34. Repair of Fatigue Damage Occurs Independent of Load-induced Strain in Rats.

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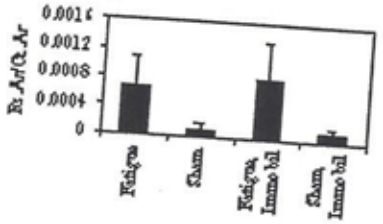
Previous work indicates that fatigue loading in bone leads to a sequence of microdamage creation, osteocyte apoptosis, and osteoclastic resorption. Osteocyte apoptosis is believed to initiate targeted remodeling and the repair of damaged bone matrix. The local mechanisms responsible for osteocyte apoptosis are unknown, but local alterations in bone strain may play a role. This study investigated the role of load-induced strain in the targeted remodeling of microdamage. The ulnae of mature rats were fatigued by applying a 20 N cyclical load until whole bone compliance increased by 25%. Following fatigue loading, rats either resumed normal load bearing or the forelimb was immediately immobilized. An additional group of rats served as sham loaded controls. Sham rats were subjected to a 3 N static load followed by immobilization or normal load-bearing. After 10 days, rats were euthanized. Sections from each ulna were examined for microdamage, resorption spaces, and osteocyte apoptosis. Both sham- and fatigue-immobilized rats had significantly ($p<0.05$) larger medullary canals than load-bearing rats, which is typical of disuse osteopenia. In contrast, intracortical resorption was significantly ($p<0.05$) elevated in fatigue-loaded rats, independent of load-bearing (Figure). Thus, these data establish that the initial fatigue event stimulates remodeling. Subsequent load-bearing activity does not serve as a signal for targeted remodeling.



SU470

PTH Regulates Expression of ODF and OPG by Different Signaling Pathway in Murine Marrow Stromal Cells.

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Osteoclast differentiation factor (ODF/RANKL/TRANCE) recently was identified as an essential osteoblast/marrow stromal-derived factor required for osteoclastogenesis from hematopoietic precursor cells. ODF is necessary for proliferation and differentiation of osteoclast progenitors, fusion of mononuclear precursors, and activation of multinuclear osteoclasts. Osteoprotegerin (OPG), a decoy receptor for ODF, inhibits osteoclastogenesis by competing for binding of ODF to receptor activator of NF-kB (RANK). PTH induction of osteoclast formation is accompanied by reciprocal up- and down-regulation of ODF and OPG, respectively(Endocrinology, 140: 3552-61, 1999). It is not yet clear how the downstream signals generated by the PTH/PTHrP receptor (PTHR)- cAMP/PKA and PLC/PKC-are involved in mediating these responses. To address these questions, we investigated effects of PTH on cAMP accumulation and ODF and OPG mRNA expression in an established conditionally transformed murine marrow stromal cell line (MS1). MS1 cells were established from mice heterozygous for PTHR gene ablation and transgenic for a temperature-sensitive transforming SV40 T antigen. In dexamethasone (Dex)-supplemented cocultures with normal spleen cells, this cell line supports osteoclastogenesis induced by PTH or $1,25(OH)_2D_3$ (Endocrinology, 139: 1952-64, 1998). For the present studies, cells were grown in alpha-MEM supplemented with 10% FBS at 33 C, and cultures then were shifted to 37 C when subconfluent. Cells were exposed to PTH or other agents, with or without Dex, for various intervals of time. PTH-dependent cAMP generation was about 1-, 10-, and 80-fold controls when assessed at 0, 3, and 7 days, respectively. In the presence of Dex, ODF expression was stimulated after 7 days of continuous PTH treatment at 37 C. On the contrary, OPG expression was inhibited. A 24 h pulse of PTH administered 6 days after transfer to 37 C also stimulated ODF mRNA expression and inhibited that of OPG. Treatment with 8Br-cAMP, a direct PKA stimulator, upregulated ODF but did not alter OPG expression. Thus, in these experiments MS1 cells exhibited a delayed appearance of cAMP responsiveness to PTH that coincided temporally with the ability of PTH to upregulate ODF expression. These findings, coupled with the direct effects of PKA activation, suggest that PTH regulation of ODF in these cells occurs via the cAMP/PKA pathway. In contrast downregulation of OPG may proceed via an alternate signaling pathway. These results suggest that the intensity of the bone resorptive response to PTH may depend upon the selective activation of different signal responses generated by the PTHR.

SU471

Parathyroid Hormone Effects in Bone: Dependence of *c-fos* and AP-1 for Osteoblast Proliferation and Gene Expression.

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Parathyroid hormone (PTH) has both anabolic and catabolic actions in bone that are, as yet, not clearly understood. The protooncogene *c-fos* and other AP-1 family members are critical transcriptional mediators in bone, and *c-fos* is rapidly upregulated by PTH. The purpose of this study was to examine the mechanisms of PTH action, and specifically the role of *c-fos* in PTH effects on osteoblast proliferation and gene expression. Mice with targeted deletion of the *c-fos* gene, (*fos* -/-), and their littermates were utilized for *in vivo* studies, or to generate primary calvarial osteblastic cells for *in vitro* studies. Cells from *fos* (-/-) mice proliferated more rapidly, than *fos* (+/+) and (+/-)($p<0.05$); however, with the addition of 0.1 microM PTH 1-34, their proliferation rate returned to control and was similar to PTH-treated *fos* (+/+; +/-) levels. The incorporation of calcium into mineralized nodules and PTH effects to inhibit mineralization were similar among genotypes (although there was a trend towards increased calcium accumulation in *fos*-/- cultures). PTH 1-34 induced steady state mRNA levels for IL-6 that peaked at 3h, declined to control levels after 8h, and were highest in *c-fos* (-/-) cells. IGF-1 mRNA levels increased with 3h of PTH 1-34 treatment and were highest in *c-fos* (-/-) versus (+/-) and (+/+) cells. PTH-stimulated MMP-13 levels were similar for (+/+) and (-/-) cells *in vitro*. *In vivo*, a single PTH 1-[illegible] microg) injection (8h) resulted in a similar upregulation of MMP-13 in all genotypes, ing that MMP-13 expression is not dependent on *c-fos*. Electrophoretic mobility ge analysis indicated that PTH 1-34 (2h), stimulated higher levels of nuclear AP-1 prote *c-fos* (+/+) than (-/-) mice. These results suggest that another factor may be either con sating for, or be more responsible than *c-fos* in mediating PTH effects on osteob Hence, PTH effects on other AP-1 family members were evaluated. Interestingly, st state mRNA for *fra*-2 was upregulated by PTH 1-34 (but not 7-34 or 53-84) *in vitro* at 20 min, peaked at 3h and remained high until after 8h when it declined to control (0h) levels. These data suggest that *c-fos* plays a role in osteoblast proliferation and differentiation, but it is not necessary for PTH effects on osteoblasts. Changes in osteoblast proliferation and differentiation in response to PTH may be mediated by more than one member of the AP-1 family of transcription factors or by other transcriptional mediators.

SU472

Reference Data for Serum PTH Should Take the Vitamin D Status into Account : Lessons of the DHEage Study.

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Subclinical vitamin D insufficiency is characterized by normal bone mineralisation but mild secondary hyperparathyroidism (SHPT), increased bone turnover, decreased BMD at the hip and thus enhanced risk of osteoporotic fracture in comparison with vitamin D sufficient subjects. However, although low levels of 25OHD are common in otherwise normal elderly people, vitamin D status is not taken into account when establishing reference values for serum PTH. We measured fasting morning serum PTH, total calcium, albumin and 25OHD in 280 healthy subjects (140 men, 140 women) aged 60-79 yr at baseline (april-june) of a one-year double blind, placebo-controlled trial of oral DHEA, the so-called DHEage study. Serum PTH was measured by means of 2 immunoradiometric assays, one recognizing the intact 1-84 PTH molecule and a big C-Mid fragment (7-84 PTH) equally (total PTH; Allegro, Nichols Institute), the other one being exclusively specific for the intact molecule (Whole PTH; Scantibodies Laboratory Inc). Results (median; 5th-95th percentile) are given according to the vitamin D status of the subjects (*:$p<0.05$ or less versus "25OH*12").

	25OHD*12 mg/L	25OHD>12 mg/L
n	167	113
tCa alb corr (mM)	2.28 (2.18-2.42)	2.29 (2.18-2.42)
total PTH (ng/L)	33 (14-71)	26 (13-46)*
whole PTH (ng/L)	24 (11-46)	20 (10-34)*

We found a high rate (59.6%) of low 25OHD in these otherwise very healthy individuals. When using the values of the vitamin D sufficient subjects (see above) as reference data for both PTH assays, approximately 25% and 75% of the subjects with low serum 25OHD had a serum PTH above the 95th percentile and above the median (whatever the kit) respectively, reflecting SHPT. This may be ignored if the reference PTH values are those obtained in the entire group (10-40 and 14-57 ng/L for the whole and the total PTH assay respectively) as usually. These results strongly suggest that vitamin D status should be taken into account when establishing reference values for serum PTH, especially in elderly subjects.