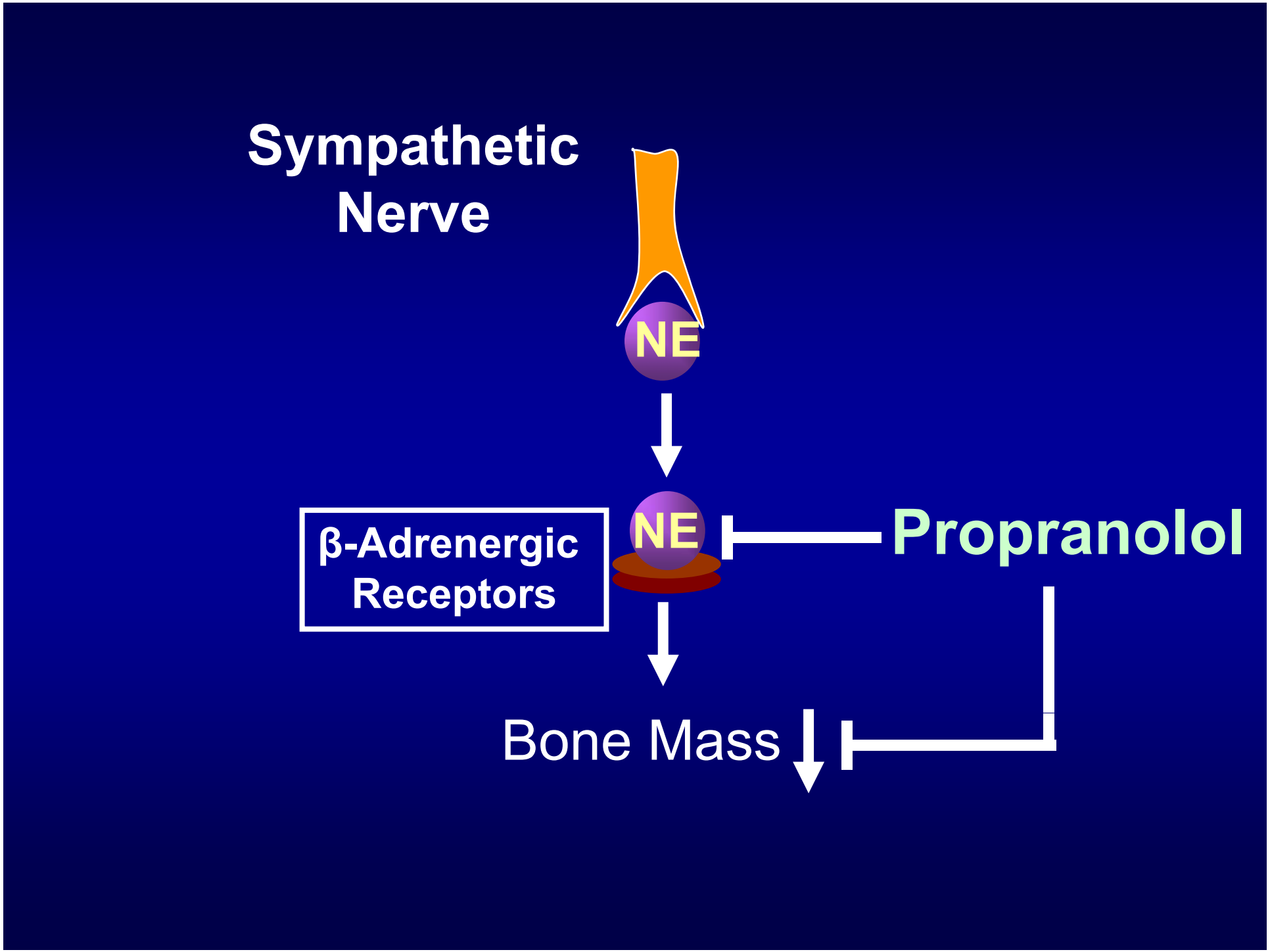
129 mice



Guanethidine Suppressed Unloading-Induced Bone Loss



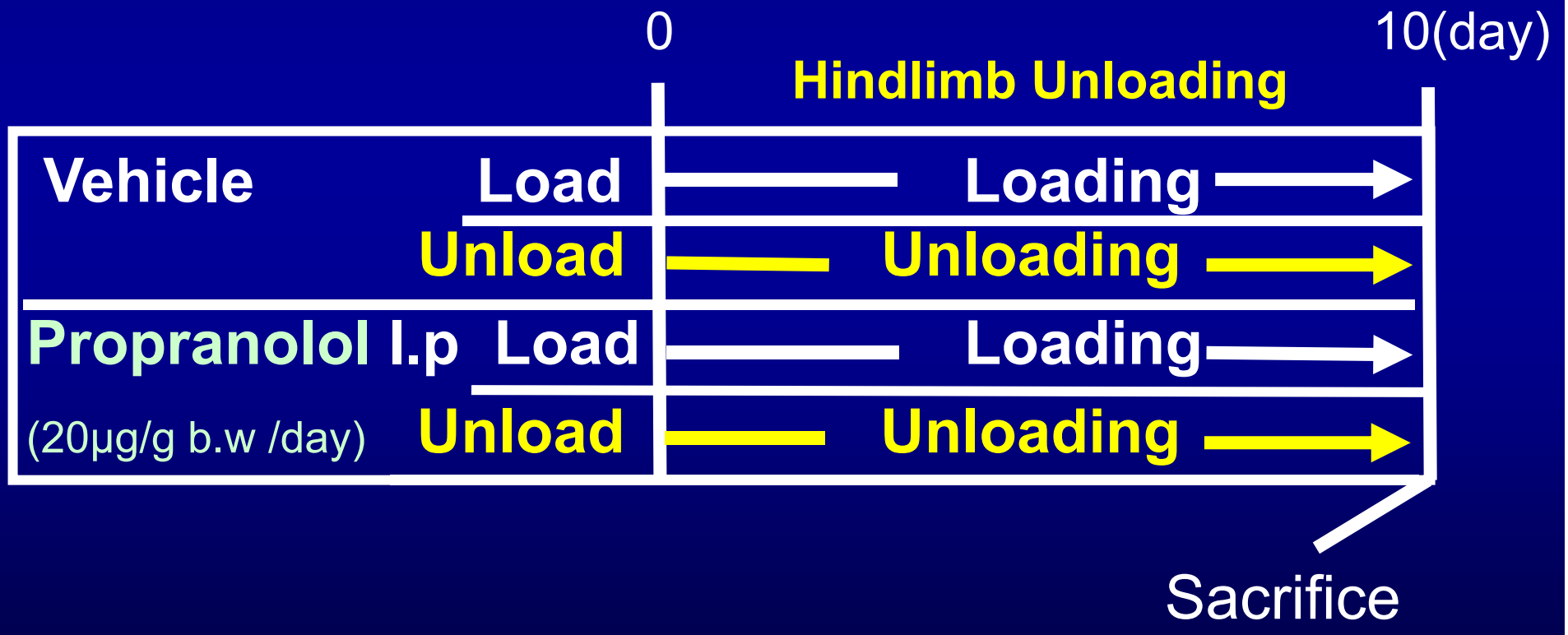


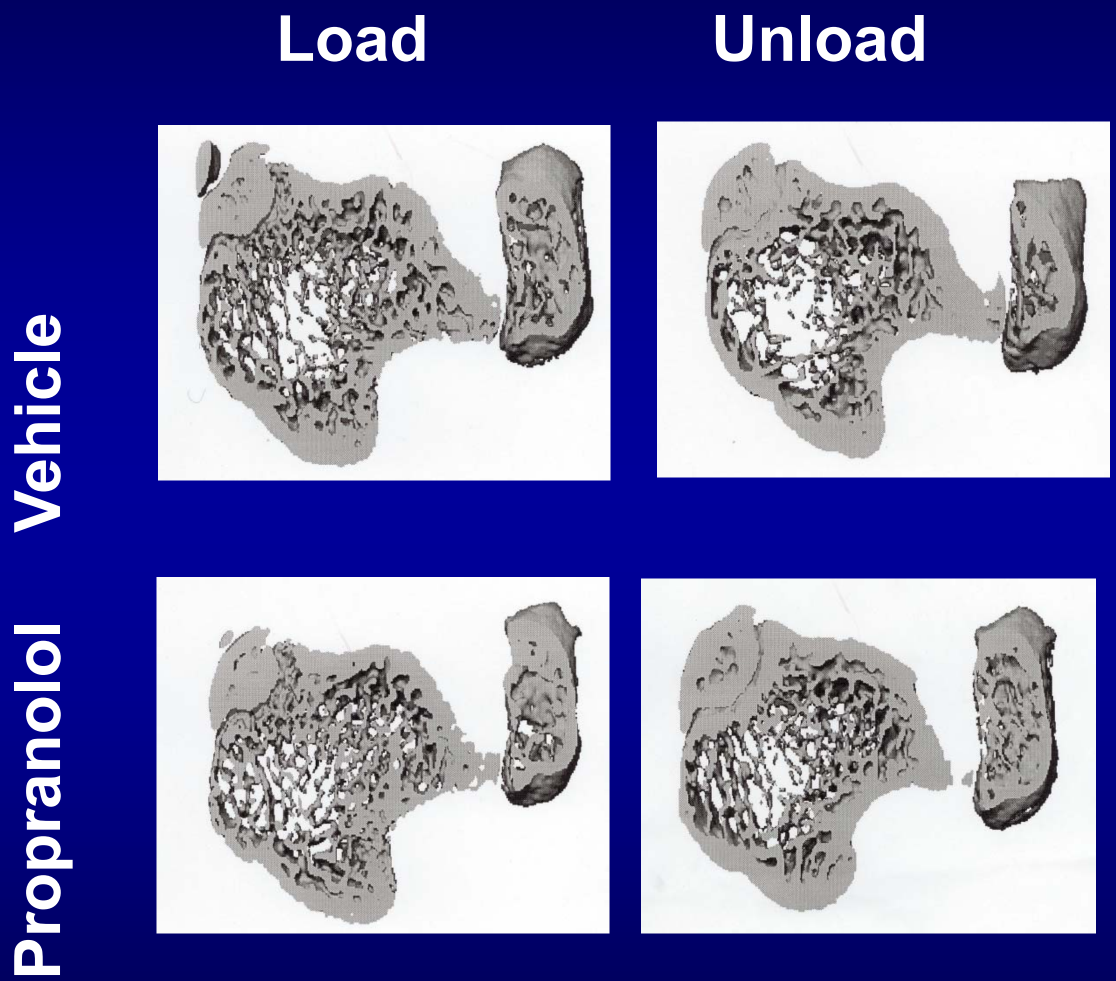

Sympathetic Nerve
NE
NE
β-Adrenergic Receptors
Propranolol
Bone Mass

β-Antagonist Propranolol Treatment

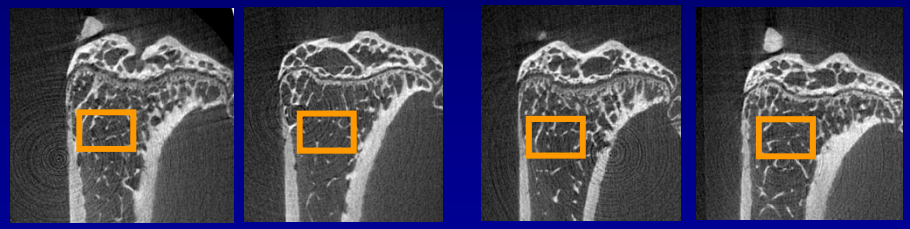
Animals: 10 week-old male

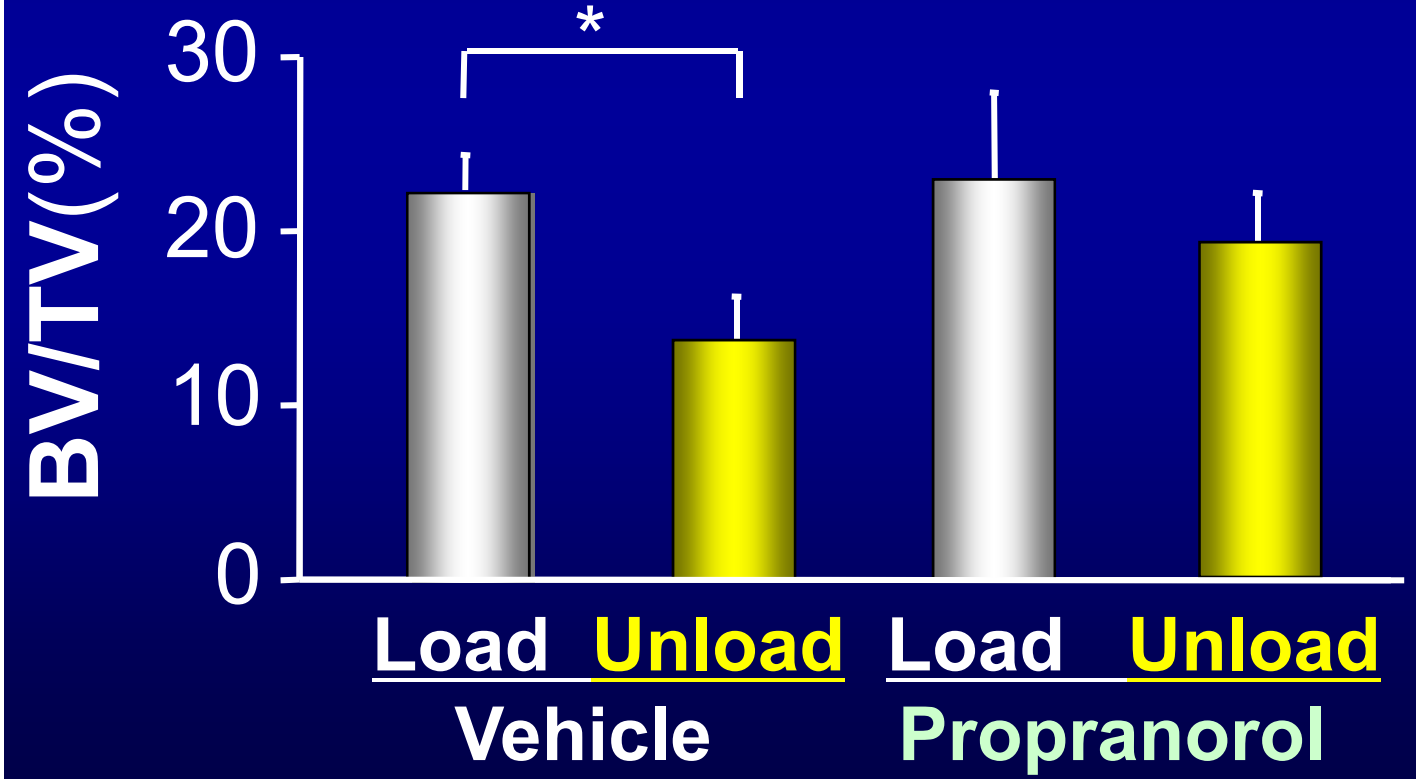
129 mice




Load
Unload
Vehicle
Propranolol

Propranorol Suppressed Unloading-Induced Bone Loss

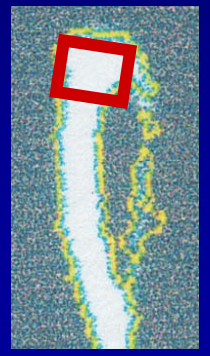




*p<0.001

10 days unload

Propranorol Suppressed Unloading-Induced Bone Loss



(g/cm^2)

BMD

0.06

0.04

0.02

0

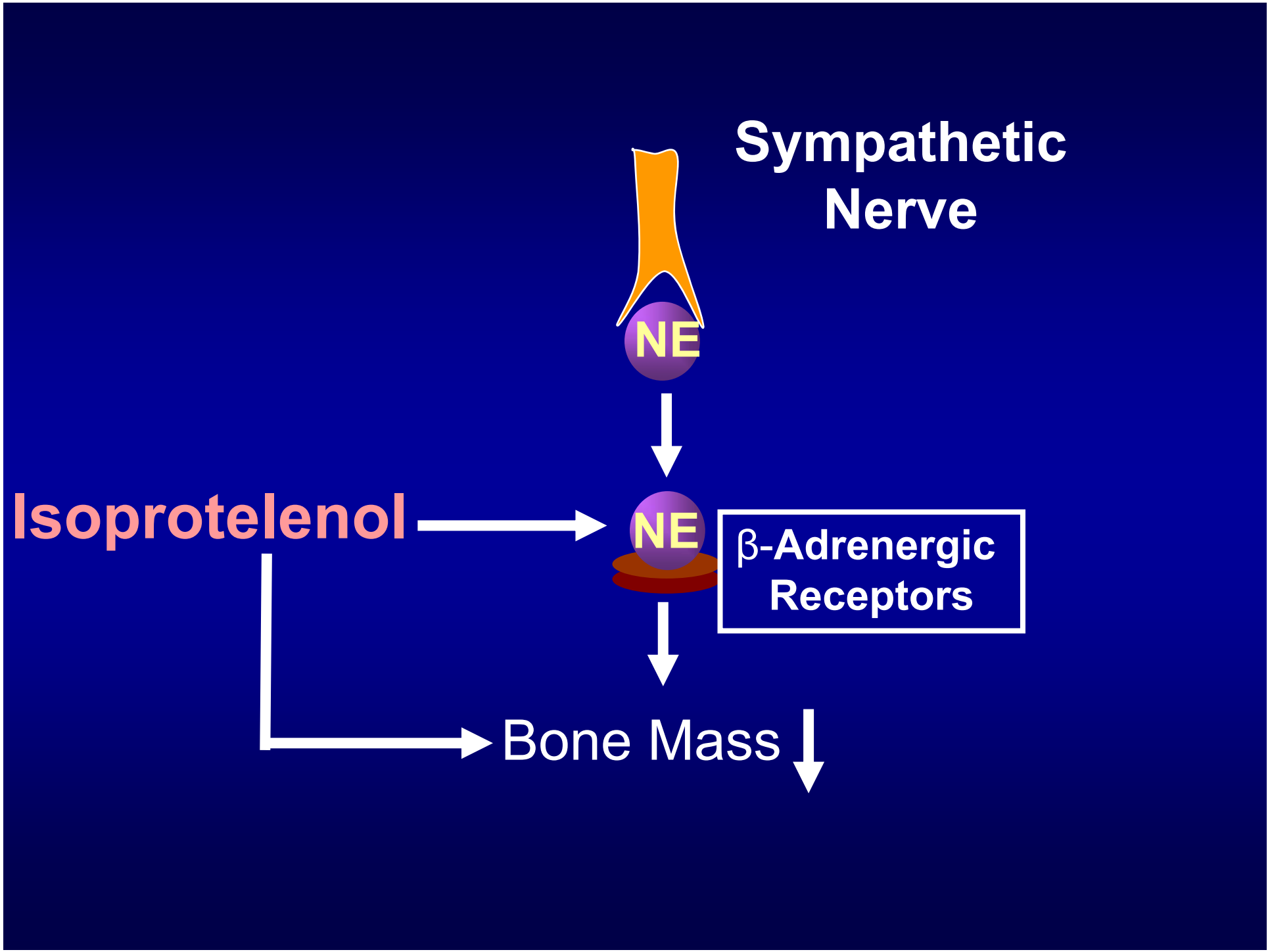
*

*p<0.001

Load Unload Load Unload

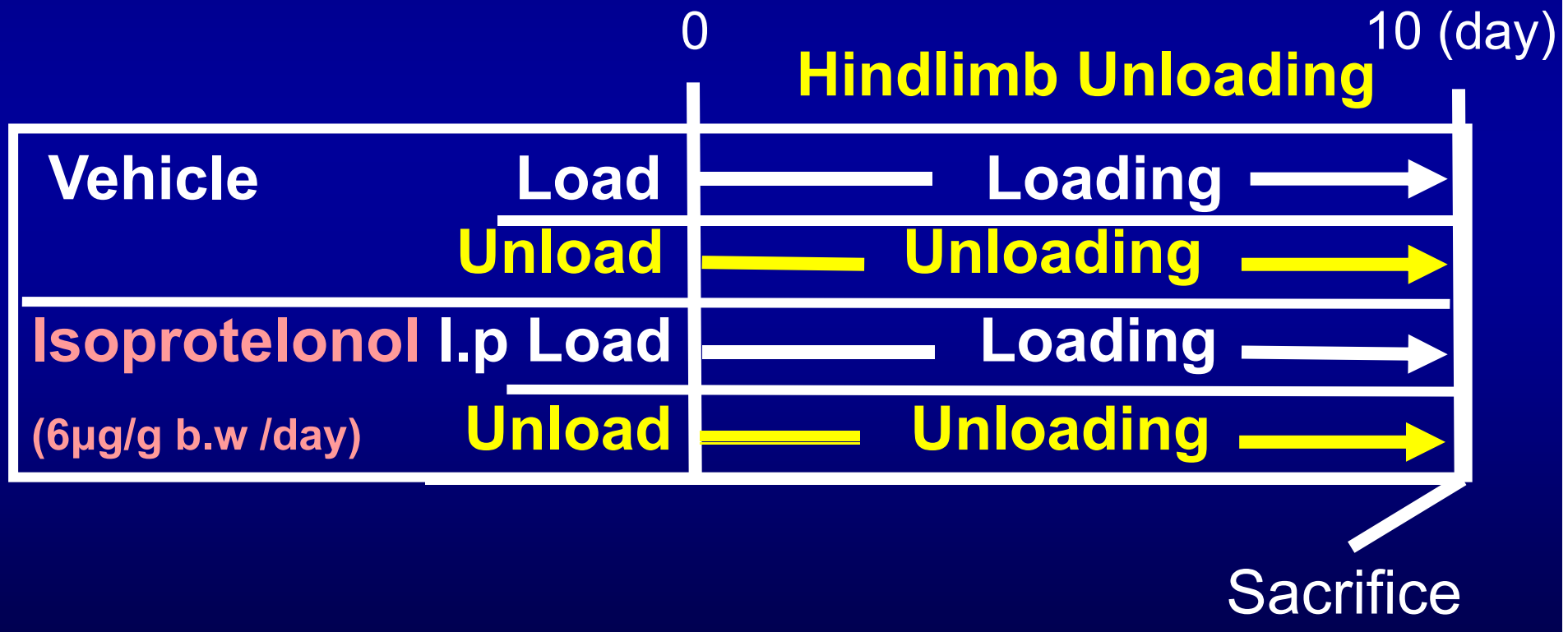
Vehicle Propranorol

Proximal End of Tibiae

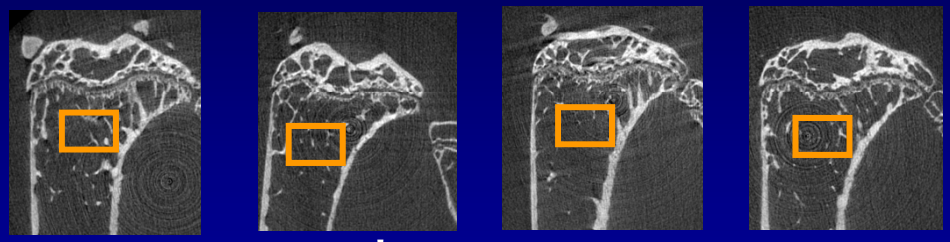

Sympathetic Nerve
NE
Isoprotelenol
NE
β-Adrenergic Receptors
Bone Mass

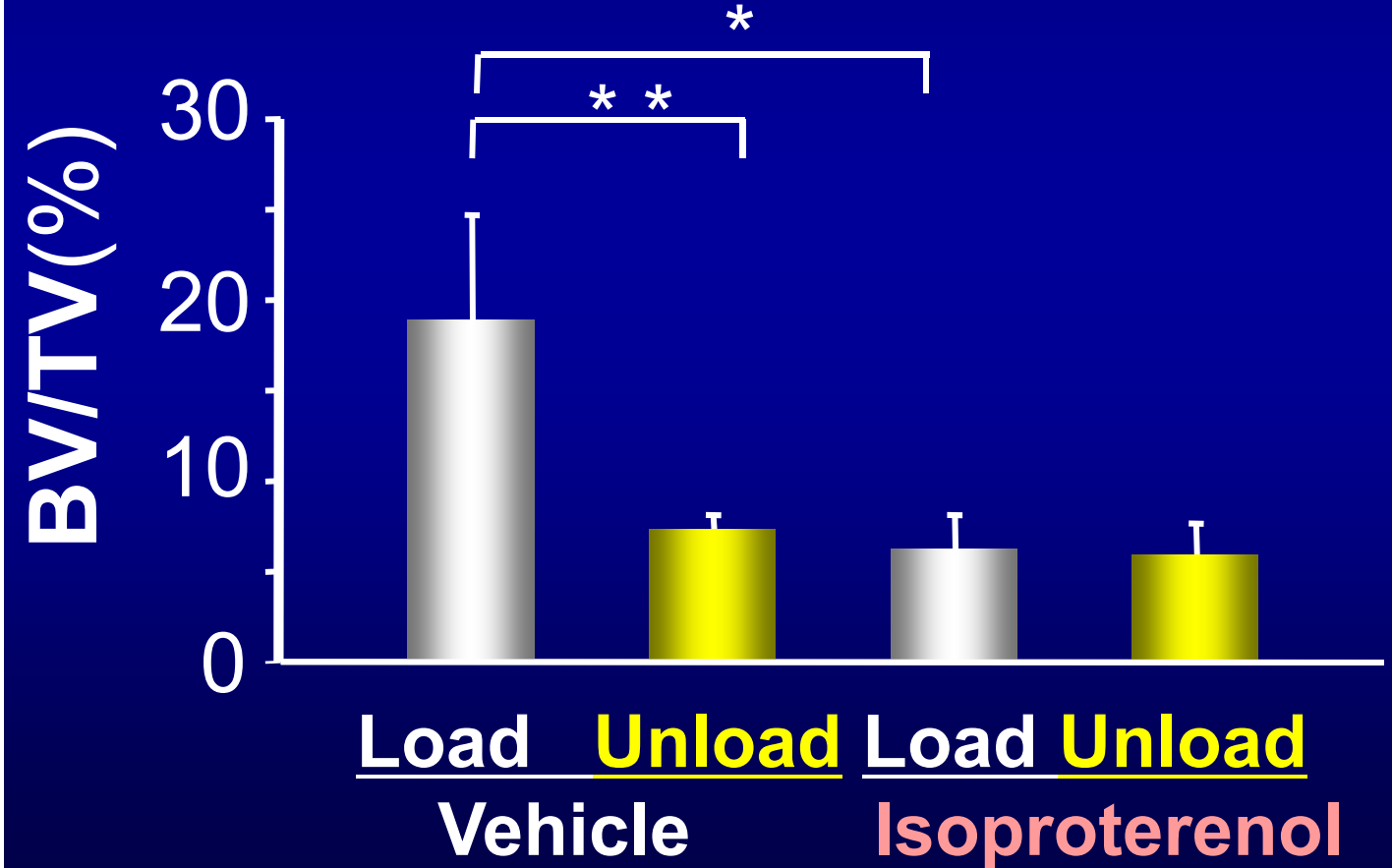
β-Antagonist Isoprotelonol Treatment

Animals: 12 week-old male
C57BL/6 mice



Isoproterenol Decreased Basal Level of Bone Mass





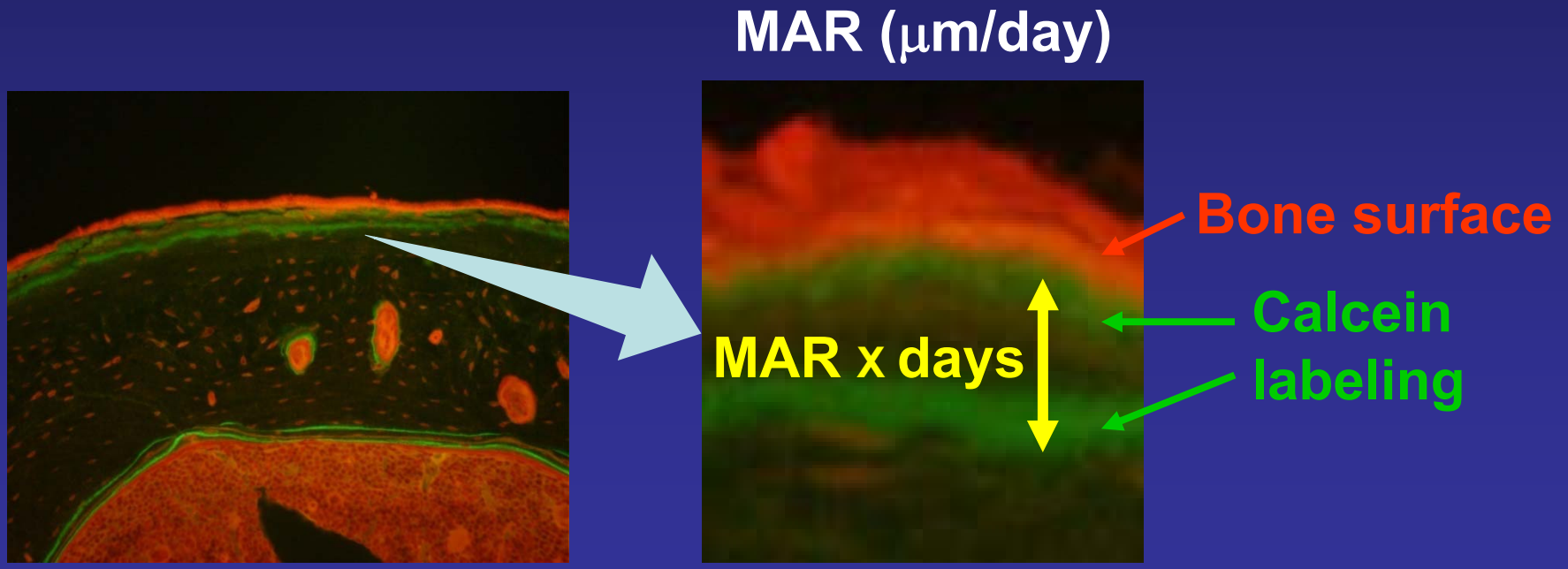
*p<0.05
**p<0.001

10 days unload

Bone Formation Marker

Osteoblast parameter

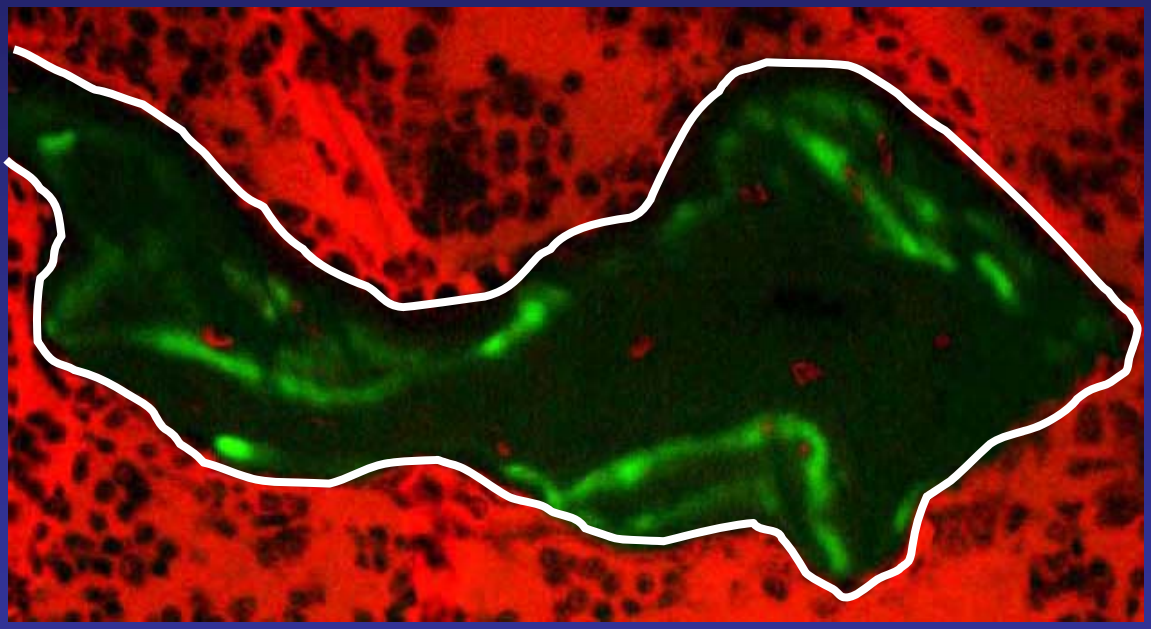
Parameter; Bone Formation Rate (BFR); 骨形成速度
Mineral Apposition Rate (MAR); 骨石灰化速度



(Parfitt *et al*., J. Bone Miner. Res. 1987)

Osteoblast parameter

BFR=MAR x (sLS/2+dLS) /BS ($mm^3/mm^2/day$)



BS; Bone surface

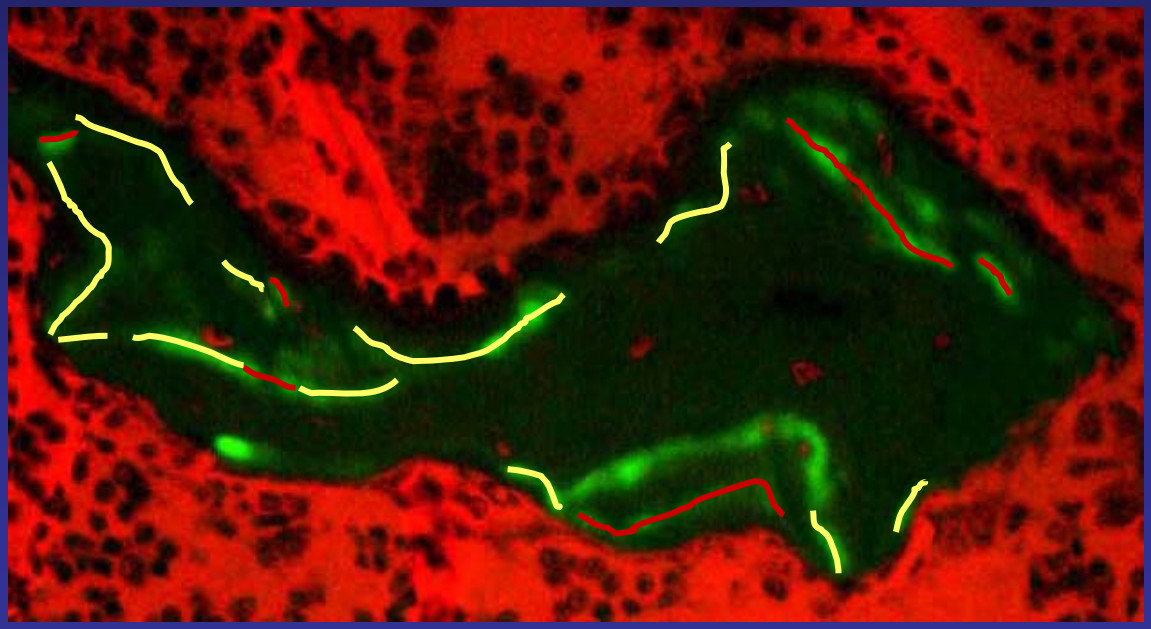
sLS; Single labeled surface

dLS; Double labeled surface

(Parfitt *et al*., J. Bone Miner. Res. 1987)

Osteoblast parameter

BFR=MAR x (sLS/2+dLS) /BS ($mm^3/mm^2/day$)

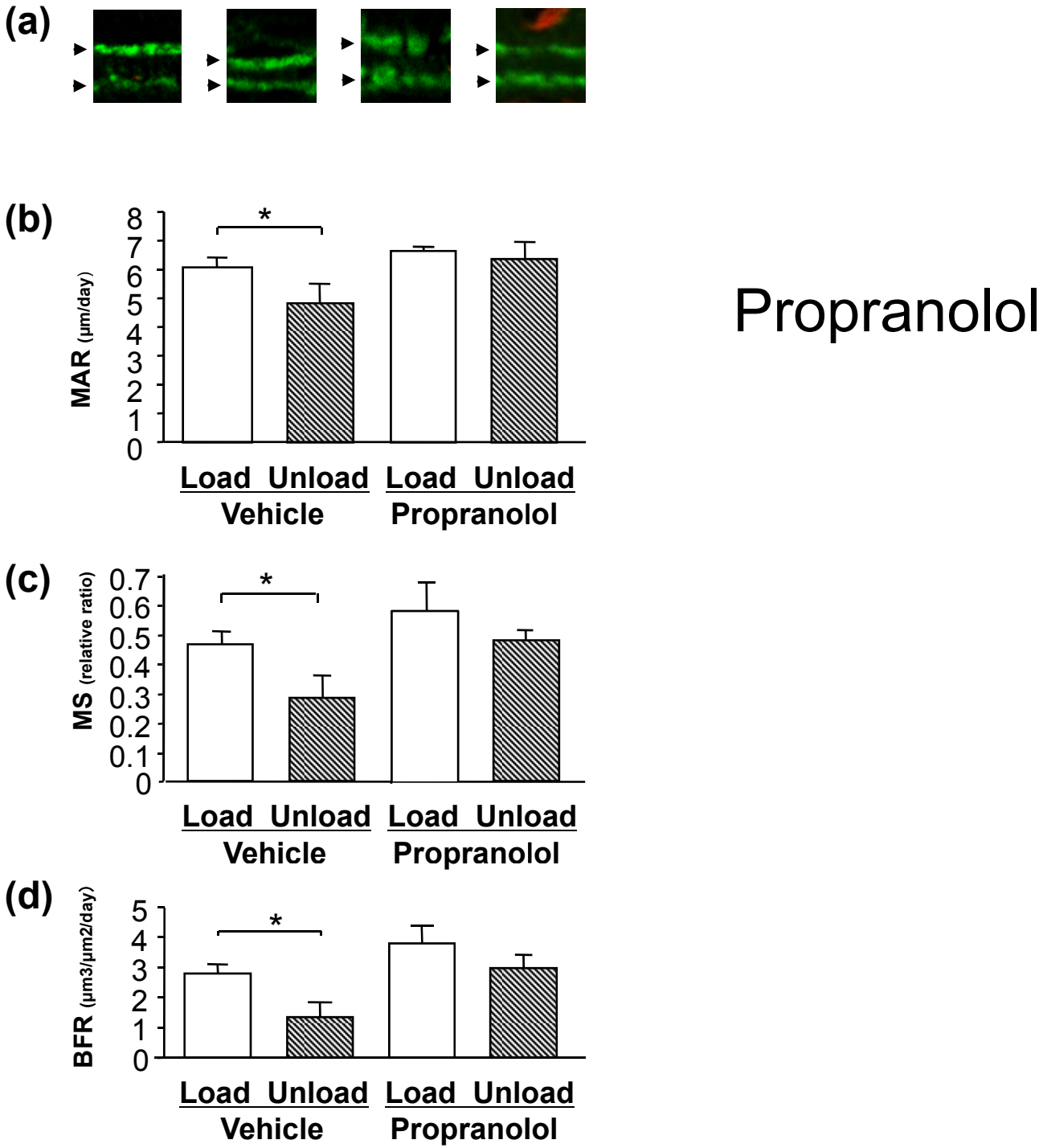


BS; Bone surface

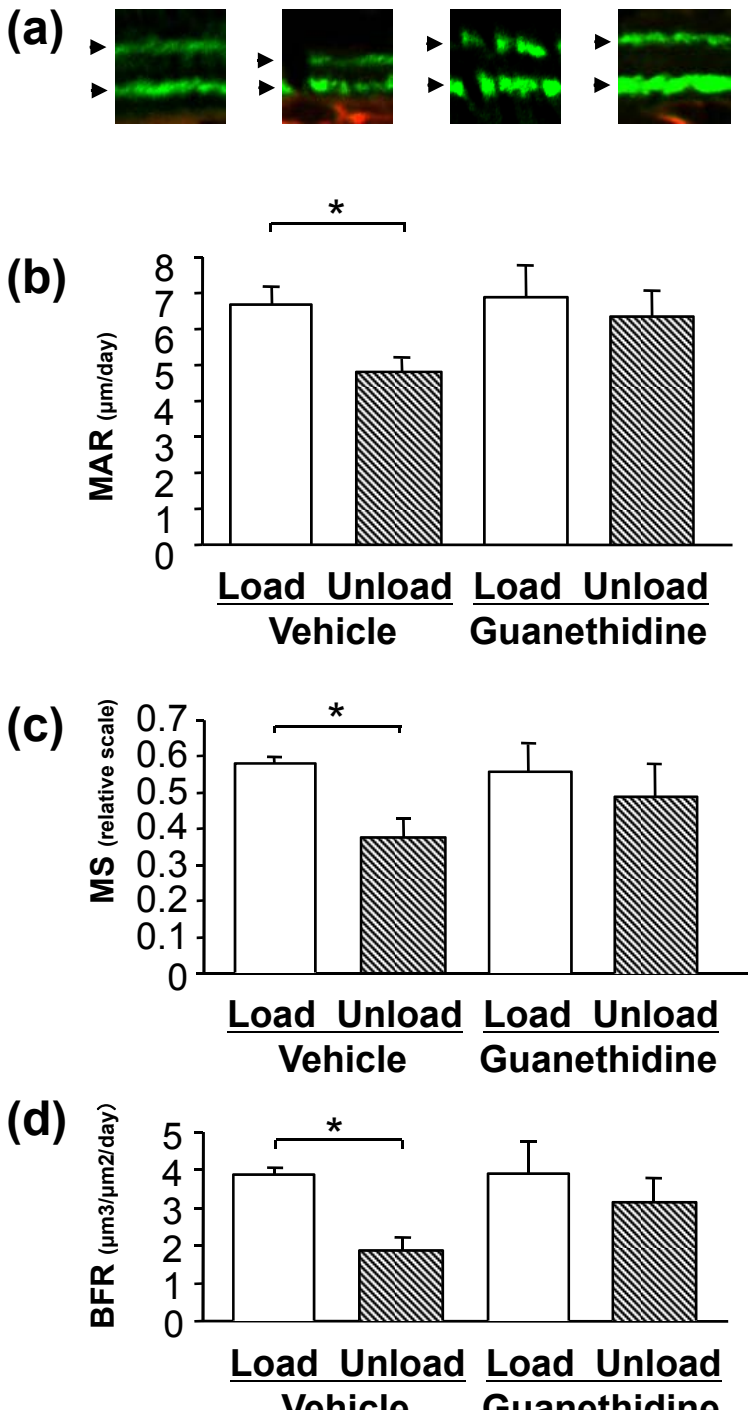
sLS; Single labeled surface

dLS; Double labeled surface

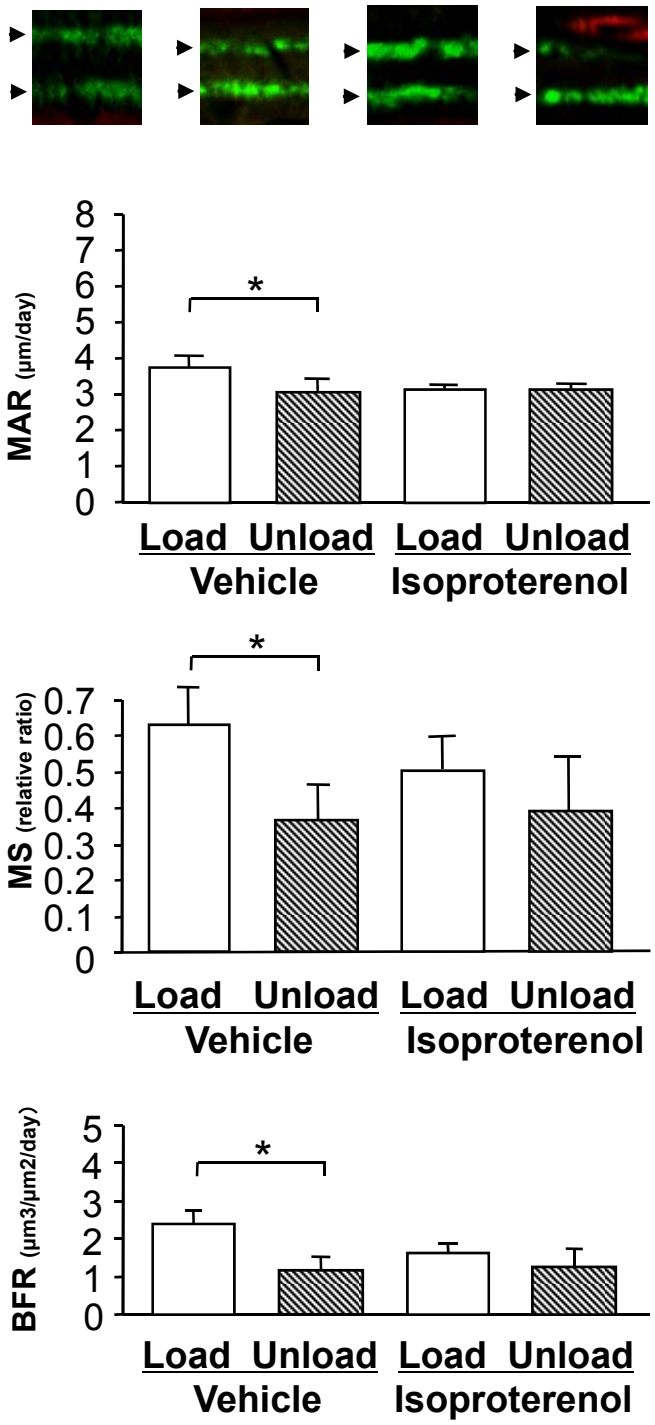
(Parfitt *et al*., J. Bone Miner. Res. 1987)



Propranolol



Guanethidine



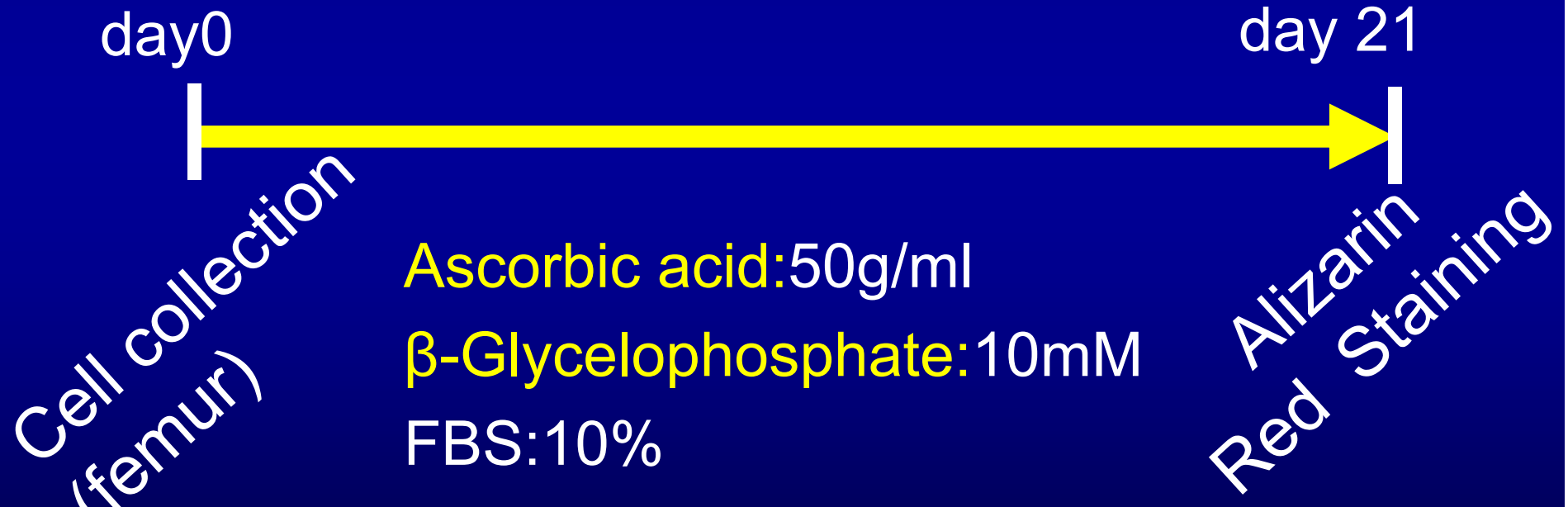
Isoproterenol

Nodule Formation

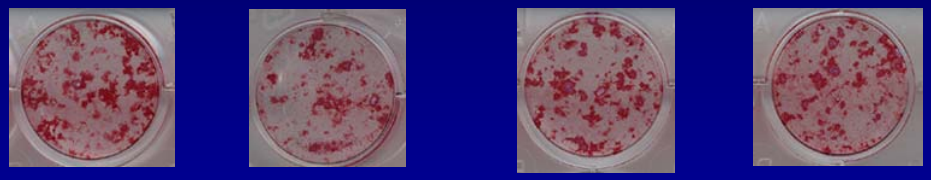
Bone Formation Analysis

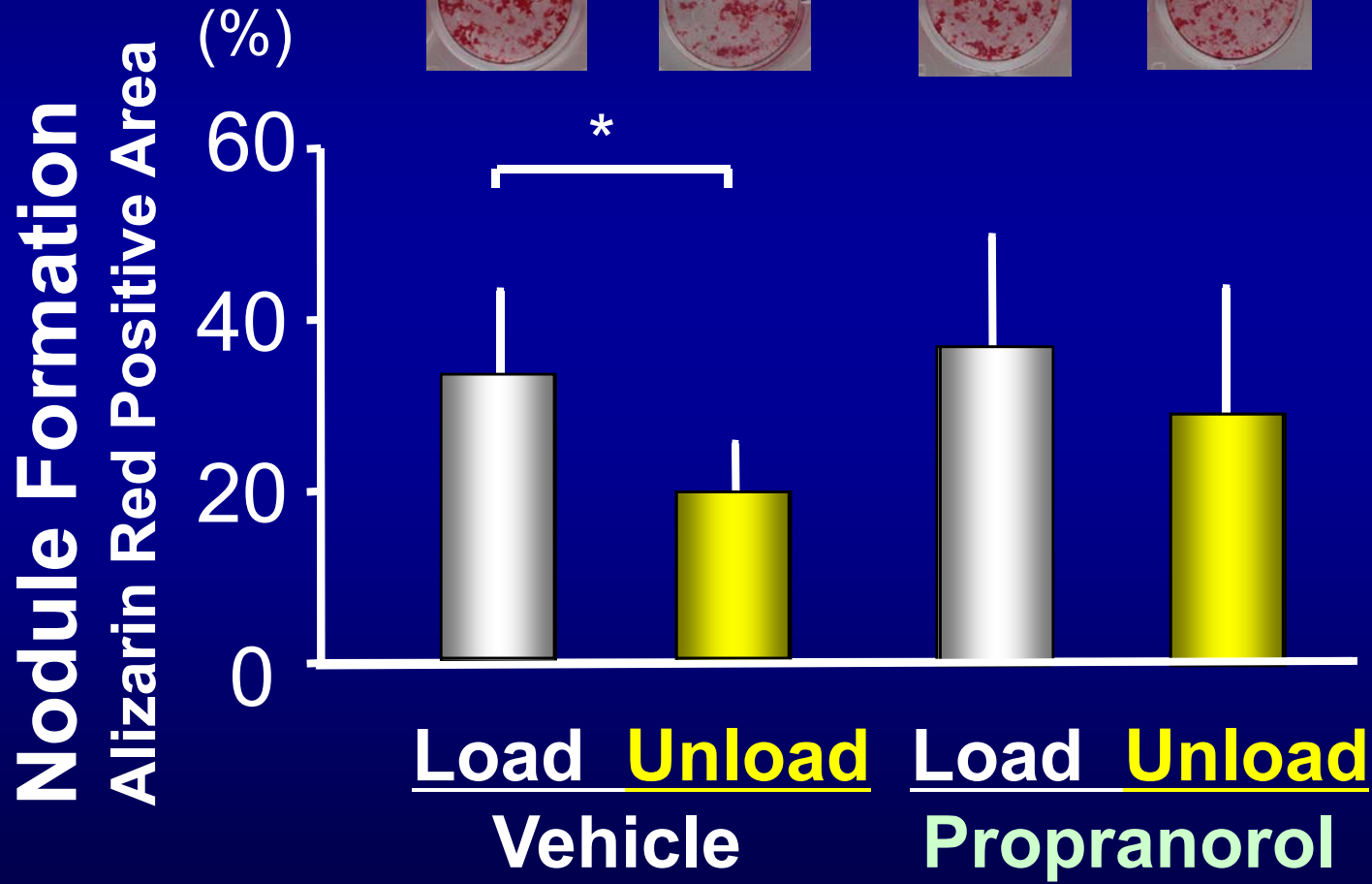
Ex Vivo Nodule Formation (Bone Marrow Cells)

- Plating Density 7.5×10^5 cells / cm^2



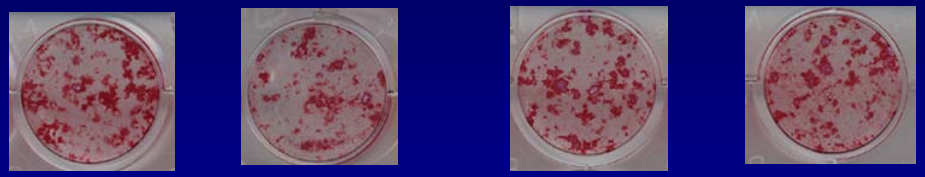
Propranorol Prevented Unloading-Induced Repression of Bone Formation

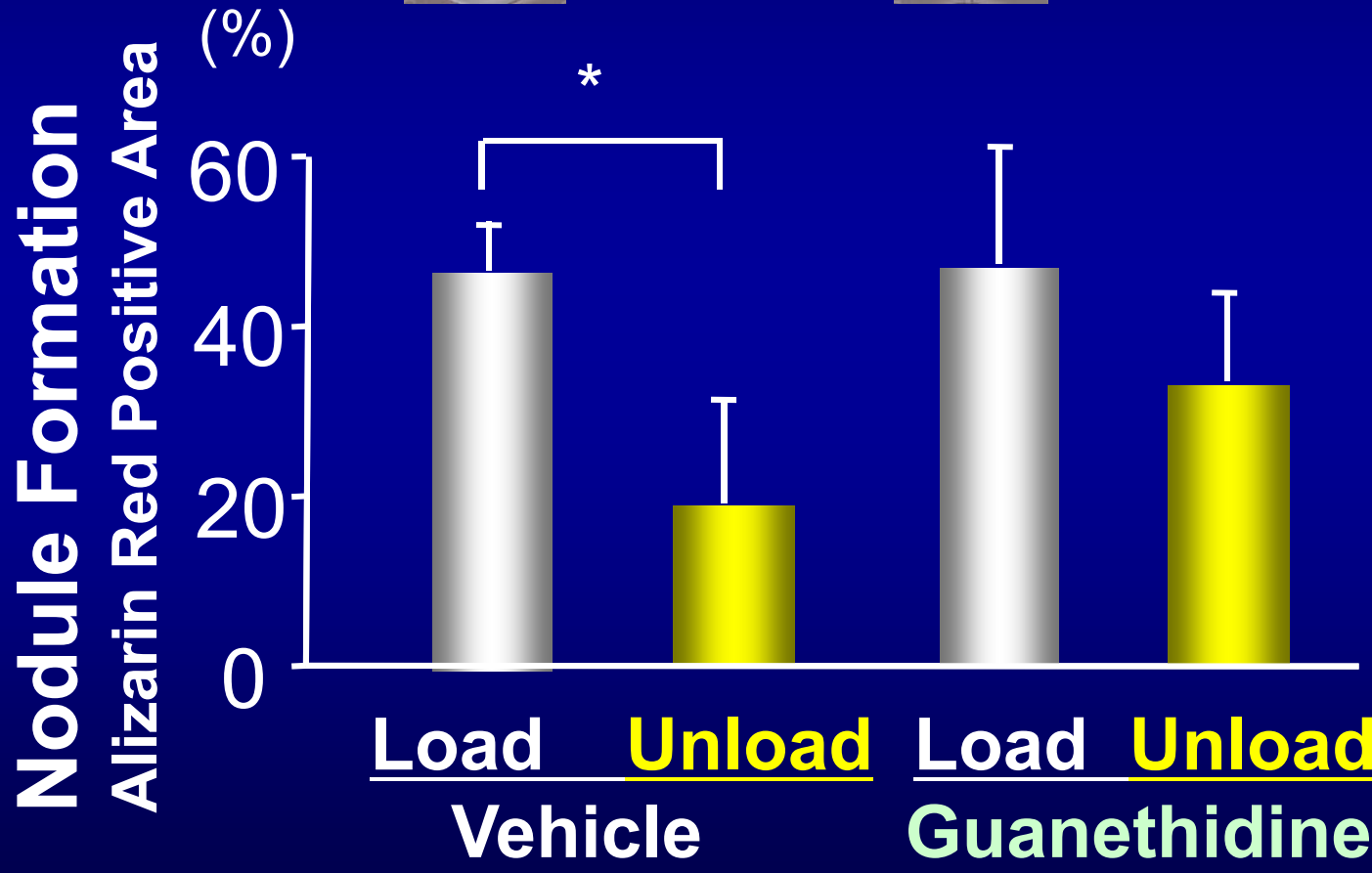




*p<0.05

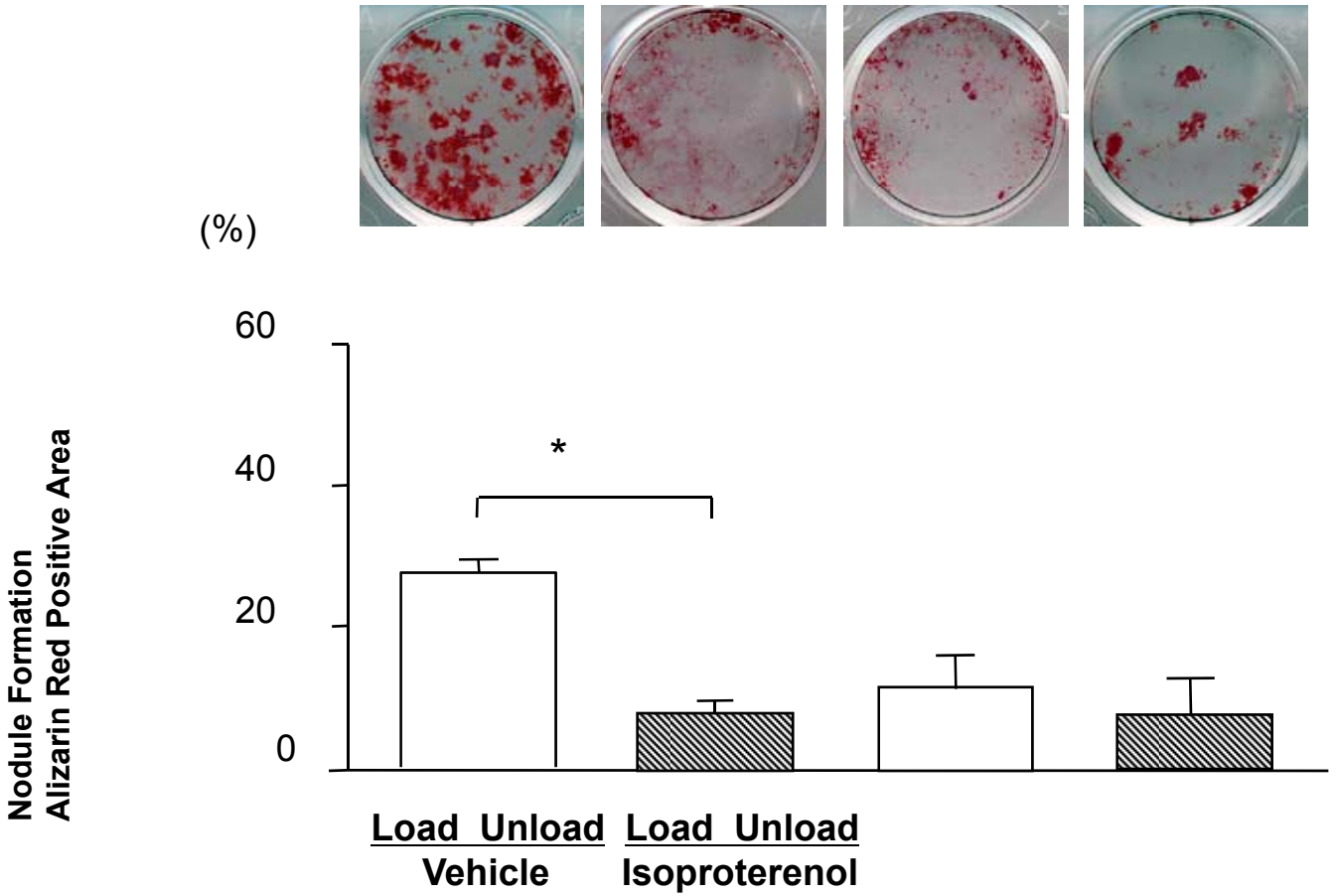
Guanethidine Prevented Unloading-Induced Repression of Bone Formation





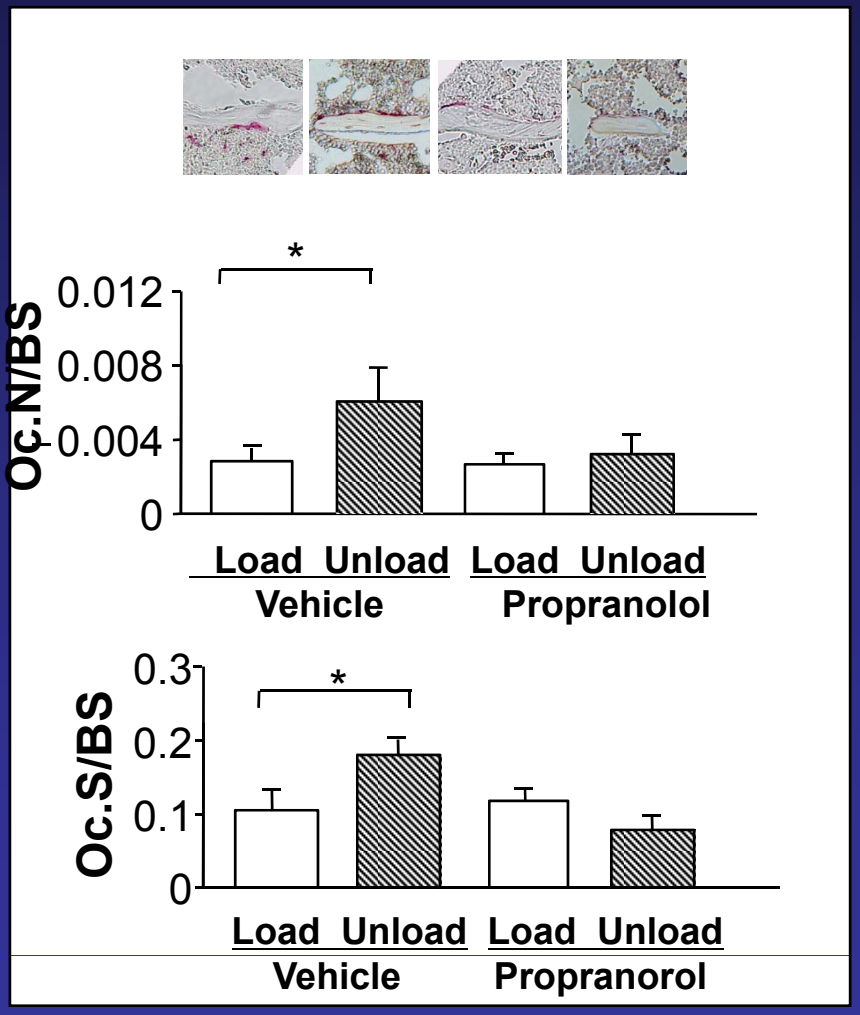
*p<0.001

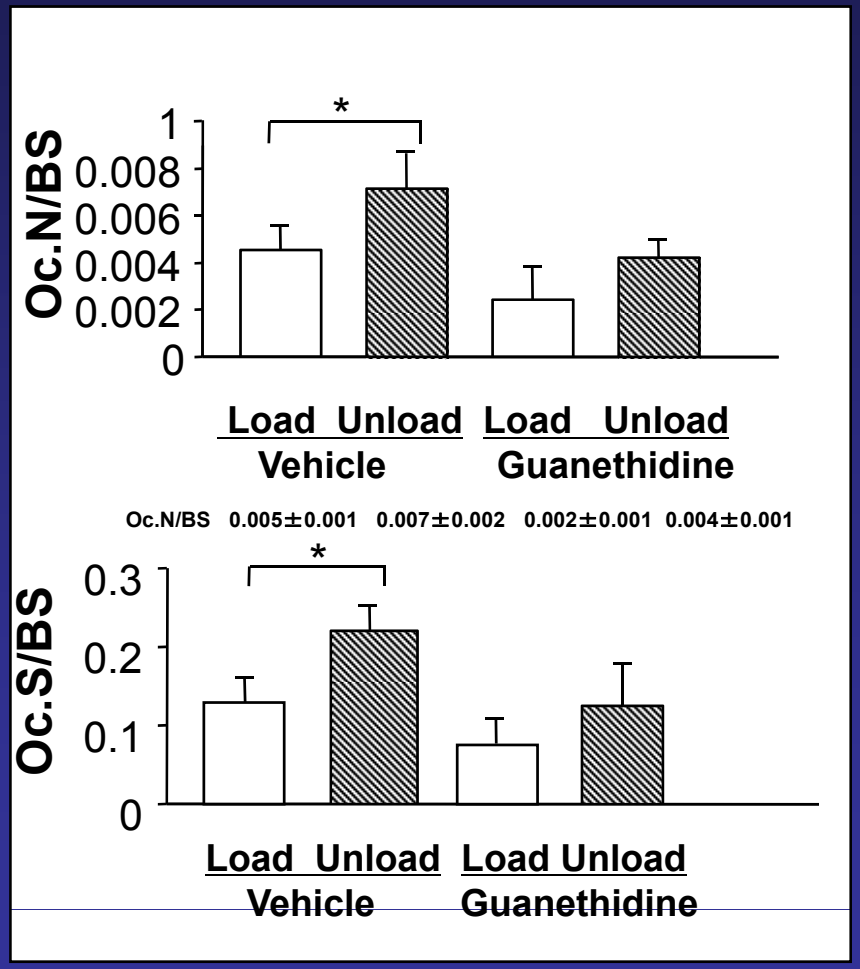
Isoproterenol



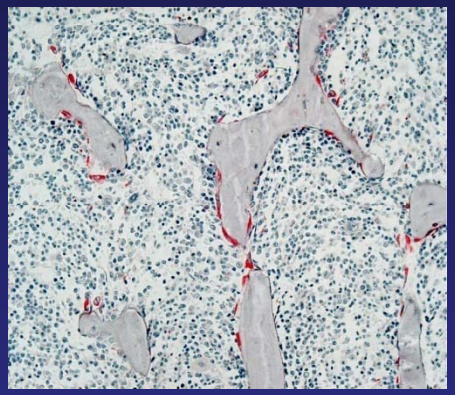
Bone Resorption Marker

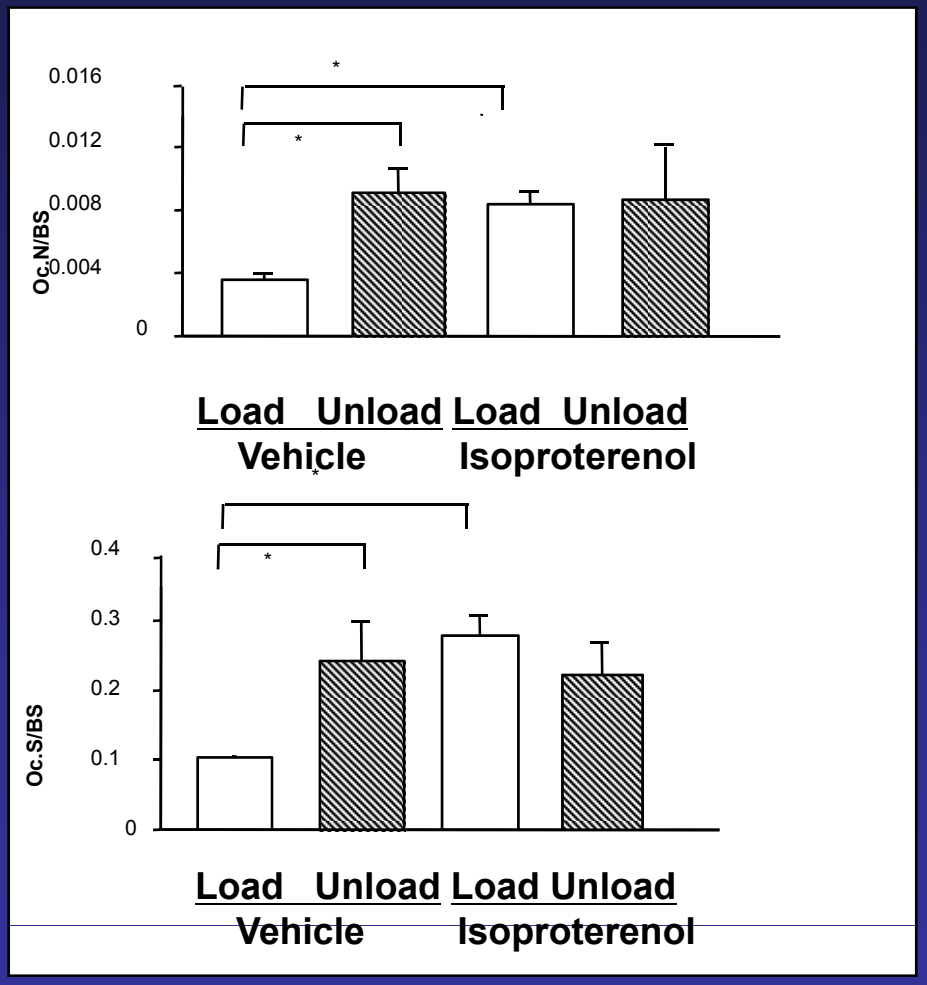
β-antagonists Propanolol and Guanethidine Suppressed Unloading-Induced Enhancement of Osteoclast Number and Surface





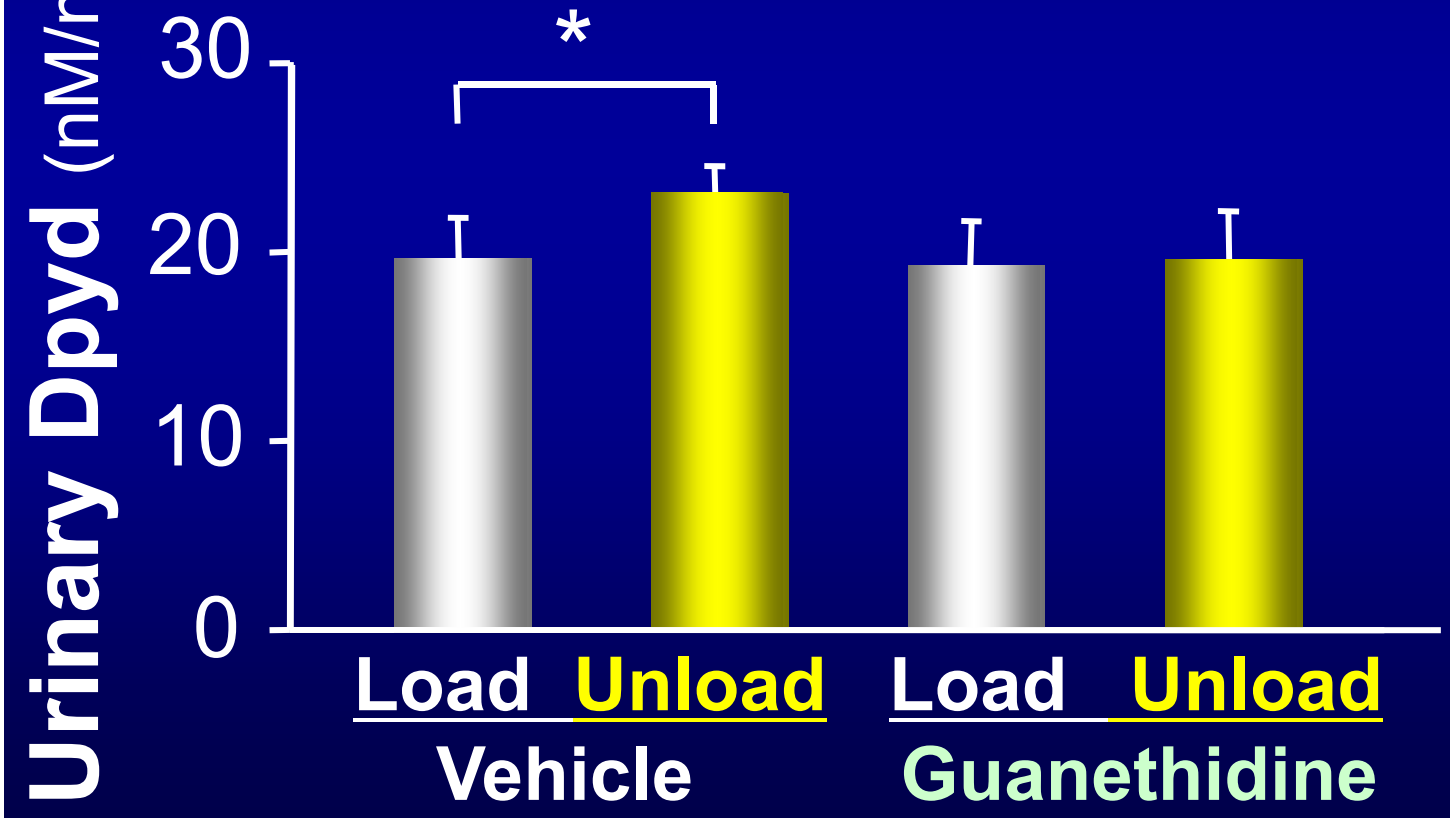
β-agonist Isoproterenol Increased Osteoclast Number and Surface as Unloading Conditions





Urinary Pyridinoline

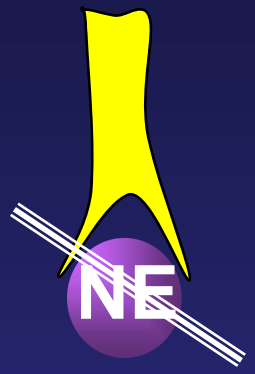
Guanethidine Suppressed Unloading-Induced Enhancement of Urinary Deoxypyridinoline



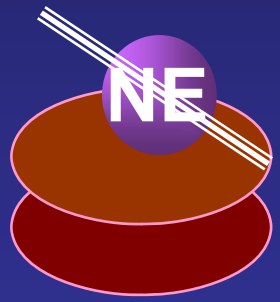
*p<0.05

2 weeks susp

Sympathetic Nerve

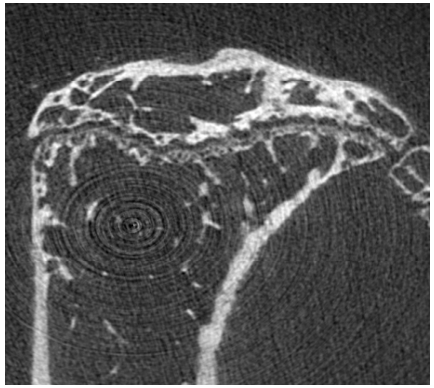


Dopamine
Beta
Hydroxylase +/-

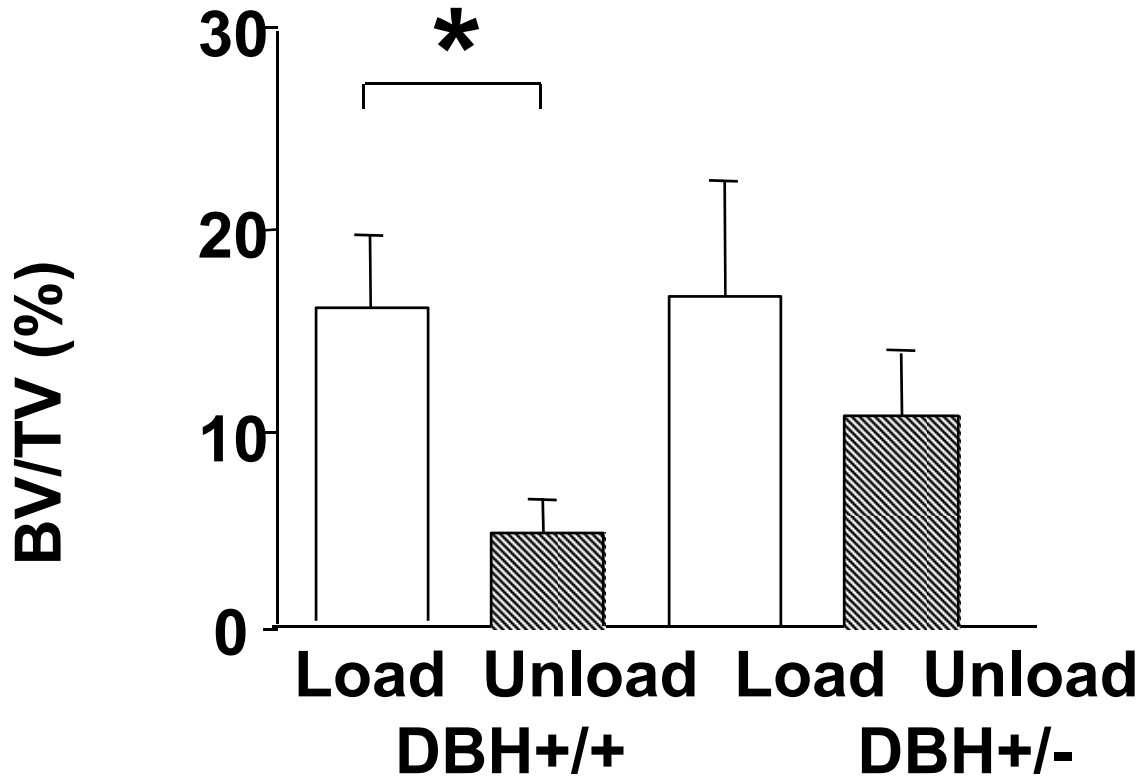


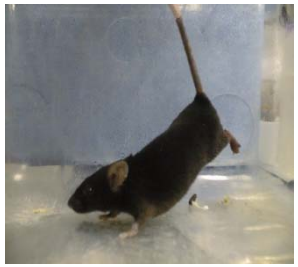
β-Adrenergic Receptors

薬剤によらない Geneticsからの　検討



骨量

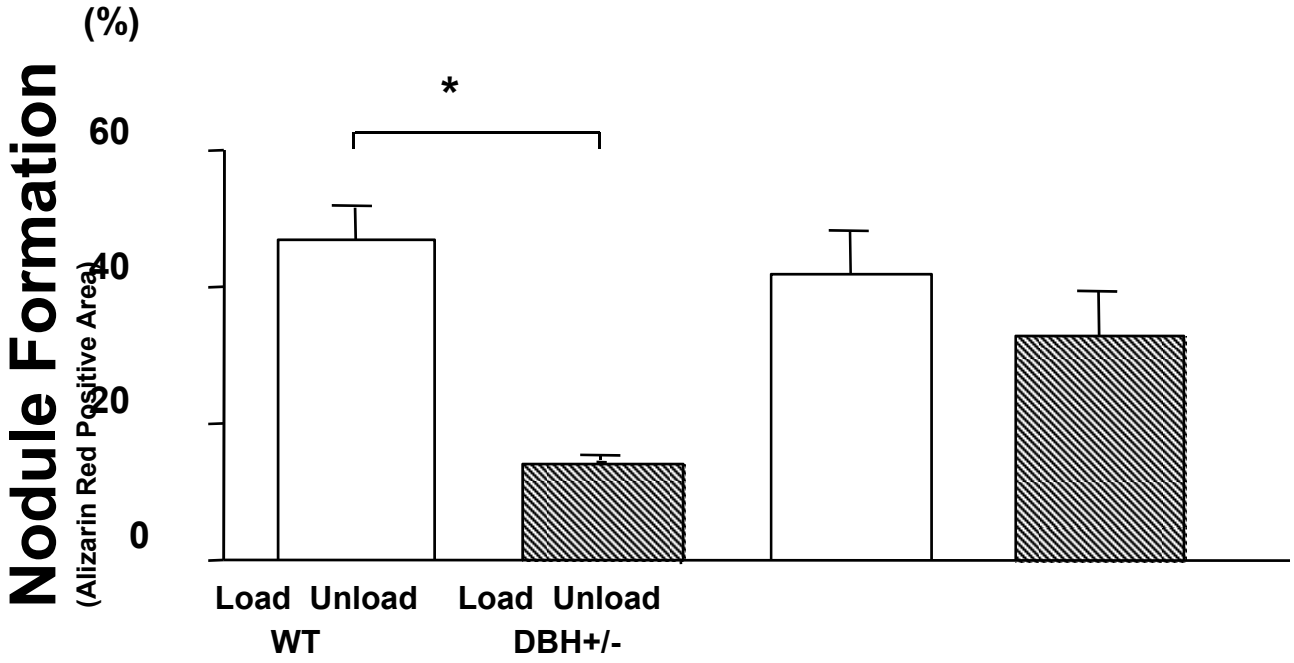


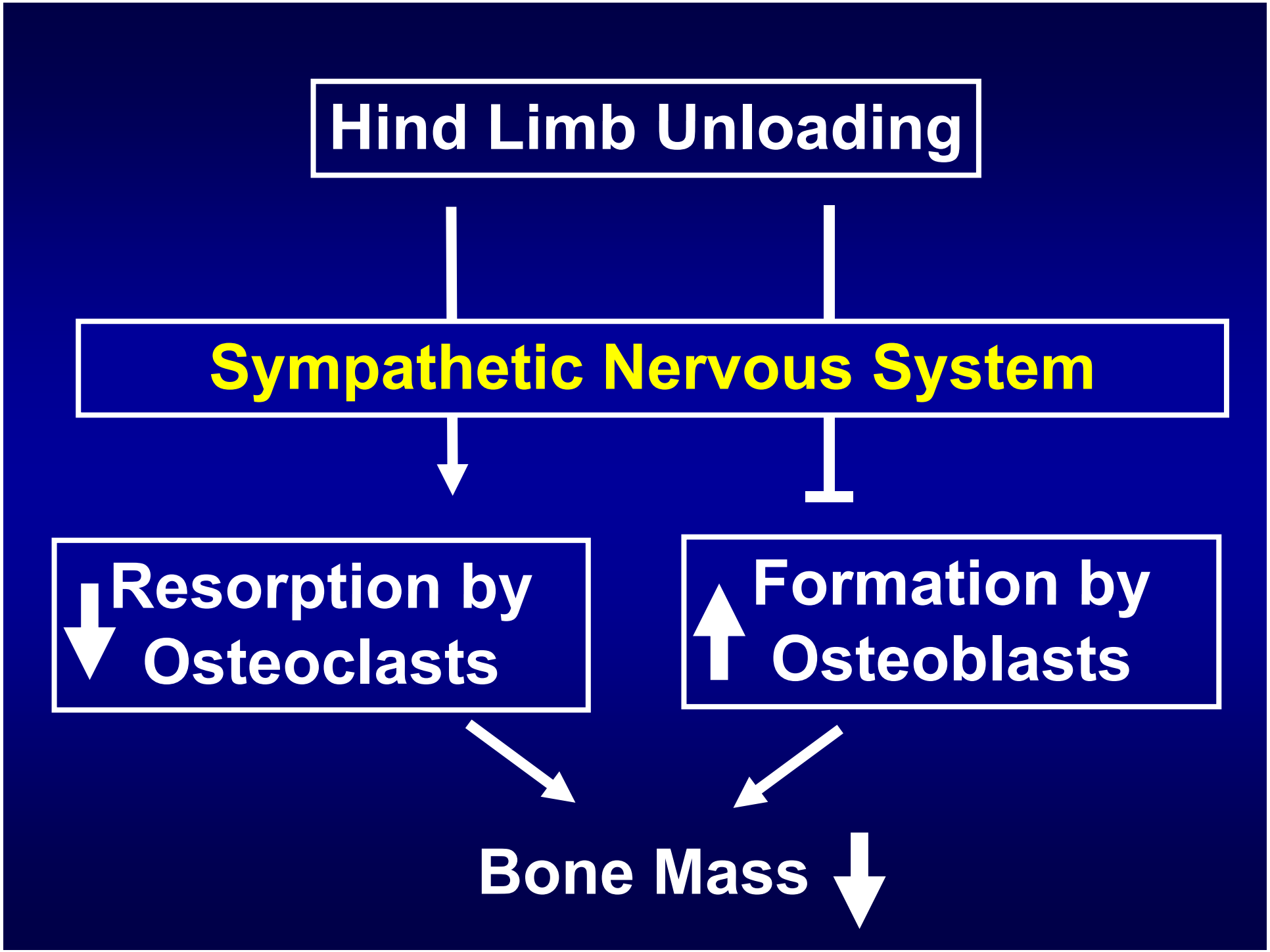


薬剤によらない Geneticsからの　検討

培養骨芽細胞の石灰化

Dopamine
Beta
Hydroxylase +/-




Hind Limb Unloading
Sympathetic Nervous System
Resorption by Osteoclasts
Formation by Osteoblasts
Bone Mass

Unloading/Sympathetic Tone