Oxidative stress and anabolic hormones in back pain: Current concept and preliminary analysis in male cohort

Edoardo Vergani,1,2 Carmine Bruno,1,2 Andrea Silvestrini,3,4 Elisabetta Meucci,2,4 Luca Proietti,4 Andrea Perna,2,4 Francesco Ciro Tamburrelli,2,4 Antonio Mancini2,4
1Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Rome; 2Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome; 3Dipartimento di Scienze Biotecnologiche di base, cliniche intensivologiche e peri-operatorie, Università Cattolica del Sacro Cuore, Rome; 4Dipartimento di Scienze geriatriche e ortopediche, Università Cattolica del Sacro Cuore, Rome, Italy

Abstract

Back Pain (BP) is a common medical problem; anabolic hormones, through the modulation of oxidative stress (OS), could influence fracture risk. We evaluated the prevalence of anabolic hormonal deficiencies and their relationship with OS in males with BP, associated or not to non-traumatic fractures. 49 males with BP, from 36 to 80 years, were divided in two groups according to radiological evidence of non-traumatic fractures; group A (n=25): non-fractured; group B (n=24): fractured. A different prevalence of hormonal deficits was observed: 24% of hypotestosteronemia in A, 0% in B; 16% of GHD in A, 29% in B; Total Antioxidant Capacity (TAC) showed a trend toward higher levels in B. In A, despite lower TAC, a significant inverse correlation was present between TAC and IGF-1. A greater prevalence of GHD in patients with vertebral fractures was seen and, in a subgroup, OS could mediate the deleterious effects of hyposecretory GH state.

Introduction

Back Pain (BP) is one of the most common medical problem, affecting at least 80% of the population. It is the fifth most common reason for all physician visits in the United States.13 BP represents the second most frequent cause of activity limitation and work absence (after upper respiratory diseases), with several economic after-effects.24 It can represent a challenging diagnostic dilemma due to its multiple etiologies, such as muscle strain, disc herniation, spinal stenosis, spondylolisthesis, cauda equine syndrome, infections, malignancy, osteoarthritis, autoimmune diseases, vertebral deformities, non-traumatic and traumatic fractures.2,5,6 The diagnosis of a vertebral fracture is not easy as it seems, in fact only 30% of all vertebral fractures comes to clinical attention.7 Frequently BP etiology remains undetermined, while several mechanisms have been hypothesized to justify its origin. Disc end plate has been considered a relevant structure in pain genesis,2 in fact there is growing evidence that the end plates are richly innervated and that innervated end plate damage may represent a common painful problem (vertebrogenic pain).8

Some important peripheral mechanisms are involved in back pain, such as:
- pathological expression of TrkA receptors by bone sensory nerve fibers;
- production of neuropeptides, such as SP, CGRP, VIP and NPY, synthesized in sympathetic nerve fibers and released from their peripheral terminals in bone periostium during inflammation and osteoporosis;
- sensitization by pH decrease during osteoclastic hyperactivity in sensory nerve fibers expressing TRPV1 and ASIC-3.

The augmented density of bone sensory nerve fibers in old population and decreased bone mass density amplifies these mechanisms.9

As regard pH, radical oxygen species (ROS) may be one of the causes of the decrease.10 On the other hand hormones are antioxidant systems modulators, as previously reviewed,11,12 in particular GH/IGF-1 axis and testosterone impairment have been related to oxidative stress (OS), defined as the result of the unbalancing between production of free radicals and antioxidant defenses in the biological systems.11 ROS greatly influence the generation and survival of osteoclasts, osteoblasts and osteocytes and loss of estrogens and androgens decrease defense against OS in bone.13

Hormones exert different effects in male bone, on cellular, architectural and metabolic levels. Estrogens have positive consequences on bone metabolism, even if other hormones, such as testosterone14 and especially GH/IGF-1,15 play pivotal roles. Hypogonadism in adults is a well-known cause of bone loss. Androgen receptors are expressed by osteoblasts, osteoclasts and osteocytes. In osteoblasts, testosterone signaling is important in trabecular bone formation (not in cortical one). This suggests an important role of testosterone in contributing to bone strength and fracture risk, since trabecular bone is determinant for both.16 The direct role of testosterone in osteoclasts is less clear, while in osteocytes testosterone is important for age-related prevention of trabecular bone resorption.17 GH/IGF-1 axis is one of the main regulator of bone remodeling and turnover.18 Childhood onset GH deficiency, besides a reduced linear bone growth and delayed skeletal maturation, leads to decreased BMD and increased fracture risk in adulthood predominantly through decreased bone formation.19 A study involving GH naïve
adults with childhood onset GHD showed a decreased lumbar and femur neck BMD measured by DXA in comparison with healthy controls; this difference disappeared after volumetric adjustments, even though GHD patients maintained significantly higher fracture risk, suggesting that BMD may not adequately predict the fracture risk in these subjects the risk of vertebral fracture is even higher if GHD is accompanied by other hormonal deficit.

GH/IGF-1 production in puberty and young adulthood, supported by an adequate androgen environment, lead to thick trabeculae formation. Thereafter, as GH/IGF-1 levels decrease during lifetime, thick trabeculae are converted into thinner, more numerous trabeculae. However, in adulthood GH deficiency syndrome is still underestimated.

Taken all these aspects together, this observational cross-sectional study was made in order to:

1. evaluate anabolic hormonal alterations in male patients with back pain,
2. explore whether different hormonal patterns can be identified in patients with or without non-traumatic fractures.
3. investigate hormonal modulation of antioxidant systems in patients with or without non-traumatic fractures.

Materials and Methods

Subjects involved in this study were admitted to the University Hospital “Policlinico Gemelli” and were enrolled after being given an explanation of purposes and nature of the study, conducted in accordance with the Declaration of Helsinki, as revised in 2013. The study protocol was approved by Review Board of the “Institute of Medical Pathology” of our Hospital and written informed consent was obtained from all patients.

We included 49 male patients with BP of unknown origin divided according to radiological evidence of fractures by RX in two groups: group A (no fractures) and group B (fractures). They were aged 36-80 years, with a BMI range 16.8-30.3 kg/m2. Criteria of exclusion were: liver or kidney chronic failure, corticosteroid therapy, hyperparathyroidism, obesity, malabsorption or other gastro-enteric diseases, traumas, neurological diseases, neoplasms and autoimmune diseases.

An endocrine evaluation including fT3, fT4, thyroid-stimulating hormone (TSH), insulin-like growth factor (IGF) -1, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), testosterone (T), steroid hormone binding globulin (SHBG), dihydrotestosterone (DHT), insulin, adrenocorticotropic hormone (ACTH) and cortisol levels was performed; bone metabolic parameters were also evaluated (25 OH-Vitamin D, calcium, phosphorus, parathormone (PTH), osteocalcin (OC), β-crosslaps, bone alkaline phosphatase. For the evaluation of antioxidant systems, blood samples were collected at 08.00 a.m., after overnight fast, immediately centrifuged and stored at -80° until assayed, to evaluate Total Antioxidant capacity (TAC). Finally, bone mineral density was assessed by DEXA.

The following methods were used for hormone assay: Radio Immunoassay (RIA) for DHT (normal range 0.30-0.85 ng/ml); ElectroChemiLuminescent method (ECLIA) for PTH (14-72 pg/ml), ACTH (10-55 pg/ml), OC (10-45 ng/ml), β Crosslaps (0.2-0.7 ng/ml); ChemoLuminescent Immunoassay (CLIA) for TSH (0.35- 2.80 μUI/ml), fT3 (2.4-4.2 ng/ml), fT4 (8.5-16.5 pg/ml), IGF-1 (80-330 ng/ml), FSH (2.5-11 mU/ml), LH (2.5-10 mU/ml), E2 (normal values <44 ng/ml), T (2.5-8.40 ng/ml), SHBG (15-65 nmol/L), cortisol (60-220 ng/ml), insulin (3-}[Orthopedic Reviews 2020; 12(s1):8686] [page 121]
20 μUI/ml), Vitamin D (31-100 ng/ml), and Chemiluminescent Microparticle ImmunoAssay (CMIA) for LH (2.5-15 mU/ml) and PRL (3.5-15.5 ng/ml). Alkaline Phosphatase was measured with IFCC AMP, calcium with Arsenazo III method, Phosphate with colorimetric assay. As IGF-1 is concerned, we also calculated the median value, using reference provided by Liaison Analyser producer (DiaSorin, Vercelli, Italy), to classify patient with low or normal IGF-1. Following this preliminary evaluation, we have performed GHRH+arginine test (Geref 50 μg i.v. + Bioarginine 20g/500 mL i.v.) in patients whose IGF-1 was under 50th percentile according to sex and age. A diagnosis of Growth Hormone Deficiency (GHD) was made when peak GH values were lower than 9 ng/ml in normal weight patients and 4 ng/ml in obese patients. Hypopitostereonemia has been defined for serum testosterone levels < 2.5 ng/ml, according to our laboratory range.

TAC was evaluated, with a modification of the method developed by Rice-Evans and Miller as previously described. The method is based on the antioxidants inhibition of the absorbance of the radical cation 2,2′-azinobis (3-ethylbenzothiazoline-6 sulphonate) (ABTS⁺) formed by interaction between ABTS (150 μM) and ferrylmyoglobin radical species, generated by activation of metmyoglobin (2.5 μM) with H₂O₂ (75 μM).

Aliquots of the frozen plasma were thawed at room temperature and 10 μl of the samples were tested immediately. The manual procedure was used with only minor modifications, i.e., temperature at 37°C to be in more physiological conditions and each sample assayed alone to carefully control timing and temperature. The reaction was started directly in cuvette through H₂O₂ addition after 1 min equilibration of all other reagents (temperature control by a thermocouple probe, model 1408 K thermocouple, Digitron Instrumentation Ltd, Scunthorpe, United Kingdom) and followed for 10 min under continuous stirring, monitoring at 734 nm, typical of the spectrophotically detectable ABTS⁺. The presence of chain-breaking antioxidants induces a lag time (the Lag phase) in the accumulation of ABTS⁺ whose duration is proportional to the concentration of this type of antioxidants. Antioxidant capacity afforded by chain-breaking antioxidants is expressed as length of Lag phase (LAG, sec). Trolox, a water-soluble tocopherol analog, was used as a reference standard and assayed in all experiments to control the system. Absorbance was measured with an Agilent 8453 UV/Vis spectrophotometer (Palo Alto, CA, USA) equipped with a cuvette stirring apparatus and a constant temperature cell holder. Measurements of pH were made with a PHM84 Research pH meter (Radiometer, Copenhagen, Denmark); the electrode response was corrected for temperature. Unless stated differently, experiments were repeated two to three times; qualitatively similar results were obtained with individual values varying < 8%. In the Lag mode, the assay mainly measures non-proteic and non-enzymatic antioxidants that are primarily extracellular chaingaining antioxidants, such as ascorbate, urate and glutathione. BMD was assessed at the neck of the right hip femur and at the lumbar spine through DXA scan with Hologic® Discovery A (Hologic, Inc., Bedford, MA, USA).

### Statistical analysis
Mean and Standard Error (SEM) were used to describe quantitative variables.

The Mann-Whitney U-Test was used to evaluate differences in hormonal and bone metabolism parameters between the two groups. Spearman correlation coefficient was used to investigate the association between LAG and IGF-1. A value of p<0.05 was considered statistically significant and the analysis was performed using Stata 13.

### Results
Table 1 shows mean±SEM values of general and ematochemical parameters and bone metabolism markers in the two groups. There are no significant differences, despite

#### Table 1. General features, serum parameters and bone metabolic features.

|             | No fractures Mean±SEM | Fractures Mean±SEM | P  |
|-------------|------------------------|--------------------|----|
| Age (yr)    | 55.7±3.1               | 60.9±2.6           | NS |
| Serum glucose (mg/dl) | 91.3±5.05           | 90.5±3.12          | NS |
| Total Cholesterol (mg/dl) | 180.3±7.6          | 180.7±7.6          | NS |
| HDLC (mg/dl) | 53.7±2.8              | 49.6±2.2           | NS |
| Triglycerides (mg/dl) | 112.1±16.1          | 138.9±24.1         | NS |
| Serum creatinin (mg/dl) | 1.12±0.2            | 1.05±0.06          | NS |
| Caemia (mg/dl)  | 9.67±0.07             | 9.67±0.08          | NS |
| Phosphatemia (mg/dl) | 3±0.15               | 2.9±0.12           | NS |
| Alkaline Phospatase (UI/l) | 107.7±15.5         | 100.6±9.7          | NS |
| E-crosslaps (ng/ml) | 0.42±0.08            | 0.42±0.05          | NS |
| Osteocalcin (ng/ml) | 15.6±1.3             | 18.9±9.8           | NS |
| 25-OH vitamin D (ng/ml) | 18.2±2.4           | 23.05±2.57         | NS |
| Parathormone (pg/ml) | 50.7±6.6             | 43.7±16.7          | NS |
| Neck T-score | -1.56                  | -1.94              | NS |
| Lumbar T-score (SD) | -1.08                | -1.58              | NS |

#### Table 2. Hormonal features.

|             | No fractures Mean±SEM | Fractures Mean±SEM | P  |
|-------------|------------------------|--------------------|----|
| IGF-1 (ng/ml) | 152.2±16.7            | 129.9±12.9         | NS |
| Testosterone (ng/ml) | 4.4±0.5              | 5.46±0.42          | NS |
| DHT (ng/ml)   | 0.37±0.06              | 0.48±0.09          | NS |
| SHBG (nmol/l) | 42.5±4.9              | 60.19±7.25         | NS |
| FSH (mIU/ml) | 7.47±2.38              | 9.67±2.74          | NS |
| LH (mIU/ml)  | 4.83±1.12              | 5.5±1.2            | NS |
| TSH (μIU/ml) | 1.83±0.32              | 1.16±0.12          | NS |
| FT3 (pg/ml)  | 3.11±0.1              | 2.97±0.12          | NS |
| FT4 (pg/ml)  | 11.6±0.4              | 11.9±0.4           | NS |
| PRL (ng/ml)  | 10.1±1.3               | 11.3±1.3           | NS |
| Cortisol (ng/ml) | 134.04±9.6       | 110.9±9.1          | NS |
| ACTH (pg/ml) | 27.5±2.9               | 25.7±3.7           | NS |
| Insulin (μIU/ml) | 14.96±3.43           | 11.3±1.9           | NS |
correlated with TAC, suggesting a protective role, acting on oxidative stress; moreover, the GH dynamics should be evaluated in this clinical scenario; longitudinal studies can confirm a prognostic role of IGF-1 versus fracture risk. Nevertheless, there are two main potential restrictions to consider in the present study. Firstly, the number of subjects in both groups is slightly small, so its statistical power is limited, thus our findings will need to be confirmed in a larger population. Secondly, this cohort-study and the power analysis cannot draw a cause-effect conclusion.

Discussion

While endocrine picture in osteoporosis is extensively reported, few data are available in BP, which can be related to different mechanisms, at cellular and molecular levels and where OS can play a significant role. For this reason, we evaluated patients with BP, not related to traumas or major skeletal diseases, comparing hormonal values in patients with or without radiological fractures. Moreover, we have correlated hormonal parameters with antioxidant capacity previously shown to be modulated by anabolic hormones.

Main findings of our study were a different prevalence of hormonal deficits in the two groups: while hypotestosteronemia is more frequent in non-fractured group, GHD is more common in fractured group, suggesting a major role of IGF-1 in bone strength. TAC shows a trend toward higher levels in fractured group, expressing a reaction to increase oxidative stress, especially in middle-aged patients. Finally, IGF-1 inversely correlated with TAC in non-fractured group. Main results in our study concern hypoactivity of GH-IGF-1 axis in patients undergoing fractures, which presented signs of increased OS. This condition was suspected by IGF-1 values under the median for age and sex and confirmed by GH dynamic evaluation. Even in unfractured patients, IGF-1 inversely correlated with TAC, suggesting a protective role of the somatotropic axis on oxidative stress. A mutual relationship is demonstrated between GH and OS.

The relationship between GH and Oxidative stress in humans has been investigated in literature showing discrepant results. Scacchi et al., moreover, showed higher peroxide levels and lower Lag phase in GHD patients, but no direct correlation with IGF-1 was present. GH treatment for 4 months restored both peroxide levels and Lag phase to control values. On the contrary Smith et al., showed in adult GHD an impaired production of O2•– by neutrophils and also reduced lipid peroxidation, evaluated both as plasma lipid hydroperoxides and LDL susceptibility to peroxidation. Both these conditions reverted after 3 months of rGH treatment, but this matched with an increase of LDL-cholesterol and triglycerides and a lower HDL-cholesterol. Hypogonadism is more frequent in non-fractured group, suggesting a minor role of testosterone rather than GH/IGF-1 axis on bone.

It is known that male hypogonadism is correlated with losses in bone quality although the connection with fracture risk is not strictly dependent on testosterone serum levels.

Discordant data were reported about testosterone levels and fracture risk, especially in older men. This discrepancy could be attributed to the evaluation of total body testosterone levels which may not necessarily reflect regional testosterone levels and/or metabolism within the bone. Interestingly and surprisingly enough, the overall results of two important studies, such as the MrOS28 and MINOS, suggested that free testosterone is not clearly associated with BMD but may have some role in fracture risk in elderly men, while bioavailable estradiol is strongly associated with both BMD and fracture risk. An interesting study on DHT treatment in wild-type and androgen receptor (AR) transgenic mice demonstrate that replacement therapy was more effective in AR mice, according to the suggestion that androgen signaling in the mature osteoblast/osteocyte can regulate osteoclastic activity to modulate bone resorption. Thus, androgen therapy is effective for the prevention of bone loss through its anti-resorptive activities, while it shows little anabolic action to restore lost bone. Moreover, in this study the response was compartment-specific, with significant elevations in cancellous bone with DHT treatment and less effective actions in cortical bone. We can summarize that in the adult, androgens acting on androgen receptors can reduce bone resorption but have little overall anabolic activity.

Conclusions

The relationships between hormones, OS and back pain need further investigation; however, we can suggest that GH can exert a protective role, acting on oxidative balance; moreover, the GH dynamics should be evaluated in this clinical scenario; longitudinal studies can confirm a prognostic role of IGF-1 versus fracture risk. Nevertheless, there are two main potential restrictions to consider in the present study. Firstly, the number of subjects in both groups is slightly small, so its statistical power is limited, thus our findings will need to be confirmed in a larger population. Secondly, this cohort-study and the power analysis cannot draw a cause-effect conclusion.

References

1. Patrick N, Emanski E, Knaub MA. Acute and chronic low back pain. Med Clin North Am. 2014;98:777-89, xii.
2. Perna A, Ricciardi L, Barone G, et al. Medical management of acute non-specific low back pain: comparison of different medical treatments, one center's retrospective analysis. J Biol Regul Homeost Agents 2018;32:121-9.
3. Ricciardi L, Sturiule CL, Pucci R, et al. Patient-Oriented Aesthetic Outcome After Lumbar Spine Surgery: A 1-Year Follow-Up Prospective Observational Study Comparing Minimally Invasive and Standard Open Procedures. World Neurosurg. 2019;122:e1041-6.
4. Steenstra IA, Verbeek JH, Heymans MW, Bongers PM. Prognostic factors for duration of sick leave in patients sick listed with acute low back pain: A systematic review of the literature. Occup. Environ Med 2005;62,12:851-60.
5. Proietti L, Schirò GR, Sessa S, Scaramuzzi L. The impact of sagittal balance on low back pain in patients treated with zygoapophysial facet joint injection. Eur Spine J 2014;23:628-33.
6. Ricciardi L, Stifano V, Proietti L et al. Intraoperative and Postoperative Segmental Lordosis Mismatch: Analysis of 3 Fusion Techniques. World Neurosurg 2018;115:e659-63.
7. Tamburrelli FC, Perna A, Proietti L et al. The Feasibility of Long-Segment Fluoroscopy-guided Percutaneous Thoracic Spine Pedicle Screw Fixation, and the Outcome at Two-year Follow-up. Malays Orthop J 2019;13:39-44.
8. Van Dieën JH, Weimans H, Toussaint HM. Fractures of the lumbar vertebral endplate in the etiology of low back pain: A hypothesis on the causative role of spinal compression in aspecific low back pain. 1999;53:246-52.
9. Loitz JC, Fields AJ, Liebenberg EC. The Role of the Vertebral End Plate in Low
10. Sipos H, Tórocsik B, Tretter L, Adam-Vizi V. Impaired regulation of pH homeostasis by oxidative stress in rat brain capillary endothelial cells. Cell Mol Neurobiol 2005;25:141-51.

11. Mancini A, Vergani E, Bruno C et al. Oxidative stress as a possible mechanism underlying multi-hormonal deficiency in chronic heart failure. Eur Rev Med Pharmacol Sci 2018;22:3936-61.

12. Vitiello R, Perisano C, Covino M, et al. Euthyroid sick syndrome in hip fractures: Valuation of vitamin D and parathyroid hormone axis. Injury 2020;9:S0020-1383:30013-9.

13. Manolagas SC. From estrogen-centric to aging and oxidative stress: A revised perspective of the pathogenesis of osteoporosis. Endocr Rev 2010;31:266-300.

14. Golds G, Houdek D, Arnason T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. Int J Endocrinol 2017;2017:4602129.

15. Chihara K, Sugimoto T. The action of GH/IGF-I/IGFBP in osteoblasts and osteoclasts. Horm Res Paediatr 1997;48:45-9.

16. Khosla S, Melton LJ, Achenbach SJ, et al. Hormonal and biochemical determinants of trabecular microstructure at the ultradistal radius in women and men. J Clin Endocrinol Metab 2006;91:885-91.

17. Sinnesael M, Claessens F, Laurent M, et al. Androgen receptor (AR) in osteocytes is important for the maintenance of male skeletal integrity: Evidence from targeted AR disruption in mouse osteocytes. J Bone Miner Res 2012;27:2535-43.

18. Mancini A, Vergani E, Bruno C, et al. Relevance of adherence monitoring in adult patients with growth hormone deficiency under replacement therapy: Preliminary monocentric data with EasypoDTM connect. Front Endocrinol (Lausanne) 2019;28:10:416.

19. Wilhelmsen L, Bengtsson B, Lappas G, et al. Increased fracture frequency in adult patients with hypopituitarism and GH deficiency. Eur J Endocrinol 1997;137:240-5.

20. Bouillon R, Koledova E, Bezlepkina O, et al. Bone status and fracture prevalence in Russian adults with childhood-onset growth hormone deficiency. J Clin Endocrinol Metab 2004;89:4993-8.

21. Mancini A, Milardi D, Bianchi A, et al. Increased total antioxidant capacity in seminal plasma of varicocele patients: A multivariate analysis. Syst Biol Reprod Med 2007;53:37-42.

22. Mancini A, Festa R, Di Donna V, et al. Hormones and antioxidant systems: Role of pituitary and pituitary-dependent axes. J. Endocrinol. Invest. 2010;33:422-33.

23. Scacchi M, Valassi E, Pincelli AI, et al. Increased lipid peroxidation in adult GH-deficient patients: Effects of short-term GH administration. J Endocrinol Invest 2006;29(8):899-904.

24. Smith JC, Lang D, McEneny J. Effects of GH on lipid peroxidation and neutrophil superoxide anion-generating capacity in hypopituitary adults with GH deficiency. Clin Endocrinol (Oxf) 2002;56:449-55.

25. Drake MT, Murad MH, Mauck KF, et al. Risk factors for low bone mass-related fractures in men: A systematic review and meta-analysis. J Clin Endocrinol Metab 2012;97:1861-70.

26. Yang Z, Zhang Y, Xu D et al. Percutaneous vertebroplasty combined with interstitial implantation of 125I seeds in banna mini-pigs. World J Surg Oncol. 2013 26;11:46.

27. Vandenput L, Labrie F, Mellström D, et al. Serum levels of specific glucuronidated androgen metabolites predict BMD and prostate volume in elderly men. J Bone Miner Res 2007;22:220-7.

28. Mellström D, Johnell O, Ljunngren Ö, et al. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 2006;21:529-35.

29. Szulc P, Beck TJ, Marchand F, Delmas PD. Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men - The MINOS study. J Bone Miner Res 2005;20:721-9.

30. Wiren KM, Zhang XW, Olson DA, et al. Androgen prevents hypogonadal bone loss via inhibition of resorption mediated by mature osteoblasts/osteocytes. Bone 2012;51:835-46.