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Influence of the dosing frequency of parathyroid hormone-(1–38) on its anabolic effect in bone and on the balance of calcium, phosphorus and magnesium

Einfluß der Frequenz der Verabreichung von PTH-(1–38) auf seine anabole Wirkung am Knochen und auf die intestinale Absorption von Calcium, Phosphor und Magnesium

Summary The effect of the frequency of administration of synthetic human parathyroid hormone fragment 1–38 [hPTH-(1–38)] on bone formation and on the balance of calcium, phosphorus, and magnesium was investigated in 32 9-week-old female Sprague-Dawley rats, using a randomly complete block design. Rats received subcutaneously during 14 days either the vehicle solution once a day or 50 µg hPTH-(1–38)/kg BW once a day at 8:00 a.m., twice a day at 8:00 a.m. and 5:00 p.m. or three times a day at 8:00 a.m., 0:30 p.m., and 5:00 p.m. The balance study was performed during the last 48 h of the hPTH-(1–38) treatment schedule after which femora, tibiae, and lumbar vertebrae were removed for the determination of the dry weight, volume, and contents of Ca, P, Mg, hydroxyproline, and DNA. PTH treatment was associated with a significant increase of the apparent intestinal absorption of Ca, P, and Mg. Mean urinary Ca excretion augmented with the increase of the frequency of dosing. Urinary Ca excretion correlated negatively with the Ca apparent intestinal absorption and with the Ca content of the tibiae in the 2 groups with the highest frequency of dosing. The mean Ca, P, and Mg balances, the mean contents of bone Ca, P, and Mg and the mean bone dry weights were significantly increased with PTH treatment. In contrast to the mean volume of tibiae which was not affected by the PTH administration, the mean volume of the fifth lumbar vertebrae increased with the treatment. With the 2 times and 3 times daily treatments, mean hydroxyproline concentrations in the femora were significantly higher than the control values. An increase of the mean hydroxyproline content of the third lumbar vertebrae was evidenced with the 1 time and 2 times daily treatment, but the mean of the highest frequency of dosing was not different from that of the control group. The DNA content of femora and of the fourth lumbar vertebrae significantly decreased with the frequency of dosing.

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Zusammenfassung Der Effekt der Frequenz der Verabreichung des synthetischen humanen Parathormon-Fragments (1–38 [hPTH-(1–38)] auf die Knochenbildung und auf die Bilanzen von Calcium, Phosphor und Magnesium wurde bei 32 weiblichen, 9 Wochen alten Sprague-Dawley Ratten mittels eines randomisierten Blockversuches untersucht. Die Ratten erhielten hPTH-(1–38) subkutan über einen Zeitraum von 14 Tagen einmal täglich um 8:00 Uhr, zweimal täglich um 8:00 und 17:00 Uhr und dreimal täglich um 8:00, 12:30 und 17:00 Uhr oder das Lösungsmittel einmal täglich. Die Bilanzuntersuchung wurde während der letzten 48 Stunden der hPTH-(1–38)-Verabreichungsperiode durchgeführt. Danach wurden die Tiere getötet und die Femora, die Tibiae und die Lendenwirbel zur Bestimmung des Trockengewichts, des Volumens und des Gehalts an Ca, P, Mg, Hydroxyprolin und DNA entnommen. Die scheinbar intestinale

Absorption von Ca, P und Mg war erhöht. Die renale Ca-Ausscheidung nahm mit der Erhöhung der Frequenz der Dosierung zu und war negativ mit der scheinbaren intestinalen Ca-Absorption und mit dem Ca-Gehalt der Knochen in den zwei Gruppen mit zweimaliger und dreimaliger täglicher Dosierung korreliert. Die Ca-, P- und Mg-Bilanzen, der Gehalt an Ca, P und Mg im Knochen und das Trockengewicht der Knochen waren nach der PTH-Verabreichungsperiode deutlich erhöht. Im Gegensatz zum Volumen der Tibiae, das von der PTH-Verabreichung unbeeinflußt war, nahm das Volumen des fünften Lendenwirbels zu. Bei zweimaliger und dreimaliger täglicher Verabreichung war der Hydroxyprolingehalt der Femora signifikant höher als derjenige der Kontrollfemora. Eine Erhöhung des Hydroxyprolingehaltes des dritten Lendenwirbels wurde nach der Verabreichung einmal und zweimal am Tag beobachtet. Die Werte der Gruppe mit der höchsten Frequenz der Dosierung unterschieden sich jedoch nicht von den Werten der Kontrollgruppe. Der DNA-Gehalt der Femora und des vierten Lendenwirbels nahmen deutlich mit der Frequenz der Dosierung ab.

Key words Parathyroid hormone – balance – calcium – magnesium – phosphate

Schlüsselwörter Parathormon – Bilanz – Calcium – Magnesium – Phosphat

Introduction

Intermittent administration of adenylate cyclase-stimulating N-terminal fragments of parathyroid hormone (PTH) induces bone formation in intact and ovariectomized rats, intact dogs, and osteoporotic humans (3, 16, 39). PTH is, thus, regarded as a promising therapeutic agent for osteoporosis in humans and the interest for its potential use has prompted the initiation of numerous investigations. The anabolic effect is dependent of the dose and duration of treatment and results in increased bone mass and improvement of biomechanical properties and structure (10, 11, 17–21, 34). Both cortex and medulla respond in rats (4, 19, 27, 38, 40) and the effect is not abolished by vitamin D deficiency (33), although oral intake of vitamin D metabolites prevents osteopenia in ovariectomized rats (5, 6). Most of the research effort has been focused on the enhancement of bone formation. However, in relation to the PTH induced augmentation of bone mineralization, the apparent intestinal absorption of Ca, P, and Mg is increased in rats (33) and that of Ca is increased in dogs (26) and in oestrogen-treated women (28). More frequent dosing, twice daily or three times daily, enhances the anabolic response in bone and also the apparent intestinal absorption of Ca despite smaller doses (29) whereas continuous administration is without effect (9, 25, 32, 35). The relationship between the apparent intestinal absorption of Ca, P, and Mg to bone mineralization and that of the bone mineral content to the mechanical compentency after intermittent PTH administration has only been partially investigated. This study was conducted to test the effects of increased frequency of dosing of synthetic human PTH fragment (1–38) [hPTH-(1–38)] on the apparent intestinal absorption and balance of Ca, P, and Mg and on the anabolic response in bone.

Material and methods

A randomly complete block design involving 32 9-week-old female Sprague-Dawley rats weighing 191.44 ± 10.11 (SD) g at the beginning of the experiment was used. The forming of 2 blocks of 16 animals was required to allow a partition of the work load. Rats were housed individually in hanging wire cages in a temperature controlled room (21 C) with a cycle of light without UV source of 14 h light/10 h dark. During the adaptation period and the experiment, the rats received ad libitum a cereal based diet (Nafag No. 890, Gossau, Switzerland) containing 8 g of calcium, 7.5 g of phosphorus, 1.8 g of magnesium, 12.3 MJ of metabolizable energy, and 1 000 IU of vitamin D/kg of diet. Free access to deionized water was allowed until the end of the trial. After an adaptation period of 7 days, rats received 50 μg/kg BW of hPTH-(1–38) (Novartis, Basle, Switzerland; a gift from Dr. J. Gasser) for 14 days subcutaneously once a day at 8:00 a.m. (n = 8), twice a day at 8:00 a.m. and 17:00 p.m. (n = 8) or three times a day at 8:00 a.m., 0:30 p.m., and 17:00 p.m. (n = 8). The hPTH-(1–38) preparation with a peptide content of 87 % was diluted in a saline solution and a volume of 0.25 ml was injected via hypodermic needles. A group of 8 control rats only received the saline vehicle once a day. A balance study was performed during the last 48 h of the hPTH-(1–38) treatment schedule at the age of 12 weeks and weights of 260.50 ± 14.69 g. Between markings with carmine red (10 %, w/v), food intake was recorded, and faeces were separated from urine using a plastic grid placed under each cage. Urine was collected on sheets of ashless filter paper (Schleicher & Schuell, Riehen, Switzerland). At the end of the trial, rats were euthanatized with sodium pentobarbital (50 mg/kg BW, ip; Vetanarcol, Veterinaria, Zurich, Switzerland).

Femora, tibiae, and lumbar vertebrae were removed, cleaned from soft tissue, and frozen until further worked up. Faeces, urine-containing filter papers, right tibiae, and the sixth lumbar vertebrae (L6) were dried at 105 °C for 96 h and ashed at 600 °C for 96 h. Extraction of Ca, P, and Mg was achieved with 1 mol/l HCl over 24 h. Extracted Ca and Mg from faeces, urine, and bones was quantitated by flame atomic absorption spectrophotometry (SpectrAA-20, Varian) after sample dilution with 1.34 % $LaCl_3$. The atomic absorption spectrophotometer was calibrated and verified with a commercially available standard (F. Hoffmann-La Roche, Basle, Switzerland and Nycomed Pharma AS, Oslo, Norway). The absorbance was measured at a wavelength of 422.7 nm for Ca and 285.2 nm for Mg and a slit of 0.5 nm. P was quantitated by a colorimetry with an automated analyser (Cobas Mira, F. Hoffman-La Roche), using a commercial phosphomolybdate kit (F. Hoffmann-La Roche).

The volume of the left tibiae and of the fifth lumbar vertebrae (L5) were determined by Archimedes' principle using 70 % ethanol and an analytic balance attachment (density determination kit ME-33360 for AE200 balance; Mettler AG, Switzerland). Hydroxyproline was measured in the right femora by colorimetry after oxydation with chloramine T and chromophore development with p-dimethylaminobenzaldehyde (8). The left femora and the fourth lumbar vertebrae (L4) were blended individually in phosphate buffered saline with an homogenizer and DNA was quantitated by fluorometry with the bis-bendimidazole method using calf thymus DNA as standard (13).

The apparent intestinal Ca absorption was obtained individually from the difference between dietary Ca intake and faecal Ca output. The Ca balance was then calculated by subtracting urinary Ca output from the apparent intestinal Ca absorption.

A two-way analysis of variance by means of the main frame computer implemented SAS procedure GLM (SAS version 6.11, SAS Institute Inc., Cary, NC, USA) evaluated the effect of the frequency of administration of hPTH-(1–38). The adequacy of the use of the parametric method was tested by distribution of residuals and quantile-quantile plots. Differences among means were tested by means of the criterion least significant difference with alpha fixed at 0.05. Pearson correlation coefficients and their respective levels of significance were calculated by use of the SAS procedure CORR.

Results

The hPTH-(1–38) treatment regimens did not affect the mean initial and final body weights and the mean food intake recorded at the end of the trial (Table 1). Results of the balance study are summarized in Table 1 and those of the bone parameters in Table 2. Treatment with hPTH-(1–38) was associated with a significant increase of the apparent intestinal absorption of Ca, P, and Mg. However, the statistical analysis did not reveal an effect of the frequency of dosing. Mean urinary Ca excretion augmented with the increase of the frequency of dosing whereas mean urinary excretions of P and Mg were not affected by hPTH-(1–38) treatment. The standard error of mean urinary Ca for the 3 times daily hPTH-(1–38) administrations was the largest and values ranged from 1.83 to 6.54 mg/day. Scatter plots of urinary calcium excretion vs. apparent intestinal absorption and urinary calcium absorption vs. calcium content in tibiae including the data of all animals revealed a similar pattern (Fig. 1). When only the data of the 2 times and 3 times daily treatment were considered, values of the daily urinary calcium excretion correlated negatively with values of the apparent intestinal Ca absorption ($r = -0.667$; $p = 0.005$; Fig. 2) and with values of the calcium content of the tibiae ($r = -0.593$; $p = 0.015$) but not with the values of the calcium content of the vertebrae (data not shown). The mean Ca and P balances were increased by hPTH-(1–38) treatment but were not affected by the frequency

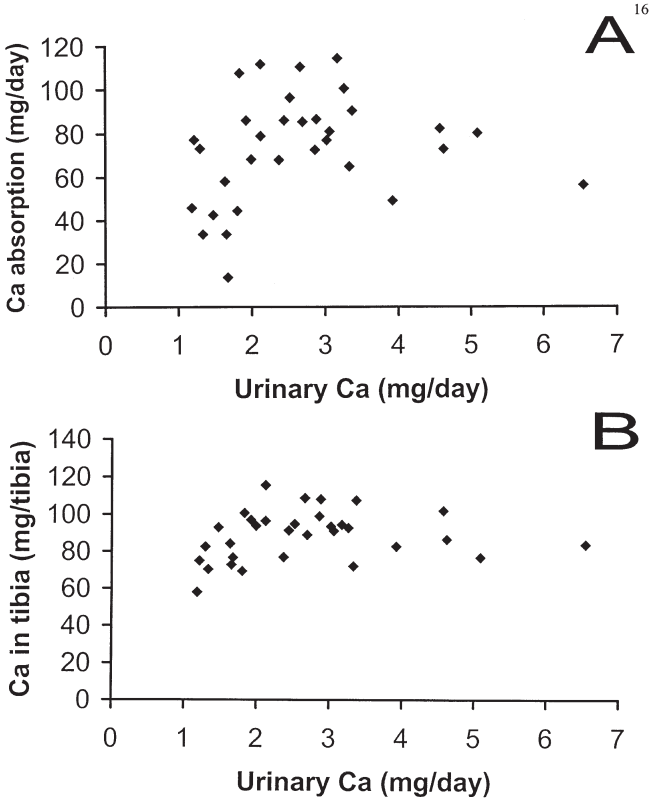

Fig. 1 Scatter plots of urinary Ca excretion vs. apparent intestinal Ca absorption (A) and urinary Ca excretion vs. Ca content in tibiae (B) in 32 3-month-old female rats treated with a vehicle solution (n = 8) or hPTH-(1–38) once daily (n = 8), twice daily (n = 8), and three times daily (n = 8).

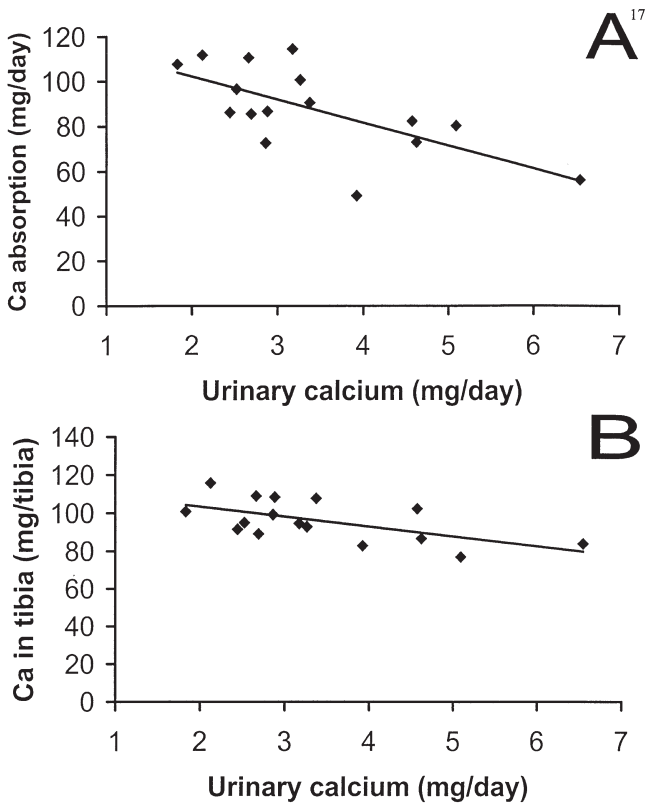

Fig. 2 Scatter plots and line of best fit of urinary Ca excretion vs. apparent intestinal Ca absorption (A) and urinary Ca excretion vs. Ca content in tibiae (B) in 16 3-month-old female rats treated with hPTH-(1–38) twice daily (n = 8) or three times daily (n = 8).

of dosing. For the Mg balance, a clear difference from the control group was only seen with the 3 times daily treatment. The mean contents of bone Ca, P, and Mg were clearly increased by hPTH-(1–38) treatment. The frequency of administration did only influence the mean P content of tibiae with the highest values for rats which had been treated 3 times daily. The mean dry weight of the tibiae was augmented by the hPTH-(1–38) treatment but was not affected by the frequency of dosing. The mean dry weight of L5 was larger after the hPTH-(1–38) treatment and dosing twice daily was associated with the heaviest mean value. In contrast to the mean volume of tibiae which was not affected by the hPTH-(1–38) treatment, the mean volume of L5 increased with the treatment and the highest frequency of administration was associated with the highest mean value. With the 2 times and 3 times daily treatments, mean hydroxyproline concentrations in the femora were significantly higher than the control values. An increase of the mean hydroxyproline content of L3 was evidenced with the 2 times daily treatment, but the mean of the highest frequency of dosing was not different from that of the control group. The DNA content of femora and L4 clearly decreased with the frequency of administration.

Discussion

As previously observed (26, 29, 33), hPTH-(1–38) treatment was associated with an increase of the apparent intestinal absorption of Ca, P, and Mg. However, in contrast to previous findings with the apparent intestinal absorption of Ca (29), the statistical analysis did not reveal an effect of the dose frequency on the apparent absorption of these minerals. Similarly, the balances of Ca and P were not affected by the frequency of dosing. This discrepancy between the results of the 2 trials may most likely be explained by the fact that in the present study the injections were done within 9 hours during the day in contrast to injections every 8 and every 12 hours in the earlier report (29). Moreover, the doses used in the earlier report were smaller. In the rats of the present study, only urinary Ca was affected by the treatment schedule. Surprisingly, urinary Ca excretion was increased with hPTH-(1–38) intermittent administration, and the increase was highest with the highest frequency of dosing. The large variation of urinary Ca excretion associated with the highest frequency of dosing indicates that the individual response of rats was quite variable. The PTH induced increase of urinary Ca must be vitamin D dependent, because PTH treatment of vitamin D deficient rats resulted in decreased urinary Ca (33). This interpretation must, however, be made with caution because the 20 % lactose and the larger Ca and P content of the vitamin-deficient diet might have been a confounding factor. In the kidney, one of the major roles of PTH is the fine-regulation of the Ca reabsorption from the thick ascending limb of Henle's loop and the distal tubule (31). It may be hypothesized that the PTH-induced augmented urinary Ca may be due to an increase in the filtered load in the glomerulus and/or related to a different response of the distal tubule in relation to the frequency of PTH administration. The first hypothesis is supported by a non significant trend for an increase of mean PTH induced apparent intestinal Ca absorption in relation to the increased frequency of dosing. However, the values of the apparent intestinal Ca absorption correlated negatively with those of urinary Ca in the two groups with the highest frequency of dosing which does not support the hypothesis. Bone Ca does not contribute to the increased filtered load because there is no evidence from these data of an increased Ca loss from bone related to an increased frequency of PTH administration. The negative correlation between the Ca content of tibiae and the urinary Ca excretion suggests that these two parameters are related by a mechanism to be determined. Serum 1,25-dihydroxy-vitamin D concentrations, which could eventually explain the relationship between the apparent intestinal Ca absorption, the Ca content of the tibiae and the urinary Ca excretion, were not determined in the present experiment. On the other hand, the urinary loss of Ca is only a small portion of the total excretion of Ca

Table 1 Effect of the frequency of the subcutaneous administration during 14 days of 50 µg hPTH-(1–38) per kg BW on the body weight, food intake, apparent intestinal absorption, urinary excretion, and balance of Ca, P, and Mg in 3-month-old female rats. Results are reported as means ± SE

Parameter	Unit	Group			
		Control n = 8	PTH once daily n = 8	PTH twice daily n = 8	PTH 3 times daily n = 8
Initial body weight	g	185.87 ± 3.32[a]	192.87 ± 3.86[a]	196.5 ± 3.90[a]	190.50 ± 2.63[a]
Final body weight	g	251.75 ± 4.25[a]	268.37 ± 7.35[a]	262.25 ± 5.22[a]	259.62 ± 4.20[a]
Food intake	g/day	24.01 ± 0.02	23.99 ± 0.02[a]	24.00 ± 0.02[a]	24.08 ± 0.02[a]
Apparent Ca absorption	mg/day	43.59 ± 6.61[a]	74.69 ± 2.59[b]	86.37 ± 6.63[b]	88.99 ± 7.24[b]
Apparent P absorption	mg/day	73.81 ± 6.16[a]	99.51 ± 2.84[b]	109.11 ± 3.92[b]	104.76 ± 5.54[b]
Apparent Mg absorption	mg/day	13.09 ± 1.15[a]	16.54 ± 0.79[b]	15.67 ± 0.77[b]	17.39 ± 1.09[b]
Ca in urine	mg/day	1.49 ± 0.08[a]	2.39 ± 0.25[b]	2.96 ± 0.18[b]	3.85 ± 0.57[c]
P in urine	mg/day	34.30 ± 2.72[a]	32.31 ± 3.27[a]	35.33 ± 4.31[a]	34.75 ± 3.33[a]
Mg in urine	mg/day	8.91 ± 0.35[a]	9.56 ± 0.50[a]	9.49 ± 0.37[a]	9.49 ± 0.36[a]
Ca balance	mg/day	42.10 ± 6.65[a]	72.30 ± 2.64[b]	83.41 ± 6.74[b]	85.13 ± 7.76[b]
P balance	mg/day	39.51 ± 5.57[a]	67.21 ± 3.15[b]	73.79 ± 3.38[b]	70.01 ± 5.61[b]
Mg balance	mg/day	4.18 ± 1.22[a]	6.97 ± 1.14[ab]	6.18 ± 0.68[ab]	7.90 ± 1.21[b]

[a,b,c] means with the same letter in the same row are not significantly different

Table 2 Effect of the frequency of the subcutaneous administration during 14 days of 50 µg hPTH-(1–38) per kg BW on the dry weight, volume, content of Ca, P, Mg, hydroxyproline, and DNA of selected long bones and vertebrae in 3 month-old female rats. Results are reported as means ± SE

Parameter	Unit	Group			
		Control n = 8	PTH once daily n = 8	PTH twice daily n = 8	PTH 3 times daily n = 8
Dry weight of tibia	g/tibia	0.33 ± 0.02[a]	0.38 ± 0.01[b]	0.39 ± 0.01[b]	0.38 ± 0.01[b]
Dry weight of L5	g/vertebra	0.16 ± 0.004[a]	0.19 ± 0.01[b]	0.21 ± 0.01[c]	0.20 ± 0.01[bc]
Volume of tibia	mL/tibia	0.31 ± 0.01[a]	0.34 ± 0.01[a]	0.33 ± 0.01[a]	0.35 ± 0.01[a]
Volume of L5	mL/vertebra	0.15 ± 0.005[a]	0.18 ± 0.01[b]	0.18 ± 0.01[bc]	0.20 ± 0.01[c]
Ca in tibia	mg/tibia	74.97 ± 3.69[a]	88.05 ± 3.36[b]	95.95 ± 3.18[b]	96.23 ± 4.62[b]
Ca in L6	mg/tibia	67.92 ± 4.27[a]	77.86 ± 3.00[ab]	84.55 ± 3.12[b]	82.17 ± 2.42[b]
P in tibia	mg/tibia	36.65 ± 14.00[a]	43.01 ± 1.39[b]	46.96 ± 1.64[bc]	47.22 ± 0.76[c]
P in L6	mg/tibia	40.45 ± 1.02[a]	50.35 ± 1.35[b]	53.81 ± 1.78[b]	50.40 ± 1.89[b]
Mg in tibia	mg/tibia	1.55 ± 0.04[a]	1.85 ± 0.07[b]	1.98 ± 0.04[b]	1.88 ± 0.05[b]
Mg in L6	mg/tibia	1.59 ± 0.09[a]	1.95 ± 0.11[b]	2.05 ± 0.07[b]	1.99 ± 0.16[b]
Hydroxyproline in femur	mg/g femur	22.02 ± 0.33[a]	22.69 ± 0.31[ab]	23.46 ± 0.25[b]	23.44 ± 0.20[b]
Hydroxyproline in L3	mg/g vertebra	26.48 ± 0.38[ab]	27.00 ± 0.39[bc]	27.69 ± 0.24[c]	25.85 ± 0.44[a]
DNA in femur	mg/g femur	8.79 ± 0.40[a]	6.51 ± 0.20[b]	3.54 ± 0.28[c]	2.71 ± 0.13[d]
DNA in L4	mg/g vertebra	9.04 ± 0.35[a]	7.34 ± 0.24[b]	4.63 ± 0.23[c]	3.25 ± 0.22[d]

[a,b,c,d] means with the same letter in the same row are not significantly different

and is unlikely to explain the relationship between the high urinary Ca excretion and the low Ca content of the tibiae. The observed effect on urinary Ca excretion with the 2 highest frequency of dosing may thus be directly related to PTH because, as in bone, the effect of intermittent administration of PTH may be quite different from that of a continuous administration. Vitamin D metabolites may likely be involved in the modulation of the PTH effect. Because urinary Ca excretion is only a minor contribution to total Ca excretion, the PTH induced changes in urinary Ca excretion is not reflected in the Ca balance.

For the Mg balance, a clear difference from the control group was only seen with the three time daily treatment confirming the results of a previous study (33) where PTH treatment once daily did not increase Mg balance in vitamin D sufficient rats receiving 0.8 % Ca, 0.5 % P, and 20 % lactose or in vitamin D deficient rats receiving 2 % Ca, 1.25 % P, and 20 % lactose. The significant PTH induced increased Mg absorption already evident at the lowest dosing frequency is not reflected in the Mg balance because urinary Mg excretion is large in proportion to Mg absorption. The mean P content of tibiae was affected by the frequency of administration in

contrast to P of vertebrae suggesting a different response in cortex and medulla because the ratio cortex to medulla is much larger in the tibiae.

In contrast to the situation in femora, the hydroxyproline concentration of L3 obtained with the highest frequency of dosing were not different from those of the control group suggesting that collagen synthesis was not stimulated in this group or that both formation and resorption were activated. This finding is reflected in the dry weight of L5 and the mineral content of L6 (nonsignificant) but not in the volume of L5 which supports the hypothesis that the processes of formation and resorption were both activated in the vertebrae. The long bones, characterized by a larger cortex to medulla ratio, did not respond similarly with the highest frequency of dosing. In bone cultures, transient exposure during several hours with PTH followed by withdrawal stimulated collagen synthesis (1, 14) whereas continuous treatment induced inhibition (12, 37). Thus, in the lumbar vertebrae of this study, the effects obtained after the 3 times daily PTH treatment may be similar to those observed after continuous administration (9, 25, 32, 35).

The decrease of the DNA content of bone which is related to the frequency of PTH dosing is unexpected because intermittent treatment with parathyroid hormone stimulates in rats the proliferation and differentiation of explanted osteoblast precursors (22) and, in bone cultures, it stimulates the replication and the synthetic activities of mature osteoblasts (1, 2, 14). In isolated osteoblasts, depending on the animal species, cell strain and experimental conditions, PTH may stimulate (15, 36) or inhibit (7, 24) proliferation mainly via the adenylate/protein kinase A pathway (23, 30). However, this experimental design does not allow to determine which cells of bone and bone marrow are affected and how the activity of the different cell types are individually modulated.

In conclusion, the parameters considered in this study were affected differently by the frequency of hPTH-(1–38) dosing. These results were obtained after a short-term treatment in growing animals that have reached sexual maturity. It is well established that the serum concentration of sex hormones influence Ca homeostasis and bone turnover. Further studies are, thus, needed to examine the response to PTH in older animals or after a long-term treatment.

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References

1. Canalis E, Centrella M, Burch W, McCarthy TL (1989) Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone culture. J Clin Invest 83:60–65
2. DeBartolo TF, Pegg LE, Shasserre C, Hahn TJ (1982) Comparison of parathyroid hormone and calcium ionophore A23187: effects on bone resorption and nucleic acid synthesis in cultured fetal rat bone. Calc Tissue Int 34:495–500
3. Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R (1993) Anabolic action of parathyroid hormone on bone. Endocrine Rev 14:690–709
4. Ejersted C, Andreassen TT, Oxlund H, Jorgensen PH, Bak B, Häggblad J, Torring O, Nilsson MHL (1993) Human parathyroid hormone (1–34) and (1–84) increase the mechanical strength and thickness of cortical bone in rats. J Bone Min Res 9:1097–1101
5. Erben RG, Kohn B, Weiser H, Sinowatz F, Rambeck WA (1990) Role of vitamin D metabolites in the prevention of the osteopenia induced by ovariectomy in the axial and appendicular skeleton of the rat. Z Ernährungswiss 29:229–248
6. Erben RG, Weiser H, Sinowatz F, Rambeck WA, Zucker H (1992) Vitamin D metabolites prevent vertebral osteopenia in ovariectomized rats. Calcif Tissue Int 50:228–236
7. Farndale RW, Sandy JR, Atkinson SJ, Pennington SR, Meghji S, Meikle MC (1988) Parathyroid hormone and prostaglandin E_2 stimulate both inositol phosphates and cyclic AMP accumulation in mouse osteoblast cultures. Biochem J 252:263–268
8. Gordeladze JO, Halse J, Djoseland O, Haugen HN (1978) A simple procedure for the determination of hydroxyproline in urine and bone. Biochem Med 20:23–30
9. Hock JM, Gera I (1992) Effects of continuous and intermittent administration and inhibition of resorption on the anabolic response of bone to parathyroid hormone. J Bone Min Res 7:65–72
10. Jerome CP (1994) Anabolic effect of high doses of human parathyroid hormone (1–38) in mature intact female rats. J Bone Min Res 9:933–942
11. Kimmel DB, Bozzato RP, Kronis KA, Coble T, Sindrey D, Kwong P, Recker RR (1993) The effect of recombinant human (1–84) or synthetic human (1–34) parathyroid hormone on the skeleton of adult osteopenic ovariectomized rats. Endocrinology 132: 1577–1584
12. Kream BE, Rowe DW, Gworek SC, Raisz LG (1980) Parathyroid hormone alters collagen synthesis and procollagen mRNA levels in fetal rat calvaria. Proc Natl Acad Sci USA 77:5654–5658
13. Labarca C, Paigen K (1980) A simple rapid and sensitive DNA assay procedure. Anal Biochem 102:344–352
14. Linkhart TA, Mohan S, Baylink DJ (1988) Bone repletion in vitro: Evidence for a locally regulated bone repair response to PTH treatment. Bone 9:371–379
15. MacDonald BR, Gallagher JA, Russell RGG (1986) Parathyroid hormone stimulates the proliferation of cells derived from human bone. Endocrinology 118:2445–2449
16. Margolis RN, Canalis E, Partridge NC (1996) Invited review of a workshop: Anabolic hormones in bone: Basic research and therapeutic potential. J Clin Endocrinol Metab 81:872–877
17. Meng XW, Liang XG, Birchman R, Wu DD, Dempster DW, Lindsay R, Shen V (1996) Temporal expression of the anabolic action of PTH in cancellous bone of ovariectomized rats. J Bone Min Res 11:421–429
18. Mosekilde L, Danielsen CC, Gasser J (1994) The effect on vertebral bone mass and strength of long term treat-

ment with antiresorptive agents (estrogen and calcitonin), human parathyroid hormone-(1–38), and combination therapy, assessed in aged ovariectomized rats. Endocrinology 134:2126–2134
19. Mosekilde L, Danielsen CC, Sogaard CH, McOsker JE, Wronski TJ (1995) The anabolic effects of parathyroid hormone on cortical bone mass, dimensions and strength – assessed in a sexually mature, ovariectomized rat model. Bone 16:223–230
20. Mosekilde L, Sogaard CH, Danielsen CC, Torring O, Nilsson MHL (1991) The anabolic effects of human parathyroid hormone (hPTH) on rat vertebral body mass are also reflected in the quality of bone, assessed by biomechemical testing: A comparison study between hPTH-(1–34) and hPTH-(1–84). Endocrinology 129:421–428
21. Mosekilde L, Thomsen JS, McOsker JE (1997) No loss of biomechanical effects after withdrawal of short-term PTH treatment in an aged, osteopenic, ovariectomized rat model. Bone 20:429–437
22. Nishida S, Yamaguchi A, Tanizawa T, Endo N, Mashiba T, Uchiyama Y, Suda T, Yoshiki S, Takahashi HE (1994) Increased bone formation by intermittent parathyroid hormone administration is due to the stimulation of proliferation and differentiation of osteoprogenitor cells in bone marrow. Bone 15:711–723
23. Partridge NC, Bloch SR, Pearman AT (1994) Signal transduction pathways mediating parathyroid hormone regulation of osteoblastic gene expression. J Cel Biochem 55:321–327
24. Partridge NC, Opie AL, Opie RT, Martin TJ (1985) Inhibitory effect of parathyroid hormone on growth of osteogenic osteosarcoma cells. Calcif Tissue Int 37:519–525
25. Podbesek R, Edouard C, Meunier PJ, Parsons JA, Reeve J, Stevenson RW, Zanelli JM (1983) Effects of two treatment regimes with synthetic human parathyroid hormone fragment on bone formation and the tissue balance of trabecular bone in Greyhounds. Endocrinology 112:1000–1006
26. Podbesek RD, Mawer EB, Zanelli GD, Parsons JA, Reeve J (1984) Intestinal absorption of calcium in greyhounds: the response to intermittent and continuous administration of human synthetic parathyroid hormone fragment 1–34 (hPTH 1–34). Clin Sci 67:591–599
27. Qi H, Li M, Wronski TJ (1995) A comparison of the anabolic effects of parathyroid hormone at skeletal sites with moderate and severe osteopenia in aged ovariectomized rats. J Bone Min Res 10:948–955
28. Reeve J, Bradbeer JN, Arlot M, Davies UM, Green JR, Hampton L, Edouard C, Hesp R, Hulme P, Ashby JP, Zanelli JM, Meunier PJ (1991) hPTH 1–34 treatment of osteoporosis with added hormone replacement therapy: Biochemical, kinetic and histological responses. Osteoporosis Int 1:162–170
29. Riond JL (1993) Modulation of the anabolic effect of synthetic human parathyroid hormone fragment-(1–34) in the bone of growing rats by variations in the dosage regimen. Clin Sci 85:223–228
30. Sabatini M, Lesur C, Pacherie M, Pastoureau P, Kucharczyk N, Fauchère JL, Bonnet J (1996) Effects of parathyroid hormone and agonists of the adenylyl cyclase and protein kinase C pathways on bone cell proliferation. Bone 18:59–65
31. Suki WN, Rouse D (1996) Renal transport of calcium, magnesium and phosphate. In: Brenner BM (ed) The Kidney, 5th ed.; WB Saunders Comp, Philadelphia, pp 472–508
32. Tam CS, Heersche JNM, Murray TM, Parsons JA (1982) Parathyroid hormone stimulates the bone apposition rate independently of its resorptive action: differential effects of intermittent and continuous administration. Endocrinology 110:506–512
33. Toromanoff A, Amman P, Mosekilde L, Thomsen JS, Riond JL (1997) Parathyroid hormone increases bone formation and improves mineral balance in vitamin D-deficient female rats. Endocrinology 138:2449–2457
34. Toromanoff A, Amman P, Riond JL (1998) Early effects of short-term parathyroid hormone administration on bone mass, mineral content and strength in female rats. Bone 22:217–223
35. Uzawa T, Hori M, Ejiri S, Ozawa H (1995) Comparison of the effects of intermittent and continuous administration of human parathyroid hormone (1–34) on rat bone. Bone 16:477–484
36. Van der Plas A, Feyen JHM, Nijweide PJ (1985) Direct effect of parathyroid hormone on the proliferation of osteoblast-like cells: A possible role of cyclic AMP. Biochem Biophys Res Comm 129:918–925
37. Vargas SJ, Raisz LG (1990) Simultaneous assessment of bone resorption and formation in cultures of 22-day fetal rat parietal bones: effects of parathyroid hormone and prostaglandin E_2. Bone 11:61–65
38. Wronski TJ, Yen CF (1994) Anabolic effects of parathyroid hormone on cortical bone in ovariectomized rats. Bone 15:51–58
39. Whitfield JF, Morley P (1995) Small bone-building fragments of parathyroid hormone: new therapeutic agents for osteoporosis. Trends Pharmacol Sci 16:382–386
40. Whitfield JF, Morley P, Willick GE, Ross V, MacLean S, Barbier JR, Isaacs RJ, Ohannessian-Barry L (1997) Comparison of the ability of recombinant human parathyroid hormone, rhPTH-(1–84), and hPTH-(1–31)NH_2 to stimulate femoral trabecular bone growth in ovariectomized rats. Calcif Tissue Int 60:26–29