BONE TURNOVER MARKERS
STILL NOT READY FOR PRIME TIME

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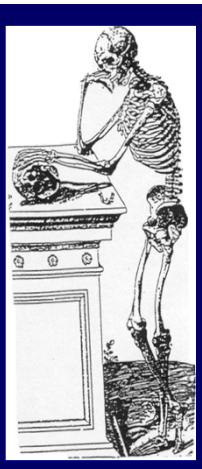
DISCLOSURES

- Stock options/holdings, royalties, company owner, patent owner, official role: OsteoDynamics co-founder, shareholder, director
- I have received honoraria for lectures from the following companies in the past year: Amgen, Merck
- I have received consulting fees from the following companies in the past year: AbbVie, Amgen, Merck, Radius, Sprout
- Through my employer, I have research support from the following companies: Merck, NPS

WHAT IS BONE TURNOVER (BONE REMODELING)

- What is bone turnover?
- What is its purpose?
- What are “markers” of bone turnover?
- What can they tell us
  - About normal physiology?
  - Disease states?
  - Effects of medications?
- How can they be used clinically?

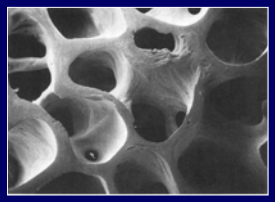
FUNCTIONS OF THE SKELETON



- Support
- Anchors for muscle attachment
- Protection of internal organs
- Cavities for blood formation
- Reservoir for minerals

DIFFERENT SKELETAL REGIONS HAVE DIFFERENT TYPE OF BONE

Cortical or compact bone makes up the outer envelope of all bones and the shafts of the long bones (appendicular skeleton)

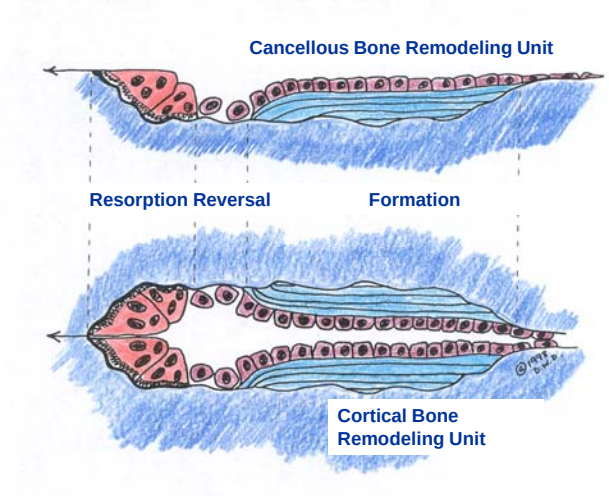


Cancellous or trabecular bone makes up the inner parts of the bones, particularly bones of the central skeleton

THE COMPOSITION OF BONE

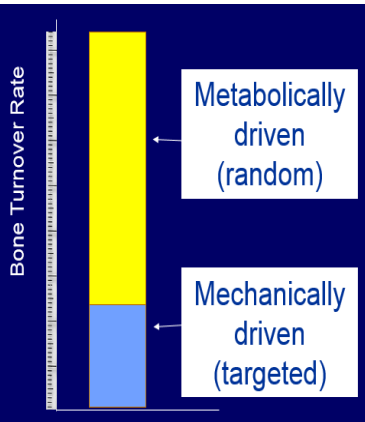
- Bone matrix: 90% is collagen, the rest is made up of other proteins (osteocalcin, osteonectin, osteopontin)
- Bone mineral: hydroxyapatite (calcium and phosphorus)
- Bone cells:
  - osteoclasts (resorption)
  - osteoblasts (formation)
  - osteocytes (regulation)

BONE REMODELING UNIT



FUNCTIONS OF BONE TURNOVER

- Ongoing calcium homeostasis and acid-base balance
- Short-term reservoir of labile minerals
- Release of growth factors
- Replacement of osteocytes
- Repair of microdamage



REMODELING *VERSUS* MODELING

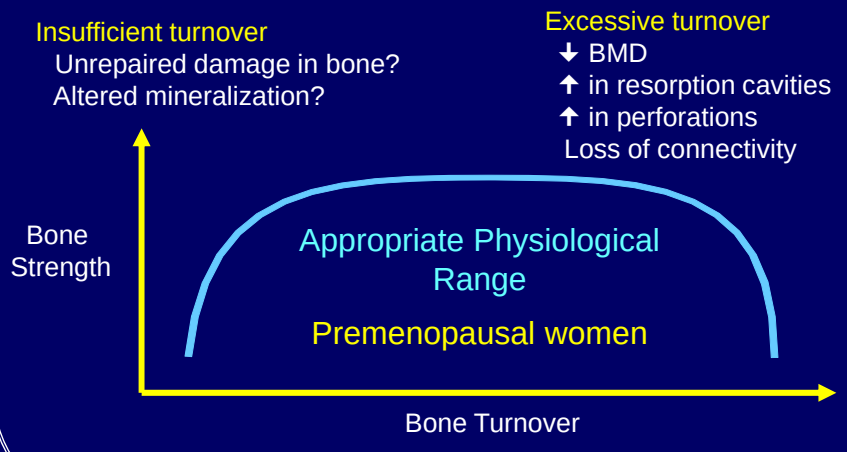
Remodeling

- Predominant form of bone turnover in skeletally mature adults
- Mediated by the coordinated activities of osteocytes, osteoclasts and osteoblasts that make up "basic multicellular units" (BMUs)

Modeling

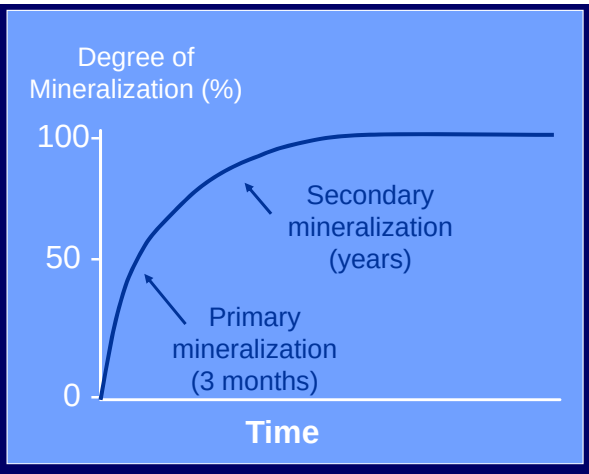
- Involves independent activities of osteoclasts or osteoblasts
- Most common during growth and in response to altered mechanical loading
- Osteoclasts and osteoblasts can alter bone mass and geometry -- to leave the skeleton as light as possible while meeting biomechanical needs

OPTIMIZING BONE TURNOVER TO MAINTAIN BONE STRENGTH



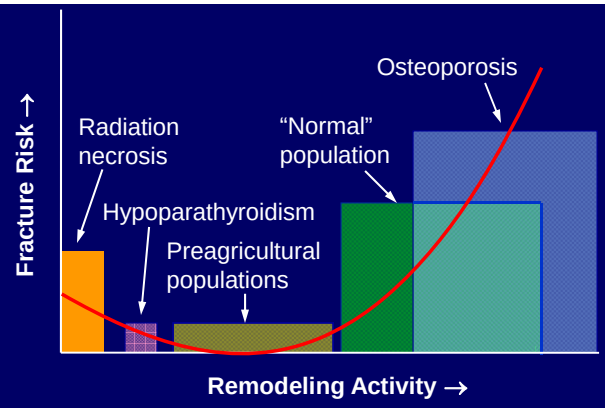
Adapted from Weinstein RS. *J Bone Miner Res* 2000:15;621

BONE TURNOVER RATE AFFECTS DEGREE OF MINERALIZATION



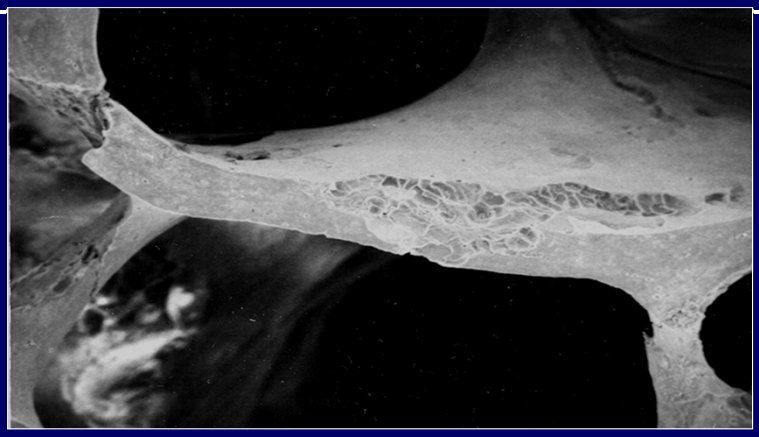
- Lower rate of bone turnover allows the slow process of secondary mineralization to occur

WHAT IS THE BEST LEVEL OF TURNOVER?



Heaney RP *Bone* 2003;33:457-465

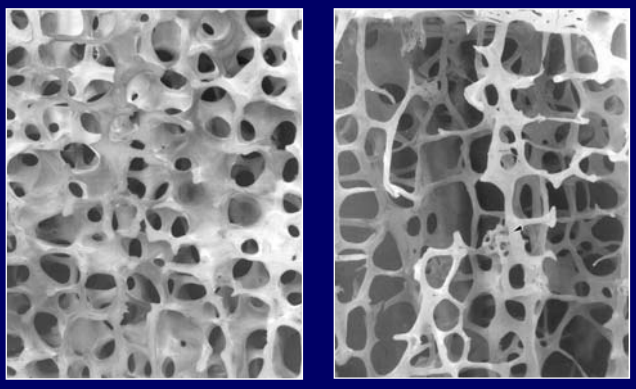
TRABECULAR PERFORATION



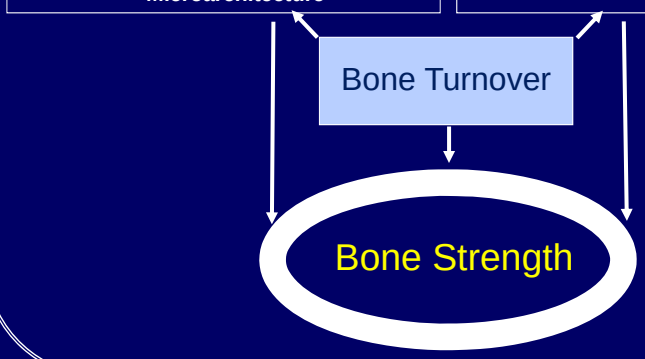
MICROARCHITECTURAL DETERIORATION

Accelerated with High Bone Turnover

Normal **Osteoporosis**



DETERMINANTS OF BONE STRENGTH

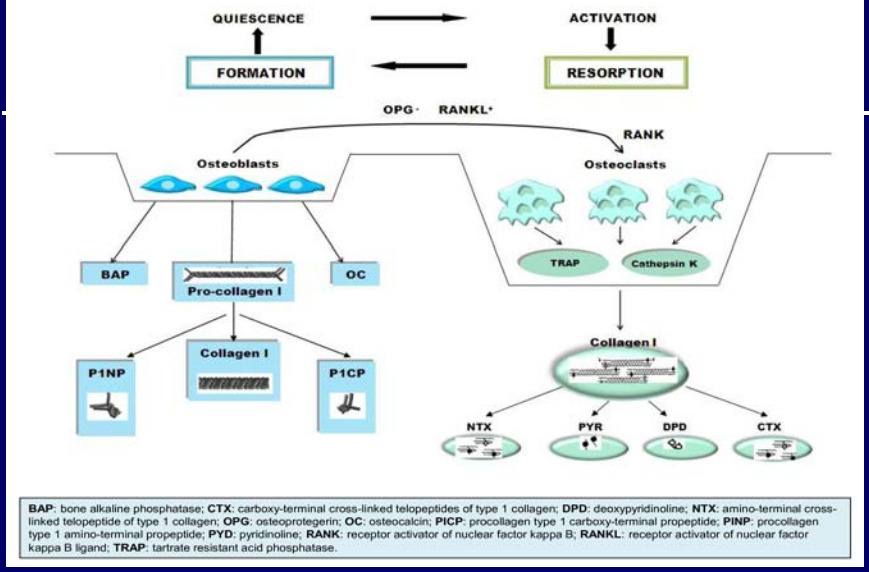


CATEGORIES OF BIOMARKERS

Table 1 Categorization of biomarkers by the US Food and Drug Administration (FDA) [26]

Biomarker category	Characteristics
Prognostic biomarker	Categorizes patients by probability for disease occurrence or progression in the absence of intervention
Predictive biomarker	Categorizes patients by their probability for a positive or negative response to a treatment
Pharmacodynamic biomarker	Demonstrates biological response has occurred in a patient after having received a therapeutic intervention
Surrogate end point biomarker	A subset of pharmacodynamic biomarkers Substitutes for a clinical efficacy end point and is expected to predict clinical benefit or harm Requires robust scientific evidence to justify qualification as a surrogate end point

Krege J et al, *Osteoporos Int* 2014;25:2159-2171



BAP: bone alkaline phosphatase; **CTX**: carboxy-terminal cross-linked telopeptides of type 1 collagen; **DPD**: deoxypyridinoline; **NTX**: amino-terminal cross-linked telopeptide of type 1 collagen; **OPG**: osteoprotegerin; **OC**: osteocalcin; **PICP**: procollagen type 1 carboxy-terminal propeptide; **PINP**: procollagen type 1 amino-terminal propeptide; **PYD**: pyridinoline; **RANK**: receptor activator of nuclear factor kappa B; **RANKL**: receptor activator of nuclear factor kappa B ligand; **TRAP**: tartrate resistant acid phosphatase.

Wheater G et al, J Transl Med 2013;11:201-2014

CAUSES OF BTM VARIABILITY

Table 1 Uncontrollable and controllable sources of pre-analytical variability according to their importance

Source	Importance	Nature of effect
Uncontrollable sources		
Age	Very important	BTM increase with age in men and women
Menopausal status	Very important	BTM increase within a few months after the last menstrual period
Gender	Very important	BTM are higher in older women than older men
Fractures	Important—limits evaluation of case control studies	BTM increase after a fracture (maximal at 2 to 12 weeks, but effect lasts for up to 52 weeks)
Pregnancy and lactation	Important	BTM are increased during pregnancy; highest levels during third trimester, even higher postpartum
Drugs	Important: corticosteroids, anticonvulsants, heparin, GnRH agonists	BTM may be decreased (glucocorticoids) or increased (anticonvulsants)
Disease	Important: thyroid disease, diabetes, renal impairment, liver disease	BTM often increased (thyrotoxicosis, chronic kidney disease)
Bed rest/immobility	Important	Bone formation markers decrease and resorption markers increase
Geography	Somewhat important	Small changes amongst countries, usually explained by differences in lifestyle
Ethnicity	Not important	Small changes, such as lower OC in African Americans vs. Caucasians
Oral contraception	Not important, except in women over 35 years	Lower values for BTM

Vasikaran S et al, Osteoporos Int 2011:22:393-420

CAUSES OF BTM VARIABILITY

Controllable sources		
Circadian	Extremely important	Most striking for bone resorption markers; highest values in second half of night and on waking; lowest values in afternoon and evening
Fasting status	Important for specific markers	Feeding results in a decrease in BTM; for example, s-CTX decreases by 20% after breakfast
Exercise	Important—chronic and acute effects	Changes occur but depend on type of exercise and age of subjects
Menstrual	Not important	Small decreases in bone resorption and increases in bone formation during luteal phase
Seasonal	Not important for individual, but maybe for longitudinal studies	Small decreases in BTM over winter
Diet	Not important	Small reduction in BTM immediately following calcium supplementation

Vasikaran S et al, Osteoporos Int 2011:22:393-420

SAMPLING FOR BONE RESORPTION MARKERS

- Diurnal variation dictates the need for a morning sample
- Effect of food dictates the need for a fasting sample

- Morning fasting blood sample (CTX)
- Second-morning fasting urine (NTX)

URINE NTX vs SERUM CTX

- Urine NTX expressed as ratio to urine creatinine, which is related to lean body mass, which in turn is related to bone mass; this is a way to normalize turnover to bone mass
- In an older, frail patient, it may be easier to collect a second-voided fasting urine at home than to bring the to the lab early for a fasting blood sample

OTHER FACTORS THAT AFFECT MARKERS

- Liver function (bone alkaline phosphatase, procollagen extension peptides, hydroxyproline cleared by the liver)
- Renal function (osteocalcin, pyridinium cross links, telopeptides cleared by the kidneys)
- Vitamin K status affects osteocalcin

INTRAINDIVIDUAL VARIATION

Least significant change
in serial results (mean ± SD)

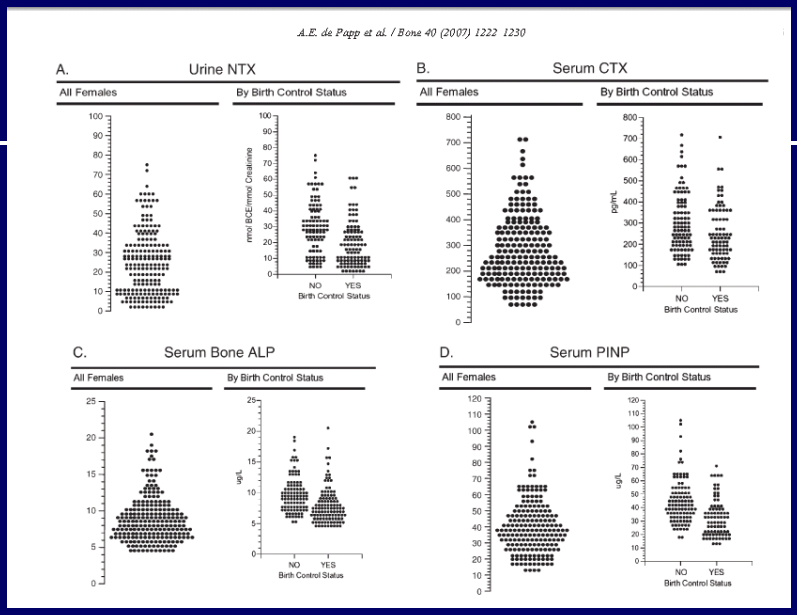
Six serum markers	17.1±4.8%
Five urine markers	38.5±7.7%

Blumsohn A et al, *J Bone Mineral Res* 1994;9 (suppl 1):S153

WHAT ABOUT DIFFERENT LABS?

Transferability of test results for a given analyte has long been a goal of the laboratory community. Achievement of transferability will require development of standardized methods that have the least possible measurement uncertainty and are traceable to reference standards… Transferability remains an elusive goal, however. Analytical hindrances include the lack of established reference measurement systems for many quantities, lack of traceability of field methods to the reference system, and lot-to-lot variability in reagents and calibrators.

Boyd JC. *Clin Chem* 2008:54:238-239



FACTORS THAT AFFECT MARKERS

- Comparator group:
  - 80 year-old woman, T-score -2.6, urine NTX 60 nmol BCE/mmol Cr, within the lab's range for postmenopausal women (4-64 nmol BCE/mmol Cr)
- Is that the right comparator?

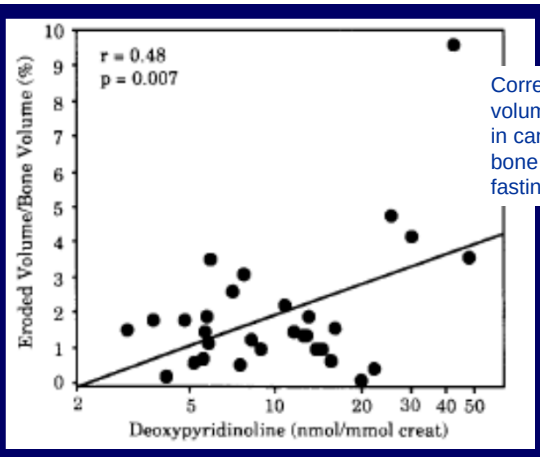
FACTORS THAT AFFECT MARKERS

- Total body bone mass:
  - Two women
    - Age 50, spine BMD 1.0 g/cm^2
    - Age 80, spine BMD 0.6 g/cm^2
  - Are you happy if they have the same serum CTX?

FACTORS THAT AFFECT MARKERS

- Bone formation vs resorption:
  - 80 year-old woman, T-score -2.6,
  - urine NTX high, 90 nmol BCE/mmol Cr
- Does it matter what her bone formation is doing?

HOW WELL DO BTMs REFLECT BONE TURNOVER?



Correlation between eroded volume/bone volume measured in cancellous + endocortical bone in 30 patients and DPD in fasting urine specimens

Roux 1995 Bone

QUESTIONS THAT BONE MARKERS MIGHT HELP ANSWER

- Does this patient have low bone mass?
- What is this patient's risk of fracture?
- Is this patient losing bone?
- How do treatments work?
- What would be the best treatment?
- Is the patient responding to treatment?
- When to start a bisphosphonate holiday?
- When to end a bisphosphonate holiday?

PROSPECTIVE STUDIES:
RESORPTION MARKERS AND FRACTURE RISK

Study (patient subgroup)	Fracture type	Marker of bone resorption	Odds ratio or relative risk (95% CI)
EPIDOS[1] (>2SD above premenopausal mean)	Hip	uCTX	2.2 (1.3, 3.6)
		uNTX	1.4 (0.9, 2.2)
		uDPD	1.9 (1.1, 3.2)
Rotterdam[2] (above median)	Hip	uDPD	3.4 (1.1, 10.6)
OFELY[3] (upper quartile)	Non-spine	uCTX	2.4 (1.1, 5.0)

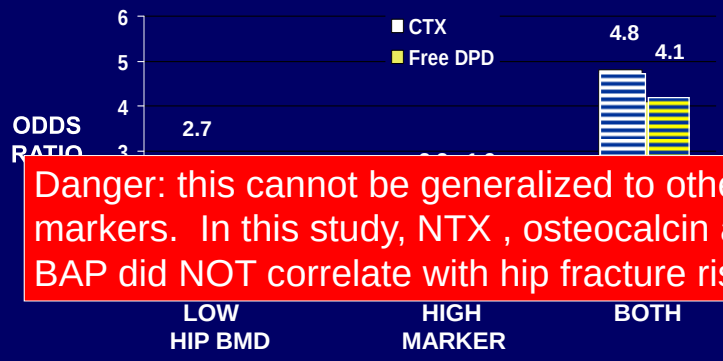
[1]Garnero P, et al. J Bone Miner Res 1996;11:1531–8
[2]Van Daele PL, et al. Br Med J 1996;312:482–3
[3]Garnero P, et al. J Bone Miner Res 2000;15:1526–36

PROSPECTIVE STUDIES: FORMATION MARKERS AND FRACTURE RISK

Study (patient subgroup)	Fracture type	Marker of bone resorption	Odds ratio or relative risk (95% CI)
EPIDOS[1] (>2SD above premenopausal mean)	Hip	OC	1.0 (0.7, 1.6)
		Bone ALP	1.1 (0.7, 1.7)
Rotterdam[2] (above median)	Hip	OC	0.3 (0.1, 1.0)
		Bone ALP	1.0 (0.4, 2.5)
OFELY[3] (upper quartile)	Non-spine	Bone ALP	2.4 (1.1, 4.9)

[1]Garnero P, et al. J Bone Miner Res 1996;11:1531–8
[2]Van Daele PL, et al. Br Med J 1996;312:482–3
[3]Garnero P, et al. J Bone Miner Res 2000;15:1526–36

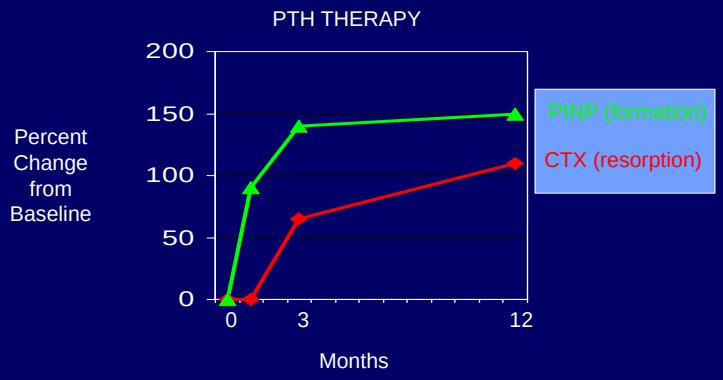
HIGH CTX AND DPD ARE RISK FACTORS FOR HIP FRACTURE



Danger: this cannot be generalized to other markers. In this study, NTX , osteocalcin and BAP did NOT correlate with hip fracture risk.

Garnero P et al, *J Bone Miner Res* 1996;11:1531

REMEMBER: BONE TURNOVER MARKERS **INCREASE** WITH TERIPARATIDE TREATMENT

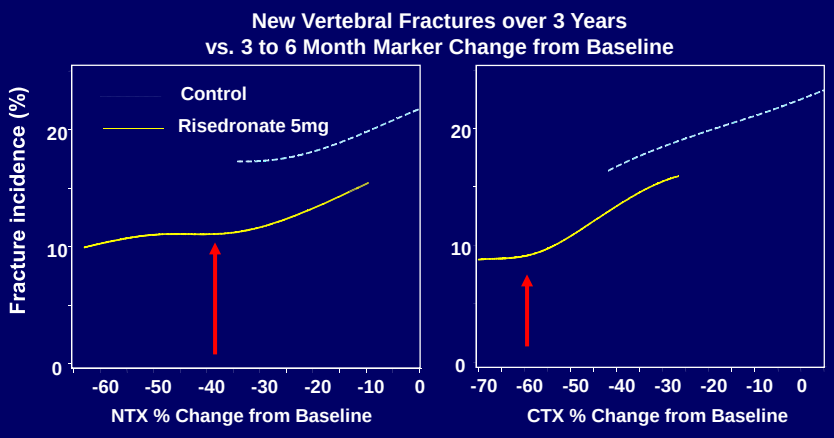


Black DM et al, *N Engl J Med* 2003;349:1207-1215

HOW BONE REMODELING FACTORS INTO THE CLINICAL TREATMENT

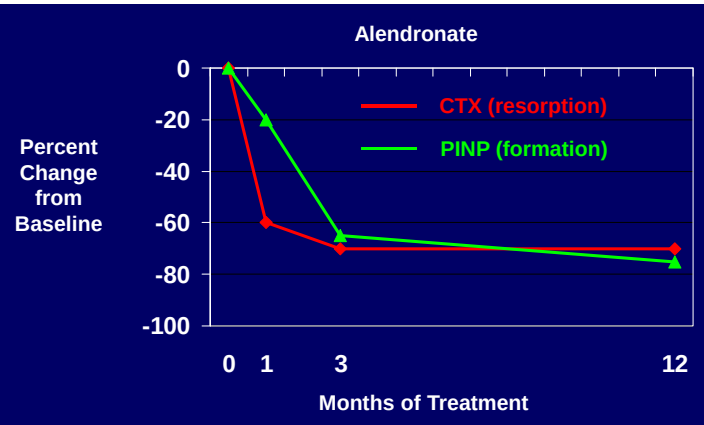
- Logic
  - Patients with high baseline markers would respond best to an antiresorptive agent
  - Patients with low baseline markers would respond best to an anabolic agent
- Experience: it doesn't matter
  - Fracture risk is high in patients with higher BTMs, but relative risk reduction with bisphosphonate is similar regardless of BTM status
  - BMD response to teriparatide is actually higher in patients with high pre-treatment BTMs

RELATIONSHIP BETWEEN CHANGE IN BONE TURNOVER MARKER AND FRACTURE INCIDENCE



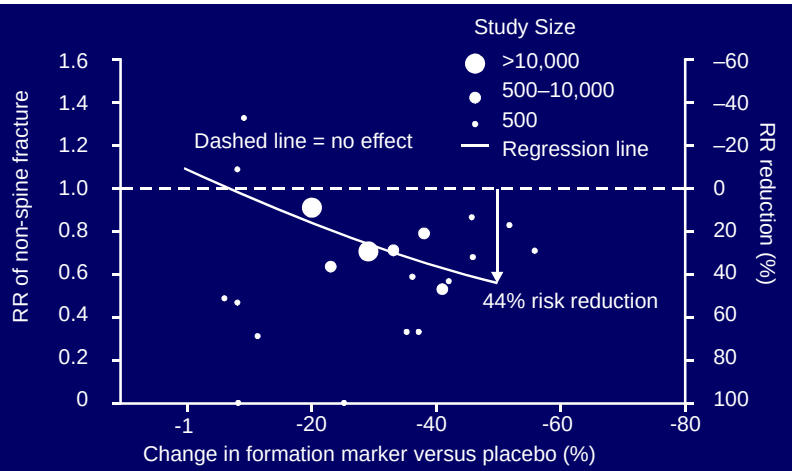
Eastell R et al, *J Bone Miner Res* 2003;18:1051-1056

RESORPTION MARKERS CHANGE MORE RAPIDLY THAN FORMATION MARKERS



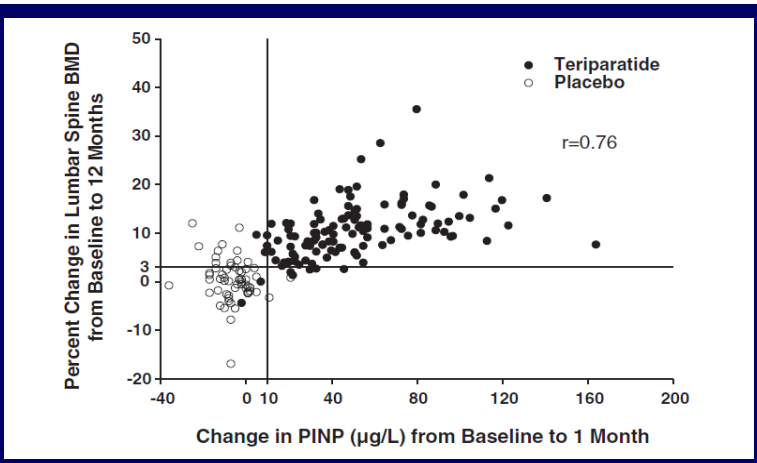
Black DM et al, *N Engl J Med* 2003;349:1207-1215

GREATER DECREASE IN MARKERS OF BONE FORMATION PREDICTS GREATER REDUCTION IN FRACTURE RISK



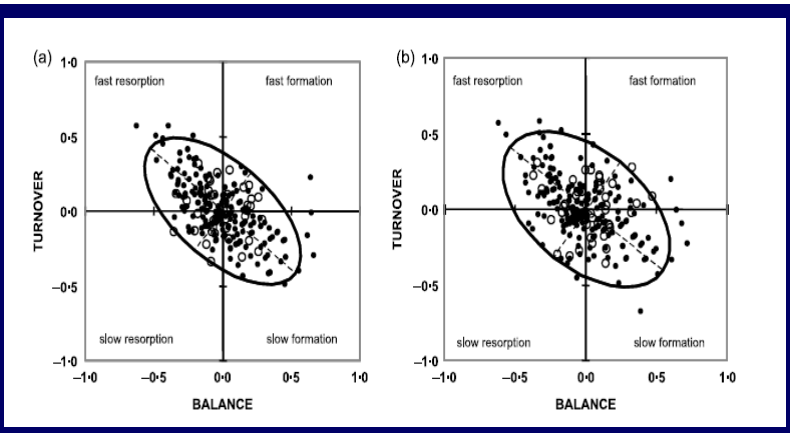
Hochberg MC, et al. J Clin Endocrinol Metab 2002;87:1586–92

CORRELATION BETWEEN P1NP AND BMD CHANGE WITH TERIPARATIDE



Krege J et al, *Osteoporos Int* 2014;25:2159-2171

COMBINING FORMATION AND RESORPTION MARKERS



Bieglmayer C et al, *Eur J Clin Invest* 2009;39:230-238

COMBINING FORMATION AND RESORPTION MARKERS

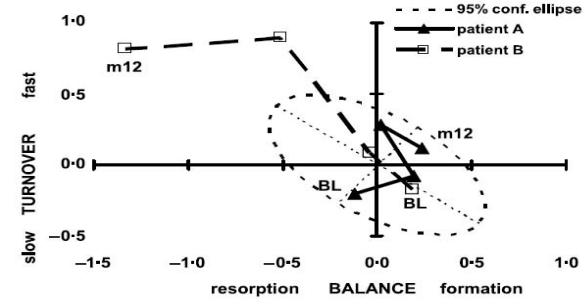


Figure 4 Bone marker plots from therapy monitoring of two patients (A and B) receiving teriparatide. CTX and OC were measured at baseline and at 1, 6 and 12 months (see BL and m12).

Bieglmayer C et al, *Eur J Clin Invest* 2009;39:230-238

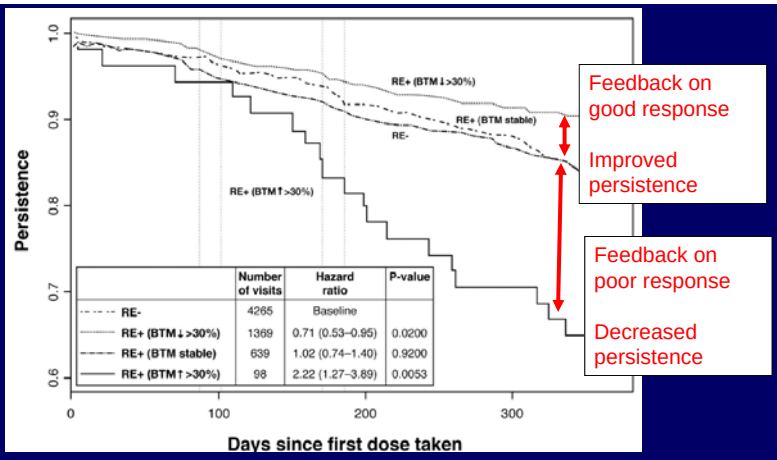
WHAT IS OPTIMAL BONE TURNOVER?

- Nobody knows, but probably lower than what is currently seen in healthy modern premenopausal women
- Neither BTMs nor iliac crest biopsies seem adequate to answer the question

HOW WOULD WE ACHIEVE OPTIMAL TURNOVER?

- Even if we knew what was optimal and could measure it in individual patients, current osteoporosis therapies are prescribed empirically and only changed if there is intolerance or failure – we do not treat to a particular target of BTM or BMD
- This would require a paradigm shift

REINFORCEMENT WITH BONE TURNOVER MARKERS
THE *IMPACT* STUDY



Delmas PD et al, *J Clin Endocrinol Metab* 2007;92:1296-1304

BONE TURNOVER MARKERS

- Bone turnover markers…
  - Predict bone loss and fracture risk in untreated patients
- With treatment…
  - Change sooner than BMD
  - Identify more “responders” than BMD
  - Explain a greater proportion of fracture reduction than change in BMD
- Can be useful in monitoring the response to treatment

BTMs IN CLINICAL TREATMENT

- Not used to select patients for treatment
- I use a formation marker to monitor treatment with teriparatide
- I use a resorption marker in patients who seem to be losing ground with antiresorptive therapy (except for those who have had a recent fracture)