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Bone Turnover in Rats Treated with 1,25-Dihydroxyvitamin D_3, 25-Hydroxyvitamin D_3 or 24,25-Dihydroxyvitamin D_3

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Female rats were given 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), 0.25 μg per 100 g body weight (bw), 25-hydroxyvitamin D_3 ($25(OH)D_3$), 1.7 μg/100 g bw or 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) 1.7 μg/100 g bw, subcutaneously three times a week for 12 weeks. Traditional variables pertaining to calcium homeostasis and growth, i.e. blood and urine calcium (Ca) and phosphate (P), serum levels of vitamin D_3 metabolites parathyroid hormone, (PTH), calcitonin (CT), prolactin (PRL) and growth hormone (GH) were measured every four weeks. This data pool was correlated with bone matrix turnover parameters, i.e. serum levels of alkaline phosphatase (ALP) and urinary hydroxyproline (u-HYP) excretion. After 12 weeks of treatment, $1,25(OH)_2D_3$ significantly enhanced serum total and ionized Ca, urine Ca and urine P, and also diminished urine cAMP due to reduced renal function (creatinine clearance). However, $25(OH)D_3$ administration had no such impact. $24,25(OH)_2D_3$ opposed the effect of $1,25(OH)_2D_3$ after 12 weeks by significantly augmenting serum P and diminishing serum levels of total Ca and ionized Ca. Cross sectional group analyses showed that circulating levels of ALP were directly related with serum $1,25(OH)_2D_3$ and inversely related to serum $24,25(OH)_2D_3$ and CT. Total u-HYP and per cent non-dialysable HYP (ndHYP) were reciprocally and positively correlated with serum PRL, respectively. However, no such relations were observed with serum GH.

It appears that rats with elevated circulating levels of $1,25(OH)_2D_3$ exhibit increased bone resorption, while augmented $24,25(OH)_2D_3$ is associated with the opposite. Apparently, high bone turnover (i.e. reduced total urinary HYP and enhanced ndHYP) is associated with high serum PRL.

KEY WORDS: Bone turnover; cholecalciferols; parathyroid hormone; calcitonin; prolactin; growth hormone; alkaline phosphatase; cAMP; hydroxyproline.

INTRODUCTION

Remodelling of bone is a complex process involving several cell lineages and a multitude of systemic and local regulatory factors (Canalis *et al.*, 1988; Huffer, 1988). Steroid hormones and vitamin D_3 analogues exert unique effect on bone

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(Stewart, 1987) and modulate the sensitivity of the osteoblast to certain systemic peptide hormones like parathyroid hormone (PTH) (Chen and Feldman, 1984; Kubota *et al.*, 1985), insulin (Hickman and McElduff, 1988), insulin-like growth factor I (IGF I) (Canalis *et al.*, 1989) and growth hormone (GH) (Canalis, 1980). The ensuing synthesis and/or release of local para- or autocrine factors further decide whether net bone gain or loss emerges (Kumegawa *et al.*, 1984; Huffer, 1988). We have previously shown *in vitro* (Klem *et al.*, 1990) and *in vivo* (Mortensen *et al.*, 1990; Mortensen *et al.*, 1992a) that $24,25(OH)_2D_3$ interferes with PTH-elicited cAMP synthesis by perturbing the coupling between the hormone receptor and the GTP-binding protein $G_s\alpha$. Furthermore, we have demonstrated that GH secretion in the rat is inversely related to serum total Ca (Mortensen, 1992b) and that circulating prolactin (PRL) is predicted by the ratio of free serum Ca to serum $1,25(OH)_2D_3$ (Mortensen, 1992b). In light of the integrated actions of hormones on bone matrix, we wanted to analyze the effects of $1,25(OH)_2D_3$, $25(OH)D_3$ and $24,25(OH)_2D_3$ administration on bone to determine which peptide hormone and/or vitamin D_3 metabolite reflected bone turnover as estimated by serum alkaline phosphatase and urinary total and non-dialysable hydroxyproline.

MATERIALS AND METHODS

Vitamin D_3 Metabolites and Chemicals

$25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ were all gifts from Leo Pharmaceutical Company (Denmark), Upjohn (Kalamazoo, MI, USA) and Hoffman-La Roche (Switzerland). The metabolites were dissolved in ethanol (96%) and diluted in 0.15 M NaCl. All animals received subcutaneous injections three times a week for 12 weeks. The dosages were as follows: $1,25(OH)_2D_3$; 0.25 μg per 100 g body weight (bw), $25(OH)D_3$; 1.7 μg per 100 g bw and $24,25(OH)_2D_3$; 1.7 μg per 100 g bw.

Animals and Sampling Procedure

44 female Wistar rats weighing 120 g were allocated to four treatment groups. The animals were placed in special metabolic cages for collection of 24 hour urine samples. Blood samples were drawn from the jugular vein after applying Hypnorm^R (Janssen, Belgium) as anaesthesia. Time for sampling was 24 hours after last injection.

Assay for Vitamin D_3 Metabolites in Serum

As previously described (Aksnes, 1980a, 1980b), serum concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ were measured by a radio immunoassay (RIA) after extraction with diethyl ether and separation by high performance liquid chromatography (HPLC).

Determination of iPHT, iCT, PRL and GH in Serum

Rooster antiserum reacting with the midregion of bovine parathyroid hormone (PTH) was used in 1:8000 dilution in a standard RIA (Gautvik *et al.*, 1979) to determine immunoreactive PTH (iPTH). A non-equilibrium RIA which employs a rabbit antihuman calcitonin (CT) antiserum (Myhre and Gautvik, 1979) was used for measurements of immunoreactive CT (iCT). Circulating levels of prolactin (PRL) and growth hormone (GH) were measured using RIAs mainly based on the reagents obtained from NIH/NIAMDD (Haug and Gautvik, 1976; Redshaw and Lynch, 1974).

Assessment of Variables in Serum and Urine Clinical Chemistry

Circulating levels of free calcium (Ca) were determined by using standard flame absorption photometry. Total Ca and Phosphate (P) in serum and urine, serum levels of alkaline phosphatase (ALP), total protein and creatinine were measured by Standard auto-analysis procedures.

Measurements of Hydroxyproline and cAMP in Urine

24 hour samples were analyzed as described previously (Gordeladze *et al.*, 1978). In brief, one hundred μl of urine was hydrolysed in 6 M HCl for two hours at 125°C in a teflon capped tube, neutralized and oxidized with chloramine T. Chromophore development was accomplished with para-dimethyl aminobenzaldehyde (p-DMAB) and heating at 100°C for 4 minutes. Absorbance was read in a Cobas-Bio automated analyzer (Hoffman-La Roche) at 562 nm. One ml of urine was dialysed against approximately 100 volumes of distilled water using cellophane tubing (Arthur Thomas Co., Phil., PA, USA) under constant stirring at 2°C for 24 hours. A complete water change was performed after 6 hours. The dialysed urine samples were evaporated to dryness at 90°C in a ventilated oven, reconstituted in 6 M HCl, hydrolysed and analyzed for hydroxyproline as described above.

When determining cAMP in urine, samples were diluted 1:500 prior to analysis by the NEN cAMP kit (Du Pont) based on the double antibody principle. First antibody was raised against succinyl cAMP methyl ester, however no derivatization of the samples was performed before incubation with this antibody.

Statistical Evaluation

The differences between groups were analyzed by the Mann–Whitney U test and the Wilcoxon rank test. Differences were considered significant when $2\alpha = p < 0.05$. Correlation matrix analysis and linear regression analyses on pooled data were performed and correlations considered significant ($r \neq 0$) when $p < 0.10$. Results are given as means ± SEM in brackets.

RESULTS

Concentrations of Circulating Vitamin D_3 Metabolites Subsequent to Injections of $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$

Administration of $1,25(OH)_2D_3$ only marginally and non-significantly enhanced $1,25(OH)_2D_3$ concentrations above controls, while concentrations of $25(OH)D_3$ and $24,25(OH)_2D_3$ were significantly ($p < 0.05$) reduced at 4 and 12 weeks of treatment. $25(OH)D_3$ injections gave a marked increase ($p < 0.05$) in $25(OH)D_3$ levels. No major alterations were observed in $1,25(OH)_2D_3$ concentrations, while $24,25(OH)_2D_3$ levels were consistently increased ($p < 0.05$). Finally, injections of $24,25(OH)_2D_3$ significantly ($p < 0.05$) elevated circulating $24,25(OH)_2D_3$ throughout the study. Concentrations of serum $1,25(OH)_2D_3$ were, however, considerably reduced ($p < 0.05$) at 8 and 12 weeks of treatment, while $25(OH)D_3$ levels were only slightly ($p > 0.05$) lower than in controls (Table 1).

Serum Levels of Calcium, Phosphate, Total Protein and Alkaline Phosphatase During Treatment with $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$

$1,25(OH)_2D_3$ treatment markedly enhanced ($p < 0.05$) serum total Ca and serum ionized Ca at 8 and 12 weeks, respectively. Administration of $25(OH)D_3$ resulted in consistently sustained total serum total Ca and serum ionized Ca. Injections with $24,25(OH)_2D_3$ for 12 weeks reduced serum levels of both total and ionized Ca ($p < 0.05$) compared to control treatment. No consistent change in serum phosphate or serum total protein could, however, be attributed to any drug

Table 1. Serum levels of $1,25(OH)_2D_3$, $25(OH)D_3$ and $24,25(OH)_2D_3$ in female rats subsequent to subcutaneous administration for 12 weeks

Serum levels of vit D_3-metabolites	Treatment groups	Treatment time			
		0 weeks	4 weeks	8 weeks	12 weeks
$1,25(OH)_2D_3$ (pmol/l)	Control	143.00 (31.05)	84.67 (25.99)	77.67 (5.49)	75.50 (12.73)
	$1,25(OH)_2D_3$		59.25 (10.85)	94.50 (14.09)	79.75 (9.16)
	$25(OH)D_3$		40.33 (8.01)	44.25 (6.42)*	77.25 (15.29)
	$24,25(OH)_2D_3$		29.25 (3.73)	40.75 (3.33)*	53.50 (11.77)*
$25(OH)D_3$ (nmol/l)	Control	49.23 (6.58)	43.28 (1.51)	34.30 (2.61)	46.43 (4.23)
	$1,25(OH)_2D_3$		16.35 (1.93)*	20.35 (1.33)	22.80 (2.32)*
	$25(OH)D_3$		62.67 (6.73)*	148.00 (7.04)*	102.73 (1.53)*
	$24,25(IG)_2D_3$		35.65 (5.62)	30.35 (1.61)	34.90 (2.95)
$24,25(OH)_2D_3$ (nmol/l)	Control	13.63 (1.53)	13.45 (1.23)	13.45 (1.09)	20.45 (1.49)
	$1,25(OH)_2D_3$		6.77 (0.84)*	7.55 (1.06)	8.35 (1.46)*
	$25(OH)D_3$		44.80 (1.90)*	95.40 (7.74)*	88.48 (6.24)*
	$24,25(OH)_2D_3$		58.08 (3.97)*	69.18 (14.46)*	53.40 (2.80)*

The vitamin D_3 analogues were administered 3 times weekly. The dosages were: $1,25(OH)_2D_3$; 0.25 μg/100 g body weight (bw), $25(OH)D_3$; 1.7 μg/100 g bw, and $24,25(OH)_2D_3$; 1.7 μg/100 g bw, while the control group received vehicle. Serum samples were analysed (see Materials and Methods) for the same compounds prior to (t_0) and after 4, 8 and 12 weeks of treatment. All values are given as mean ± SEM (brackets) ($n = 2 - 4$). *Significant ($p < 0.05$) differences (treated vs. control rats, Wilcoxon rank test).

Table 2. Serum concentrations of total and ionized calcium, phosphate, total protein and alkaline phosphatase in female rats subsequent to subcutaneous administration of $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$

Serum parameters	Treatment groups	Treatment time			
		0 weeks	4 weeks	8 weeks	12 weeks
Total Calcium (mmol/l)	Control	2.62 (0.04)	2.65 (0.05)	2.60 (0.00)	2.59 (0.01)
	$1,25(OH)_2D_3$		2.60 (0.20)	2.70 (0.10)*	2.92 (0.10)*
	$25(OH)D_3$		2.60 (0.00)	2.45 (0.05)	2.60 (0.03)
	$24,25(OH)_2D_3$		2.50 (0.00)	2.45 (0.05)	2.43 (0.02)*
Ionized Calcium (mmol/l)	Control	1.41 (0.03)	1.52 (0.05)	1.45 (0.02)	1.44 (0.03)
	$1,25(OH)_2D_3$		1.42 (0.09)	1.58 (0.06)*	1.59 (0.06)*
	$25(OH)D_3$		1.27 (0.03)	1.32 (0.00)	1.44 (0.02)
	$24,25(OH)_2D_3$		1.33 (0.04)	1.41 (0.06)	1.33 (0.02)*
Phosphate (mmol/l)	Control	3.00 (0.18)	2.25 (0.05)	1.80 (0.10)	1.56 (0.11)
	$1,25(OH)_2D_3$		1.75 (0.05)	2.95 (0.55)	1.82 (0.24)
	$25(OH)D_3$		1.90 (0.10)	2.05 (0.35)	1.87 (0.13)
	$24,25(OH)_2D_3$		1.55 (0.35)	1.35 (0.05)	2.19 (0.17)*
Total protein (g/l)	Control	52.40 (0.60)	59.00 (0.00)	61.00 (3.00)	65.57 (1.13)
	$1,25(OH)_2D_3$		58.00 (2.00)	62.00 (2.00)	57.00 (0.89)*
	$25(OH)D_3$		54.50 (1.50)	58.00 (2.00)	59.14 (1.16)*
	$24,25(OH)_2D_3$		57.50 (1.50)	61.50 (0.50)	59.71 (0.94)*
S-ALP (U/l)	Control	473.80 (52.83)	267.00 (6.00)	294.50 (130.50)	189.00 (28.98)
	$1,25(OH)_2D_3$		361.00 (156.00)	112.00 (5.00)	156.00 (41.75)
	$25(OH)D_3$		283.00 (56.00)	152.50 (9.50)	157.43 (17.17)
	$24,25(OH)_2D_3$		162.50 (16.50)	153.50 (18.50)	128.43 (18.24)*

The vitamin D_3 dose regimens are described in Materials and Methods and in legend to Table 1. The serum analyses were all performed according to Nordic Standard on a multi-channel automatic analyzer (see Materials and Methods). Results are computed as mean ± SEM (brackets) ($n = 2 - 7$). *Significantly different ($p < 0.05$) from controls (Wilcoxon rank test).

Abbreviations: alkaline phosphatase (ALP).

Table 3. Circulating levels of immunoreactive parathyroid hormone (iPTH) and immunoreactive calcitonin (iCT) in female rats given $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$ subcutaneously for 12 weeks

Serum parameters	Treatment groups	Treatment time			
		0 weeks	4 weeks	8 weeks	12 weeks
iPTH (μg/l)	Control	0.76 (0.10)	0.57 (0.03)	0.73 (0.15)	1.01 (0.11)
	$1,25(OH)_2D_3$		0.61 (0.03)	0.69 (0.09)	1.03 (0.00)
	$25(OH)D_3$		0.54 (0.04)	0.70 (0.07)	1.26 (0.24)
	$24,25(OH)_2D_3$		0.47 (0.03)	0.57 (0.06)	1.27 (0.23)
iCT (μg/l)	Control	0.10 (0.00)	0.14 (0.00)	0.14 (0.00)	0.17 (0.01)
	$1,25(OH)_2D_3$		0.14 (0.00)	0.15 (0.00)	0.15 (0.00)
	$25(OH)D_3$		0.14 (0.00)	0.14 (0.00)	0.17 (0.02)
	$24,25(OH)_2D_3$		0.15 (0.01)	0.16 (0.00)	0.14 (0.00)

Immunoreactive PTH and CT were measured with standard RIA techniques using polyclonal antibodies (see Materials and Methods). Mean group values are given with SEM in brackets ($n = 5 - 11$). *Significantly different ($p < 0.05$) from controls (Wilcoxon rank test).

effect. At 12 weeks of vitamin D_3 administration, serum total protein was marginally, but significantly ($p < 0.05$) reduced. Serum ALP proved to be consistently suppressed during 12 weeks with either vitamin D_3 metabolite treatment, although only significantly ($p < 0.05$) with administration of $24,25(OH)_2D_3$ after 12 weeks (Table 2).

Serum Levels of Immunoreactive Parathormone (iPTH) and Calcitonin (iCT) After $1,25(OH)_2D_3$, $25(OH)D_3$, or $24,25(OH)_2D_3$ Treatment

No consistent between-group alterations in serum iPTH and iCT were observed, except for a slight non-significant ($p > 0.05$) increase in circulating levels of iPTH at 12 weeks with either $25(OH)D_3$ or $24,25(OH)_2D_3$ treatment (Table 3).

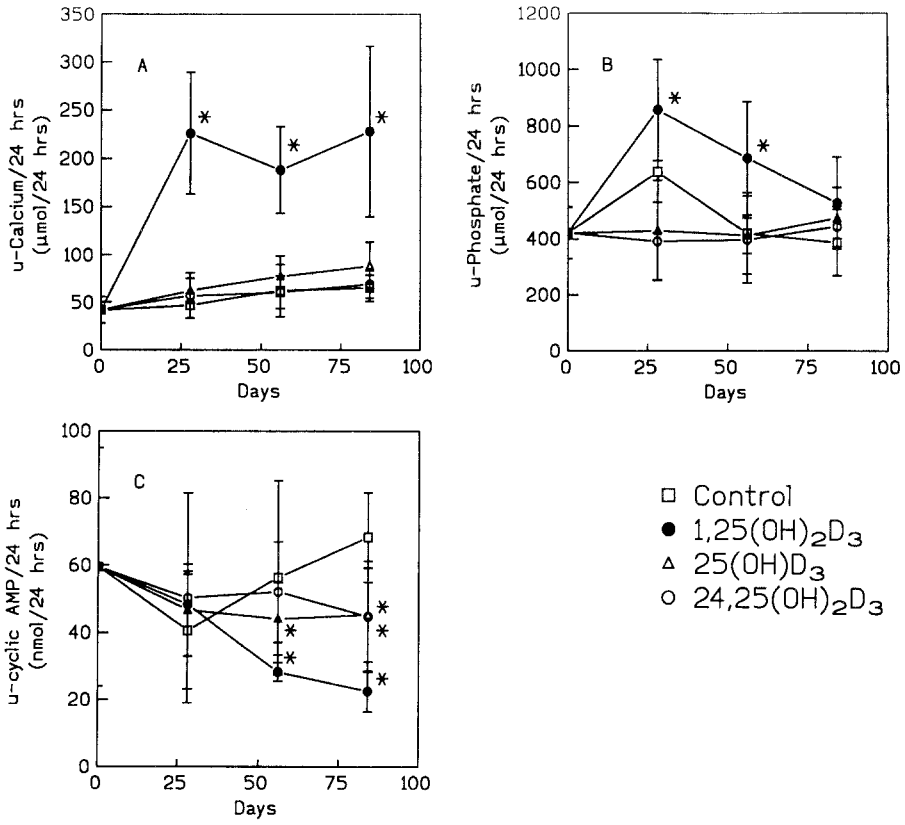

Fig. 1. Uninary excretion of calcium (panel A), phosphate (panel B) and cyclic AMP (panel C) in female rats chronically treated with subcutaneous $1,25(OH)_2D_3$, $25(OH)D_3$ for $24,25(OH)_2D_3$ for 12 weeks. Vitamin D_3 dosage regimens are described in Materials and Methods and legend to Table 1. 24 hour urine samples were collected prior to drug administration and at 4, 8 and 12 weeks. Results are given as µmol/24 hrs (calcium and phosphate) or nmol/24 hrs (cyclic AMP) and depicted as mean ± SEM ($n = 7$). *Significantly different ($p < 0.05$) from controls (Wilcoxon rank test).
Abbreviations: cyclic AMP = 3′–5′ cyclic adenosine monophosphate.

Urinary Excretion of Ca, P and cAMP Subsequent to Treatment with $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$

After 4 weeks of treatment with $1,25(OH)_2D_3$, both 24 hour urinary Ca ($p < 0.05$) and urinary phosphate ($p < 0.05$) were enhanced and the alterations persisted throughout the study (Figs. 1A & B). Contrastingly, cyclic AMP excretion was decreased ($p < 0.05$) at 8 and 12 weeks (Fig. 1C). Administration of $25(OH)D_3$ resulted in minor variations ($p > 0.05$) in the urinary excretion of both Ca and P (Figs. 1A & B), while cAMP was significantly ($p < 0.05$) diminished (Fig. 1C). Rats treated with $24,25(OH)_2D_3$ displayed no alterations ($p > 0.05$) in the urinary parameters assessed.

Urinary Total Hydroxyproline (HYP) and Non-dialysable Hydroxyproline (ndHYP) After Administration of $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$

All HYP measurements displayed large intra- and inter-individual variations. Total urinary HYP turned out to be marginally suppressed ($p > 0.05$) by both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ administration at 8 and 12 weeks, while $25(OH)D_3$ treatment consistently enhanced ($p > 0.05$) HYP excretion (Fig. 2A). Neither vitamin D_3 metabolite exerted a consistent effect on per cent urinary

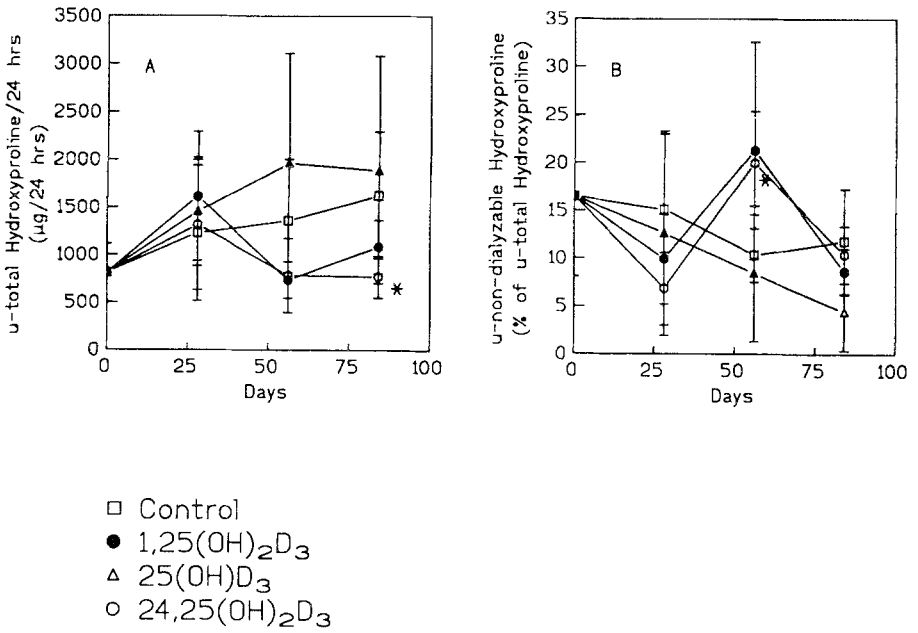

Fig. 2. Urinary total (A) and non-dialysable (B) hydroxyproline (HYP) (in female rats receiving subcutaneous $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$ for 12 weeks. Total and non-dialysable HYP contents were analysed in 24 hours urine specimens applying the micro-method by Gordeladze *et al.* (1978) (see Materials and Methods). All results are calculated as μg HYP/24 hours or per cent non-dialysable HYP relative to total. The results are expressed as mean ± SEM ($n = 7$). *Significantly different ($p < 0.05$) from controls (Wilcoxon rank test).

ndHYP, except for $25(OH)D_3$, which gradually reduced it ($p > 0.05$) throughout the whole treatment period (Fig. 2B).

Scatter Plots of Circulatory Hormones and Bone Turnover Variables

Serum $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ were directly and reciprocally related to serum ALP (Fig. 3A & B), respectively. iCT appeared to be negatively correlated to serum ALP (Fig. 3C), while urinary total HYP and per cent ndHYP were reciprocally and directly related to serum PRL (Figs. 3D & E), respectively.

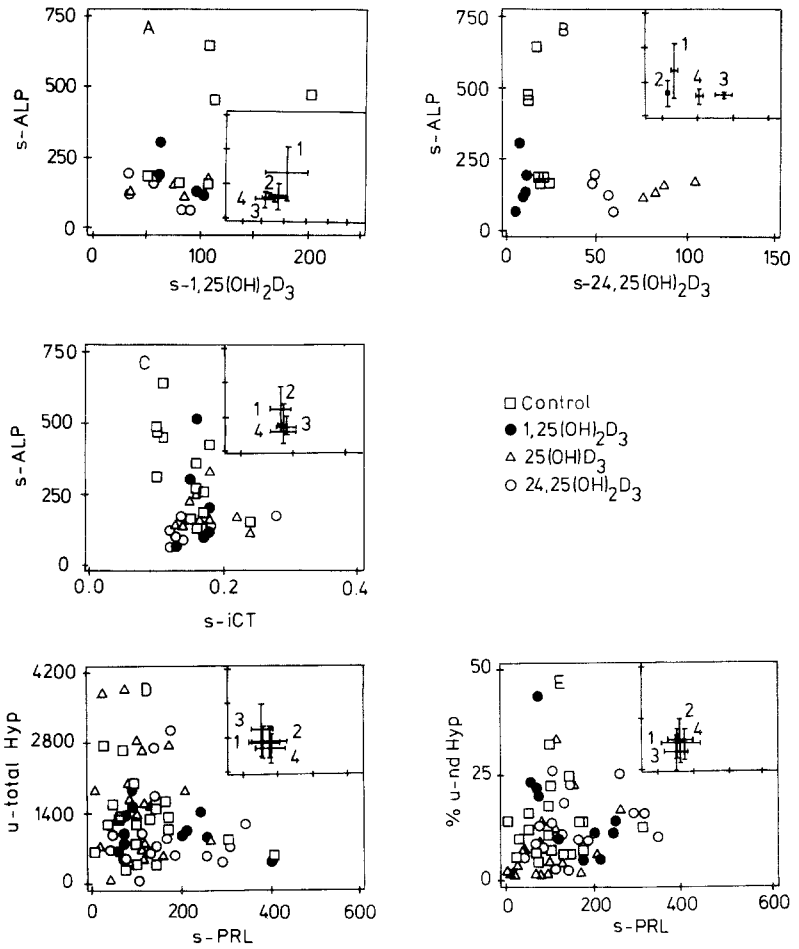

Fig. 3. Scatter and bivariate distribution plots (inserted) between circulating hormones and variables pertaining to bone turnover in rats receiving subcutaneous $1,25(OH)_2D_3$, $25(OH)D_3$ or $24,25(OH)_2D_3$ for 12 weeks. The control group received vehicle.

Correlation matrix analyses were performed and relevant parameters (r, p and n) listed in Table 4. The regression coefficient r is considered statistically different from zero when $p = \alpha < 0.10$ (one-tailed test).

Corresponding bivariate distribution plots are shown as inserts in all panels. Other matrix correlations (*data not shown*), will be discussed in the following.

DISCUSSION

In accordance with the effect of vitamin D_3 on the gut (DeLuca, 1988; Suda, 1990), $1,25(OH)_2D_3$ administration enhanced serum concentrations of total and ionized calcium as well as urinary calcium and phosphate excretion. However, urinary cAMP excretion was diminished probably due to impairment of the renal function as exemplified by lowered creatinine clearance, since a manifest suppression of immunoreactive parathyroid hormone (iPTH) was not observed. $24,25(OH)_2D_3$ treatment yielded opposite effects signifying that this vitamin D_3 metabolite actively is able to funnel calcium to the skeleton, since also alkaline phosphatase (ALP) was reduced as previously shown in normo- and hypercalcaemic dialysis patients (Popovtzer, 1991).

Contrary to serum $1,25(OH)_2D_3$, circulating $24,25(OH)_2D_3$ levels measured 24 hours subsequent to injections were significantly increased. This vitamin D_3 metabolite is known to accumulate in bone tissue (Dickson, 1987) where it is supposed to play a role in endochondral ossification (Corvol *et al.*, 1980; Sömjen *et al.*, 1987). Previously, $24,25(OH)_2D_3$ has been shown to suppress the hypercalcaemic effect of $1,25(OH)_2D_3$ in uraemic rats (Rubinger *et al.*, 1990). This is proposed to be due to a reduction in osteoclast number and activity (Yamato *et al.*, 1991; Popovtzer, 1991). The molecular mechanism for this effect entails a direct inhibitory action of $24,25(OH)_2D_3$ on osteoblastic PTH-receptor coupling to the GTP-binding protein $G_s\alpha$ (i.e. adenylate cyclase activation)

Table 4. Correlation matrix analyses

VARIABLES	PANEL	r	$p = 2\alpha$	n
$1,25(OH)_2D_3$ vs ALP	A	0.50	0.02	20
$24,25(OH)_2D_3$ vs ALP	B	−0.34	0.08	20
iCT vs ALP	C	−0.29	0.04	47
PRL vs total HYP	D	−0.20	0.06	80
PRL vs % ndHYP	E	0.21	0.05	77

Abbreviations: ALP = alkaline phosphatase, iCT = immunoreactive calcitonin, PRL = prolactin, total HYP = total urinary hydroxyproline, % ndHYP = per cent non-dialysable hydroxyproline, 1 = control group, 2 = $1,25(OH)_2D_3$ treatment, 3 = $25(OH)D_3$ treatment, 4 = $24,25(OH)_2D_3$ treatment.

resulting in perturbation of the catabolic paracrine signal to the osteoclast (Klem *et al.*, 1990). However, since serum iPTH was only marginally affected by either form of drug treatment, a more direct action of $24,25(OH)_2D_3$ on osteoclastic function could not be ruled out. Serum ALP has traditionally been regarded as a marker of bone formation (Teitelbaum and Bullaugh, 1979; Fritsch *et al.*, 1985) despite the fact that, in renal osteodystrophy, increasing ALP levels have been taken to signify progression of bone disease (Salusky *et al.*, 1988). Good correlations between bone turnover and ALP have been found in primary hyperparathyroidism (Broadus *et al.*, 1980) and acromegaly (Duda *et al.*, 1988). In this paper, serum ALP is shown to be positively correlated with serum $1,25(OH)_2D_3$. This is in concordance with the conditions prevailing in patients with primary hyperparathyroidism who display elevated serum $1,25(OH)_2D_3$. Furthermore, we found reciprocal correlations between serum $24,25(OH)_2D_3$ and serum ALP on one hand and between serum $24,25(OH)_2D_3$ and serum calcitonin (CT) on the other. This indicates that ALP in this model reflects net bone degradation, since circulating levels of $24,25(OH)_2D_3$ (which is hypocalcaemic) and CT (which blocks resorption through osteoclast inhibition) (Raisz, 1983; Nicholson *et al.*, 1986) are inversely correlated to the enzyme. Hence, ALP proves to distinguish well between biological effects of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on bone.

Total hydroxyproline (total HYP) serves as a degradation index of the extracellular matrix, although the applicability is somewhat limited due to lack of specificity and sensitivity (Robins, 1982; Delmas, 1990). Total HYP was inversely related to serum prolactin (PRL), which may implicate this hormone as a regulator of bone turnover. Elevated PRL is known to serve as an immunomodulator, adversely affecting mitogenesis of both T-cells and macrophages (Nagy *et al.*, 1983; Bernton *et al.*, 1988; Jara *et al.*, 1991). Consequently, ensuing loss of osteoclast recruitment with a reduced matrix degradation is expected. However, no direct action of PRL on bone tissue has been demonstrated (Ciccarelli *et al.*, 1988). In contrast, during normal lactation in the rat, PRL has been postulated to be responsible for enhanced serum calcium through direct mobilization from bone (Rasmussen, 1977) and absorption from the gut by regulating renal $25(OH)D_3$-1α-hydroxylase activity (Pike *et al.*, 1979). Similarily, hyperprolactinaemic male rats, with unaltered levels of PTH, glucocorticoid or sex steroids, have been shown to suffer from reduced cortical bone mass and bone calcium contents (Fiore *et al.*, 1988). However, PRL also positively predicted urinary ndHYP, which represents the N-terminus of newly synthesized collagen molecules (Askenasi *et al.*, 1976). The increase in ndHYP may solely be due to a reduction in total HYP and/or be attributed to enhanced *de novo* matrix synthesis. As an immunomodulator, PRL would affect collagen synthesis through a reduction in macrophage-derived tumour necrosis factor (TNF_α) which *per se* blocks collagen synthesis (Bertolini, 1986).

Finally, serum growth hormone (GH) proved to be inversely correlated to serum calcium. These results invoke a positive regulatory role for GH through insulin-like growth factor I (IGF-I) which is known to increase osteoblast proliferation, collagen deposition and mineralization (Canalis, 1980).

In summary, we have described an animal model system which, in terms of clinical chemistry pertaining to bone turnover, readily discriminates between biological effects of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. Apart from exerting direct effects on bone, the vitamin D_3 metabolites apparently also affect bone turnover via influence on PRL and GH secretion. The mechanism of PRL on bone metabolism is not known.

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