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THE ROLE OF 1,25-DIHYDROXYVITAMIN D IN THE INHIBITION OF BONE FORMATION INDUCED BY SKELETAL UNLOADING

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Abstract

Skeletal unloading results in osteopenia. To examine the involvement of vitamin D in this process, the rear limbs of growing rats were unloaded and alterations in bone calcium and bone histology were related to changes in serum calcium (Ca), inorganic phosphorus (P_i), 25-hydroxyvitamin D (25-OH-D), 24,25-dihydroxyvitamin D ($24,25(OH)_2D$ and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Acute skeletal unloading induced a transitory inhibition of Ca accumulation in unloaded bones. This was accompanied by a transitory rise in serum Ca, a 21% decrease in longitudinal bone growth ($P< 0.01$), a 32% decrease in bone surface lined with osteoblasts ($P< .05$), no change in bone surface lined with osteoclasts and a decrease in circulating $1,25(OH)_2D$ from 130 ± 10 pg/ml to 53 ± 11 pg/ml. No significant changes in the serum concentrations of P_i, 25-OH-D or $24,25(OH)_2D$ were observed. After 2 weeks of unloading, bone Ca stabilized at approximately 70% of control and serum Ca and $1,25(OH)_2D$ returned to control values. Maintenance of a constant serum $1,25(OH)_2D$ concentration by chronic infusion of $1,25(OH)_2D$ (Alza osmotic minipump) throughout the study period did not prevent the bone changes induced by acute unloading. These results suggest that acute skeletal unloading in the growing rat produces a transitory inhibition of bone formation which in turn produces a transitory hypercalcemia leading to a temporary decrease in serum $1,25(OH)_2D$. No evidence could be found for a direct involvement of $1,25(OH)_2D$ in the bone changes induced by skeletal unloading.

Introduction

Skeletal unloading results in osteopenia (1-11). Humans confined to bed or immobilized by paralysis (5,7), or individual limbs of animals immobilized by casting or nerve/tendon section lose bone mineral (1,3,4,6,8,10). Weightlessness, as experienced during space flight (12-14), and simulated weightlessness (9,11) also induce osteopenia. Despite numerous studies, the pathogenesis of this bone loss remains unclear.

Regardless of how skeletal unloading is accomplished, the development of osteopenia appears to follow a common course. In the adult, skeletal unloading results in a rapid loss of bone initially followed by a period in which bone mass stabilizes. Minaire et al. have shown, for example, that acute immobilization of adult humans as a result of spinal cord injuries results in a rapid loss of bone for a period of approximately 25 weeks (7). After this time, trabecular bone volume stabilizes at roughly 67% of normal and appears to remain at that level indefinitely. In the growing animal, skeletal unloading does not cause a loss of bone per se but rather appears to produce a temporary inhibition of bone formation (3,10). This transitory reduction in bone formation results in an osteopenic bone when compared to age-matched, normally loaded bones.

We have observed a similar phenomenon using the suspended rat model to produce skeletal unloading (15). Within 5-7 days of skeletal unloading by tail suspension, there is a significant inhibition of ^{45}Ca and ^{3}H-proline uptake by bone. When compared to normally loaded bone, bone formation rate at the tibiofibular junction and total bone Ca are also significantly reduced. Between days 7 and 15 of unloading the uptake of ^{45}Ca and ^{3}H-proline return to normal and although total bone Ca remains abnormally low, the rate of Ca accumulation with time and bone formation rate at the tibiofibular junction return to normal.

To examine the relationship between these characteristic changes in bone metabolism following acute skeletal unloading and vitamin D metabolism we measured the serum concentrations of 25-hydroxyvitamin D (25-OH-D), 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) at various times after skeletal unloading. We also determined the effect of chronic infusion of $1,25(OH)_2D_3$ on the bone changes associated with unloading.

Materials and Methods

Skeletal unloading was accomplished using the suspended rat model as previously described (11). Male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA), weighing 124-149 gm, were randomized into control (pair-fed) and experimental (suspended) groups. Experimental animals, suspended by their tails such that both rear limbs, pelvis and caudal portion of the spinal column were unloaded while the fore limbs remained weight bearing or loaded, were fed ad libitum standard rat chow (Wayne Lab Blox, F-6) containing 1.2% Ca, 0.99% P and 4.41 I.U. vitamin D_3/g of food. Control (nonsuspended) animals were pair-fed to the suspended animals. All rats were maintained on a 12 hour light, 12 hour dark cycle and were the same age at the time of sacrifice.

To determine the effect of skeletal unloading on vitamin D metabolism, rats were sacrificed after 2, 5, 7, 10, 12 and 15 days of suspension and compared to pair-fed nonsuspended animals. All rats were injected i.p. with the tetracycline derivative demeclocycline (Declomycin) 24 hours prior to sacrifice to label calcifying tissues (16). At the time of sacrifice, blood was collected from the dorsal aorta and plasma obtained for measurement of total Ca, inorganic phosphate (P_i), 25-OH-D, $24,25(OH)_2D$ and $1,25(OH)_2D$. Plasma Ca was measured

using atomic absorption spectroscopy after dilution of plasma with aqueous $LaCl_3$ (1/50), and plasma P_i was measured using the method of LeBel et al. (17). The vitamin D metabolites were measured according to the method of Horst et al. (18). The right tibia, lumbar vertebra (L-1) and right humerus were removed, cleaned of adhering tissue, extracted and hydrolyzed in HCl (15). Calcium in the hydroylate was measured using atomic absorption spectroscopy (15).

The left tibiae from animals suspended or pair-fed for 5 days were processed for quantitative bone histomorphometry. The bone specimens were fixed in 10% phosphate-buffered formalin (pH 7.4) for 24 hours, dehydrated in ethanol, and embedded undecalcified in methyl methacrylate. Longitudinal sections of 4 μm thickness were cut with an AO Autocut/Jung 1150 microtome and stained according to Goldner's method (19). Bone parameters were measured at a magnification of 400X with a Merz eyepiece reticle (20). Two sections of the proximal tibial metaphysis, equivalent to approximately 14 mm^2 of trabelular bone tissue, were sampled in each animal. This area was standardized in relation to the growth plate-metaphyseal junction.

The number of points superimposed over mineralized tissue (calcified cartilage and bone) and bone marrow were recorded. The fractional area of mineralized tissue, commonly referred to as trabecular bone volume, was determined by dividing the number of points lying over minuralized tissue by the total number of points. Intersections of semicircular reticle lines with the bone bone-marrow interface were classified as resting, osteoblast, or osteoclast surface. Resting surface is defined as trabecular bone surface without adjacent osteoblasts or osteoclasts. Osteoblast surface is defined as trabecular surface lined with osteoblasts, whereas irregular or scalloped trabecular surface with adjacent osteoclasts is classified as osteoclast surface. Osteoblast surface

(%) was determined by dividing the number of intersects with bone surfaces lined by osteoblasts by the total number of intersects. Osteoclast surface (%) was calculated in a similar manner. The numbers of osteoblasts and osteoclasts adjacent to trabecular bone surfaces of the proximal tibial metaphysis were also quantified. These data are expressed as number of bone cells per mm trabecular bone perimeter. This latter parameter was determined by multiplying the total number of intersects by the grid constant d, which is equal to the distance between grid points (20).

Unstained, 10 μm-thick sections of the proximal tibial metaphysis were collected for measurement of longitudinal bone growth. The distance between the growth plate-metaphyseal junction and the fluorescent tetracycline band that parallels the growth plate was quantified with a calibrated eyepiece micrometer (21) at 5 equally-spaced sites per section. These measurements were performed under ultraviolet illumination at a magnification of 200X in 2 sections per animal. The rate of longitudinal bone growth was calculated by dividing the distance between the growth plate-metaphyseal junction and the tetracycline band by the time interval between administration of the tetracycline label and sacrifice.

To establish a constant elevated concentration of $1,25(OH)_2D$ in the serum throughout the period of skeletal unloading, subcutaneously implanted Alza osmotic minipumps (Model 2002, Alza Corp., Palo Alto, CA) were used to infuse $1,25(OH)_2D_3$ (a gift from Dr. M. Uskokovic, Hoffmann LaRoche, Nutley, NJ) at a constant rate of 75 pmoles/day (12 μl/day) for 2 weeks. Control animals were infused with vehicle (1.25% ethanol in propylene glycol). Pumps were implanted such that each animal was infused for a total of 13 days before sacrifice (i.e. animals sacrificed after 2 days of suspension were implanted 11 days before suspension, while animals sacrificed after 12 days of suspension were

implanted 1 day before being suspended). This insured that all animals received the same total dose of vehicle or $1,25(OH)_2D$. To prove that the serum concentration of $1,25(OH)_2D$ is maintained constant during the infusion period, we measured serum $1,25(OH)_2D$ at 2, 7, and 14 days after pump implant in both suspended and nonsuspended animals. The serum concentrations at these time points were 206 ± 12 pg/ml (n = 5), 204 ± 11 pg/ml (n = 5) and 179 ± 14 pg/ml (n = 5) respectively, and did not differ between suspended and nonsuspended rats.

All data are given as mean ± SE and analyzed, where appropriate, using analysis of variance or Students t-test.

Results

Unloading of the tibia and lumbar vertebra (L-1) result in an inhibition of Ca accumulation in bone (Fig. 1). This occurs despite normal or near normal gain in body weight. At the time of sacrifice, the mean body weights of the suspended and pair-fed control animals were not significantly different except for a slight (5.8%, $P<.01$) decrease in body weight observed in the 12 day suspended animals. The humeri of suspended animals, which remain loaded in this model, did not differ from the humeri of control animals in terms of Ca accumulation (data not shown). Since the animals used in this study are growing, bone calcium is increasing in both loaded and unloaded bones (data not shown). However, the rate of increase becomes less in unloaded bones after 2-5 days of suspension. This diminished rate of Ca accumulation accounts for the growing difference in total bone Ca between loaded and unloaded limbs between days 2 and 10 of suspension. After 10-15 days of suspension, the difference in

bone Ca between loaded and unloaded bones tends to stabilize at 70-90% of the control level (Fig. 1, 3c, 3e), although complete stabilization may not occur until day 21 (15). Total tibial and lumbar vertebral Ca decreased from 42.9 ± 1.8 mg to 32.0 ± 2.3 mg ($P<.005$) and from 16.7 ± 1.0 mg to 11.9 ± 0.8 mg ($P<.005$) respectively after 15 days of suspension.

The results of histological examination of tibia from control and suspended animals 5 days after beginning suspension are shown in Table 1. Although no significant difference in trabecular bone volume was seen, a 32% reduction in osteoblast surface and a 39% reduction in osteoblasts per mm trabecular surface was observed in bones from suspended rats. No difference in the osteoclast number was seen. Longitudinal bone growth was reduced by 21%.

The serum concentrations of Ca, $1,25(OH)_2D$ and $24,25(OH)_2D$ during the 2 week course of skeletal unloading are shown in Figure 2. Two days after unloading, serum Ca was elevated but quickly returned to normal and maintained a concentration not significantly different from control animals for the remainder of the study period (Fig. 2A). A dramatic fall in the serum concentration of $1,25(OH)_2D$ occurred within 2 days of skeletal unloading and persisted to day 5 (Fig. 2B). Five days after unloading, serum $1,25(OH)_2D$ had fallen from a control value of 130 ± 10 pg/ml to a low of 53 ± 11 pg/ml ($P<.01$). During the next 10 days, the concentration of $1,25(OH)_2D$ in the serum gradually increased, returning to control levels by day 15 of suspension.

Although the changes were small, the serum concentration of $24,25(OH)_2D$ tended to increase during the first 5 days of suspension and then gradually decreased toward control levels during the next 10 days (Fig. 2C). The serum concentration of 25-OH-D was the same in loaded (overall mean = 19.4 ± 1.6 ng/ml) and unloaded (overall mean = 19.6 ± 1.2 ng/ml) animals at all times.

The serum concentrations of $1,25(OH)_2D$ in animals chronically infused with either vehicle (Fig. 3A) or $1,25(OH)_2D_3$ (Fig. 3B) are shown in Figure 3. Animals infused with vehicle demonstrated the expected initial fall and subsequent return to normal in serum $1,25(OH)_2D$ concentration. (Note that because of pump limitations, animals in these experiments were only suspended for 12 days and therefore the serum concentration of $1,25(OH)_2D$ has not quite returned to normal by the last day of the experimental period.) Animals infused with $1,25(OH)_2D_3$ all had the same serum concentration of $1,25(OH)_2D$ (overall mean = 198 $\pm$ 9 pg/ml) regardless of whether they were suspended or not.

The serum concentration of Ca was uniformly increased in all $1,25(OH)_2D_3$ infused animals compared to vehicle infused animals (11.58 $\pm$ 0.12 mg/dl vs. vehicle infused of 10.26 $\pm$ 0.07 mg/dl, $P < .001$) and no transitory rise in serum calcium was observed at day 2 of suspension. The concentration of inorganic phosphorus in the serum was unaffected by $1,25(OH)_2D_3$ infusion and the same in both suspended and nonsupended animals (overall mean = 8.3 $\pm$ 0.01 mg/dl).

By day 12 of suspension, the Ca contents of the tibia (48.5 $\pm$ 1.6 mg) and lumbar vertebra (14.8 $\pm$ 0.6 mg) from vehicle infused suspended animals were significantly less ($P < .05$) than their pair-fed controls (54.8 $\pm$ 1.1 mg and 17.3 $\pm$ 0.7 mg) by 11.6% and 14.5% respectively (Fig. 3C and 3E). The Ca contents of the tibia and lumbar vertebra from $1,25(OH)_2D_3$ infused animals behaved in almost an identical manner when unloaded. By day 12 of suspension, the Ca contents of the tibia (49.0 $\pm$ 1.3 mg) and lumbar vertebra (15.5 $\pm$ 0.4 mg) from $1,25(OH)_2D_3$ infused suspended animals were significantly less ($P < .05$) than their pair-fed controls (60.4 $\pm$ 1.6 mg and 18.3 $\pm$ 1.1 mg) by 18.9% and 15.3% respectively.

Note that the fall in bone Ca appears to be more rapid in the animals depicted in Figure 3 than in Figure 1. Although the reason for this difference

is not clear, this kind of inter-experiment variation has been seen previously (unpublished results). The exact time at which the effects of unloading can be observed varies between 2 and 7 days.

Body weight, total tibial Ca and tibial Ca per unit body weight are given in Table II. Body weights of $1,25(OH)_2D_3$ infused animals, regardless of whether they were suspended or not, were significantly ($P<.001$) lower than vehicle infused animals (multivariate analysis). Total tibial Ca and total tibial Ca per unit body weight, on the other hand, were significantly ($P<.001$) higher in $1,25(OH)_2D_3$ infused than in vehicle infused animals (multivariate analysis). Total vertebral Ca (data not shown) was also higher in $1,25(OH)_2D_3$ infused animals.

Discussion

Skeletal unloading, whether in the adult or during periods of growth, alters bone metabolism in such a way as to decrease the mass of the unloaded bone. Although the sequence of events leading to this abnormality are unknown, the loss of bone following acute skeletal unloading in the adult or the inhibition of bone formation following unloading in the growing animal exhibit characteristic temporal patterns. Regardless of the means by which unloading is accomplished (casting, nerve/tendon section, bed rest), the pattern of bone loss in the adult is marked by an initial rapid decrease in bone mass followed by a period in which bone mass stabilizes and no further loss occurs. In the growing animal, the pattern of altered bone metabolism is marked by an initial inhibition of bone formation followed by a period in which bone formation returns to normal.

We observe the same pattern when skeletal unloading is accomplished using the suspended rat model. Unloading of the hind limbs of growing rats by tail suspension produces a transitory change in the rate of calcium accumulation in the unloaded bones (tibia and vertebra). This is seen first as an increasing difference in total bone Ca between loaded and unloaded bones, followed by a period in which the difference in Ca between loaded and unloaded bone stabilizes. This pattern is well illustrated by the lumbar vertebra in the experiment depicted in Fig. 1B and, although not as obvious in the tibia (Fig. 1A), has been clearly demonstrated in both bones of animals suspended for 4 weeks (15). After 2 weeks of suspension, both loaded and unloaded bones grow in mass and accumulate Ca at approximately the same rate.

The transitory dimunition in calcium accumulation in unloaded bones could be the result of a change in bone formation or resorption or both. The significant decrease in osteoblast numbers and longitudinal bone growth coupled with the absence of change in the osteoclast population suggest, that in the growing rat, skeletal unloading inhibits bone formation. This is consistent with previous studies from our laboratory demonstrating a significant inhibition of ^{3}H-proline and ^{45}Ca uptake into unloaded bones after 5-10 days of suspension (15). After 2 weeks of suspension, bone formation returns to normal as judged by uptake of ^{3}H-proline and ^{45}Ca (15), and bone histology (unpublished results). Landry and Fleisch observed the same phenomenon in nerve sectioned rats, although with a slightly different time course (3). As judged by bone weight and tetracycline uptake, bone formation was initially inhibited after nerve section, but returned to normal within 4 weeks. Klein et al. present further evidence to support the hypothesis that acute skeletal unloading in growing animals primarily influences bone formation (10).

Acute skeletal unloading in growing rats not only induces changes in bone metabolism but also vitamin D metabolism. In particular, the serum concentration of $1,25(OH)_2D$ falls dramatically within 5 days of unloading. Because this change in serum $1,25(OH)_2D$ appears to occur as rapidly or more rapidly than the changes in bone, it raises the question as to the cause and effect relationship between the changes in bone and vitamin D metabolism. For example, the cephlad fluid shift associated with skeletal unloading, as effected by suspension, may result in redistribution of serum Ca between bound and ionized forms and in turn change parathyroid hormone secretion (PTH) and vitamin D metabolism. Since $1,25(OH)_2D$ can increase osteoblast numbers (22-24), a fall in serum $1,25(OH)_2D$ could conceivably lead to a reduction in the osteoblast population, and in turn, bone formation. This sequence of events however is unlikely for two reasons. First, if the change in serum $1,25(OH)_2D$ concentration were inducing the bone changes, then all bones, loaded and unloaded, would be affected. This is not the case. The humerus (a loaded bone in our model system) from suspended rats, despite the change in serum $1,25(OH)_2D$, does not exhibit inhibition of bone formation (15). Second, if the fall in serum $1,25(OH)_2D$ were responsible for the inhibition of bone formation, then maintenence of a high constant serum concentration of $1,25(OH)_2D$ would be expected to prevent the changes. As seen from Figure 3, this does not occur.

More consistent with our observations is the hypothesis that acute skeletal unloading directly reduces bone formation which in turn influences vitamin D metabolism. A reduction in bone formation would reduce the demand for Ca from the serum pool. Because a major portion of the skeleton is unloaded in our model (both rear limbs, pelvis and the caudal part of the spinal column), an acute reduction in Ca flux from the serum pool into bone would be expected to cause a transitory rise in serum Ca. This in fact occurs (Fig. 2A). The rise

in serum Ca would be expected to decrease circulating PTH and the combined effects of a lower serum PTH concentration and hypercalcemia would in turn be expected to decrease renal production of $1,25(OH)_2D$ and therefore the serum concentration of $1,25(OH)_2D$. As bone formation returns to normal (day 10-15), the demand for Ca by the skeleton increases. The resulting Ca drain on the serum pool stimulates $1,25(OH)_2D$ production and a return to normal in the serum concentration of $1,25(OH)_2D$. This sequence of events is in fact supported by evidence from other laboratories. Stewart et al. have shown in patients immobilized by spinal cord injuries that the serum concentration of $1,25(OH)_2D$ 5-21 weeks after injury is significantly reduced (25). At the same time, urinary Ca excretion is increased, serum Pi and the renal phosphorus threshold are increased, and nephrogenous cAMP and serum immunoreactive PTH are decreased. Consistent with our results, the loss of bone Ca appears to trigger a fall in serum PTH and in turn a fall in serum $1,25(OH)_2D$.

The decrease in total bone Ca observed in animals chronically infused with $1,25(OH)_2D$ (Table II) is consistent with a report by Gallagher et al. indicating that chronic administration of $1,25(OH)_2D$ can increase trabecular bone volume (26). This phenomena is currently under further investigation.

In summary, our data suggest that acute skeletal unloading during periods of growth produces a transitory inhibition of bone formation, and a temporary reduction in the serum concentration of $1,25(OH)_2D$ but does not have a prominent effect on bone resorption. No evidence could be found for a direct involvement of $1,25(OH)_2D$ in the inhibition of bone formation due to unloading. On the contrary, the change in serum $1,25(OH)_2D$ appears to reflect a transitory decrease in the skeletal demand for Ca, a direct result of the inhibition of bone formation.

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FIGURE LEGENDS

Figure 1 Effect of acute skeletal unloading on the Ca content of the tibia (Fig. 1A) and lumbar (L-1) vertebra (Fig. 1B) of growing rats. The hind limbs of animals (6 per group) were unloaded (by tail suspension) for 2, 5, 7, 10, 12 and 15 days, and the Ca contents of the tibia and vertebra were compared to pair-fed nonsuspended control rats. Data are presented as per cent of control (mean ± SE). Total tibial Ca in the control groups was not significantly different (overall mean = 42.9 mg = 100%, Fig. 1A) nor was total vertebral Ca in the control groups (overall mean = 16.7 mg = 100%, Fig. 1B). [a] $p < 0.05$, [b] $p < 0.005$, Students t-test for comparison between suspended and control groups, Bonferroni method.

Figure 2 Effect of acute skeletal unloading on the serum concentrations of Ca (Fig. 2A), $1,25(OH)_2D$ (Fig. 2B), and $24,25(OH)_2D$ (fig. 2C) of growing rats. The hind limbs of animals (6 per group) were unloaded (by tail suspension) for 2, 5, 7, 10, 12, 15 days, and the serum concentrations of Ca, $1,25(OH)_2D$ and $24,25(OH)_2D$ compared to pair-fed control rats. No differences in serum Ca, $1,25(OH)_2D$ or $24,25(OH)_2D$ were seen between the control groups. For this reason, these data were combined to form an overall control (represented by the respective values at day 0 of suspension). All data are presented as mean ± SE. [a] $p < 0.01$, [b] $p<.05$, Students t-test for comparison between suspended and control groups, Bonferroni method.

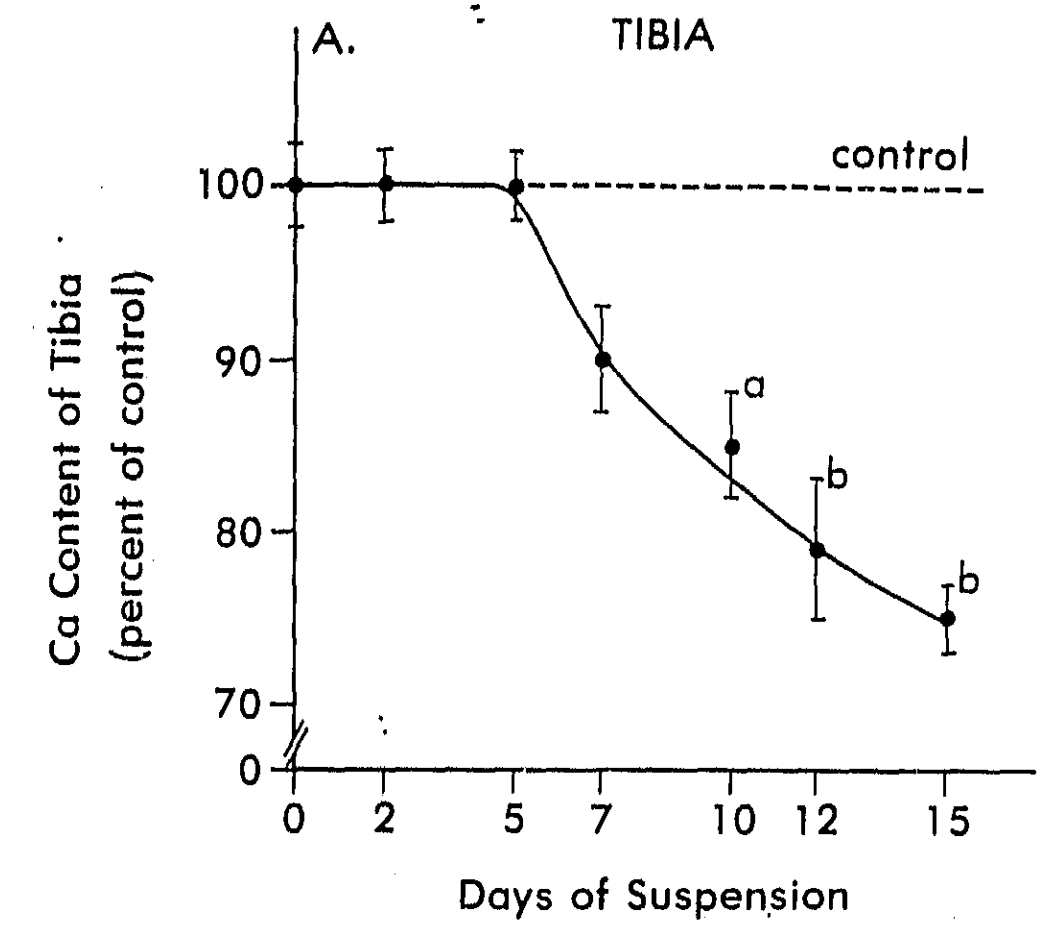
Figure 3 Effect of acute skeletal unloading on the serum concentrations of $1,25(OH)_2D$ (Fig. 3A, B) and on the Ca contents of the tibia (Fig. 3C, D) and lumbar vertebra (Fig. 3E, F) of growing rats infused with either vehicle (Fig. 3B, D, F), or $1,25(OH)_2D_3$ (75 pmole/d, Fig. 3A, C, E) The hind limbs of animals (6 per group) were unloaded by tail suspension for 2, 5, 8 and 12 days. Data (mean $\pm$ SE) are presented as in Figures 1 and 2. Total tibial and vertebral Ca in control groups were not significantly different. Overall mean tibial Ca in vehicle infused and $1,25(OH)_2D_3$ infused control animals were 55.8 mg (100%, Fig. 3C) and 61.0 mg (100%, Fig. 3D) respectively. Overall mean vertebral Ca in vehicle infused and $1,25(OH)_2D$ infused control animals were 17.3 mg (100%, Fig. 3E) and 18.3 mg (100%, Fig. 3F) respectively. [a] $p < 0.05$, [b] $p < .01$, [c] $p < 0.005$, Student's t-test for comparison between suspended and control groups, Bonferri method.

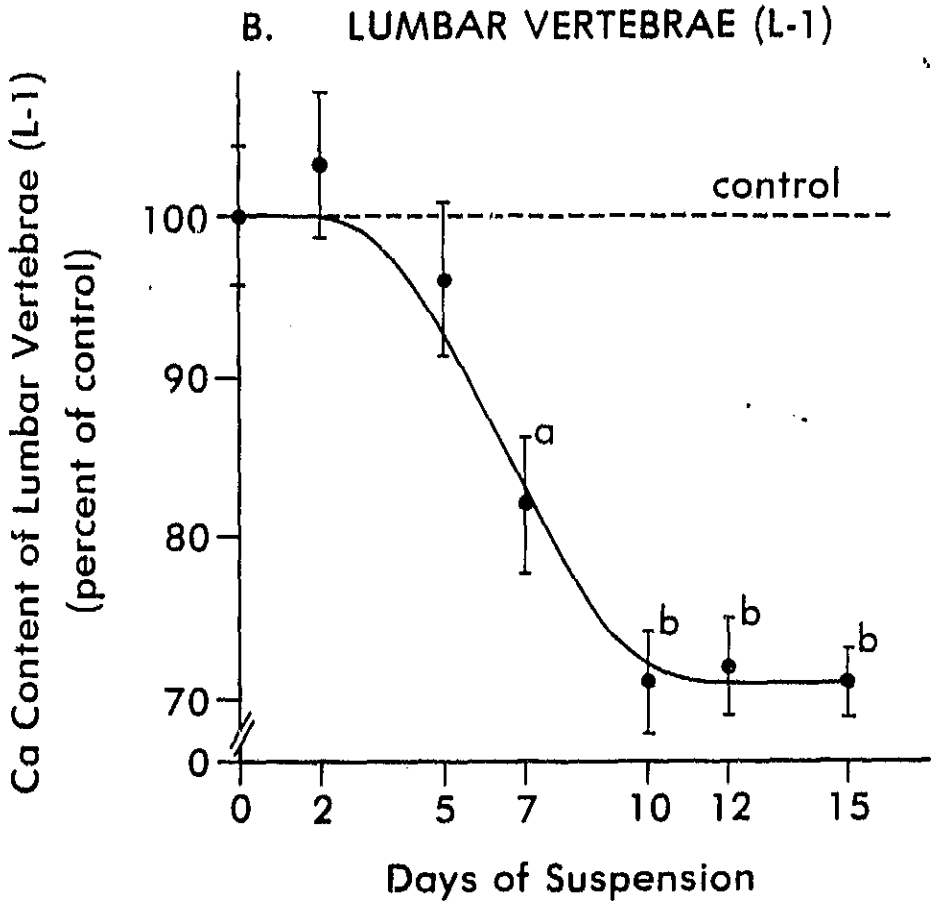
TABLE II. Effect of suspension and chronic $1,25(OH)_2$ D infusion on body weight and tibial Ca.

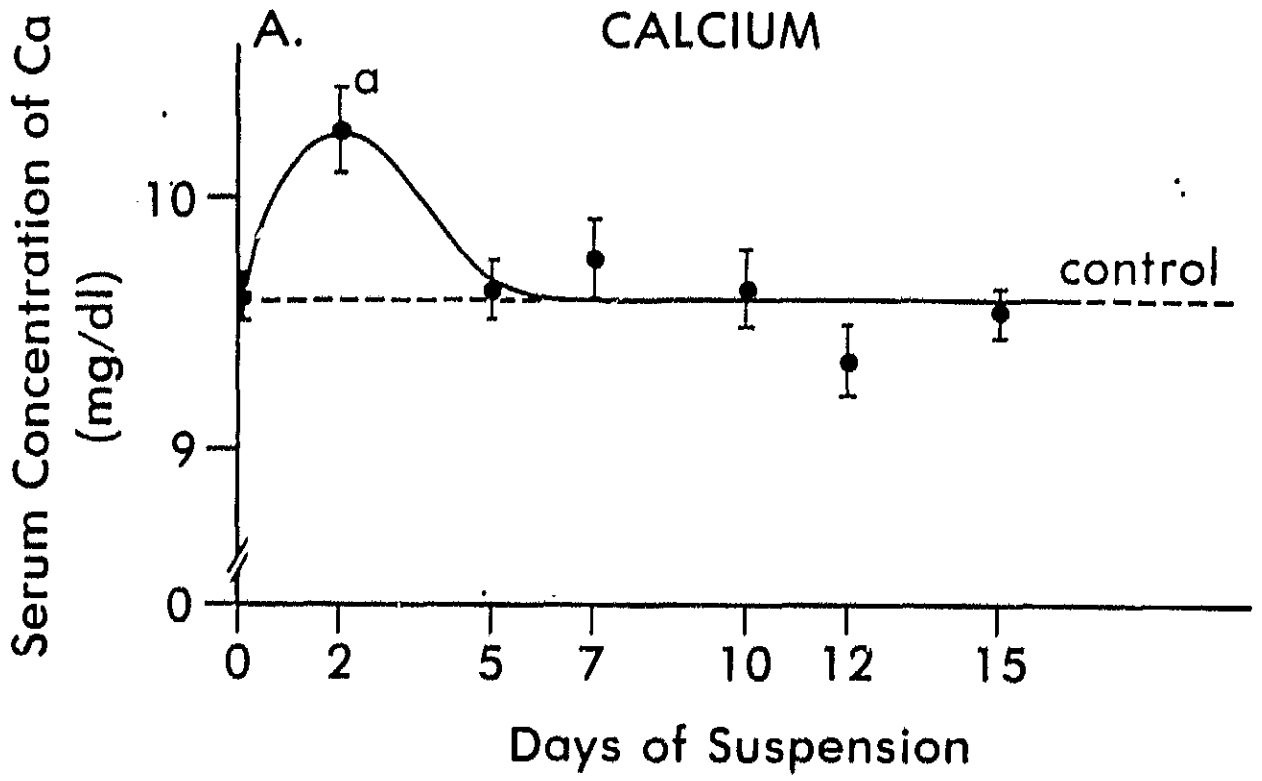
Group	Vehicle Infused			$1,25(OH)_2D_3$ Infused		
	Body weight (g)	Total bone Ca (mg)	Bone Ca / Body weight (mg/g)	Body weight[e] (g)	Total bone[e] Ca (mg)	Bone Ca[e] / Body weight (mg/g)
Control 2/5 day	251 ± 9	56.9 ± 2.3	.226 ± .005	247 ± 9	61.6 ± 1.7	.251 ± .011
Suspended 2 day	258 ± 6	54.3 ± 1.3	.211 ± .006	240 ± 6	58.5 ± 1.1	.236 ± .009
Suspended 5 day	256 ± 10	51.7 ± 0.8	.203 ± .006[a]	242 ± 9	56.1 ± 2.3	.233 ± .015
Control 8/12 day	239 ± 7	54.8 ± 1.1	.230 ± .006	225 ± 8	60.4 ± 1.6	.269 ± .004
Suspended 8 day	248 ± 6	49.3 ± 1.3[c]	.199 ± .007[b]	224 ± 8	51.6 ± 1.7[d]	.232 ± .010[b]
Suspended 12 day	241 ± 7	48.5 ± 1.6[c]	.203 ± .012	220 ± 7	49.0 ± 1.3[d]	.223 ± .009[c]

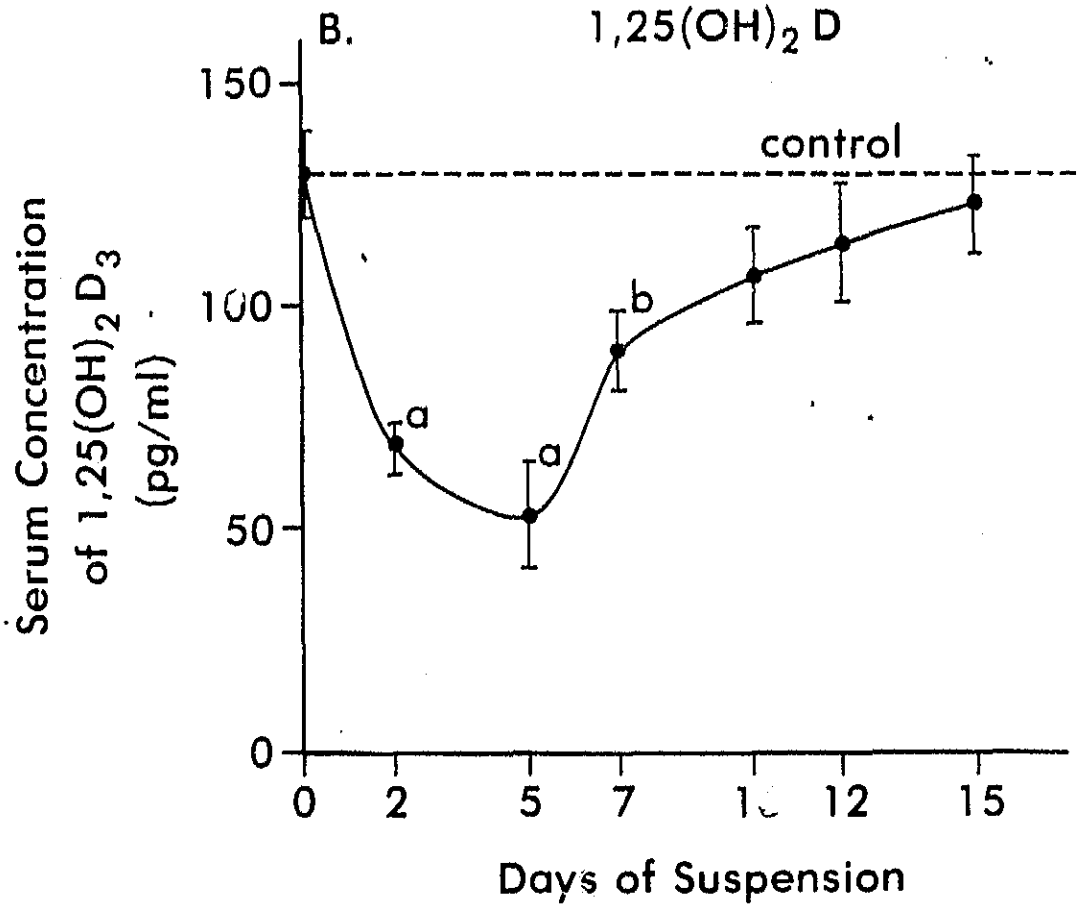
[a] $P < .05$, [b] $P < .02$, [c] $P < .01$, [d] $P < .005$, compared to control, Student's t-test, Bonferroni method

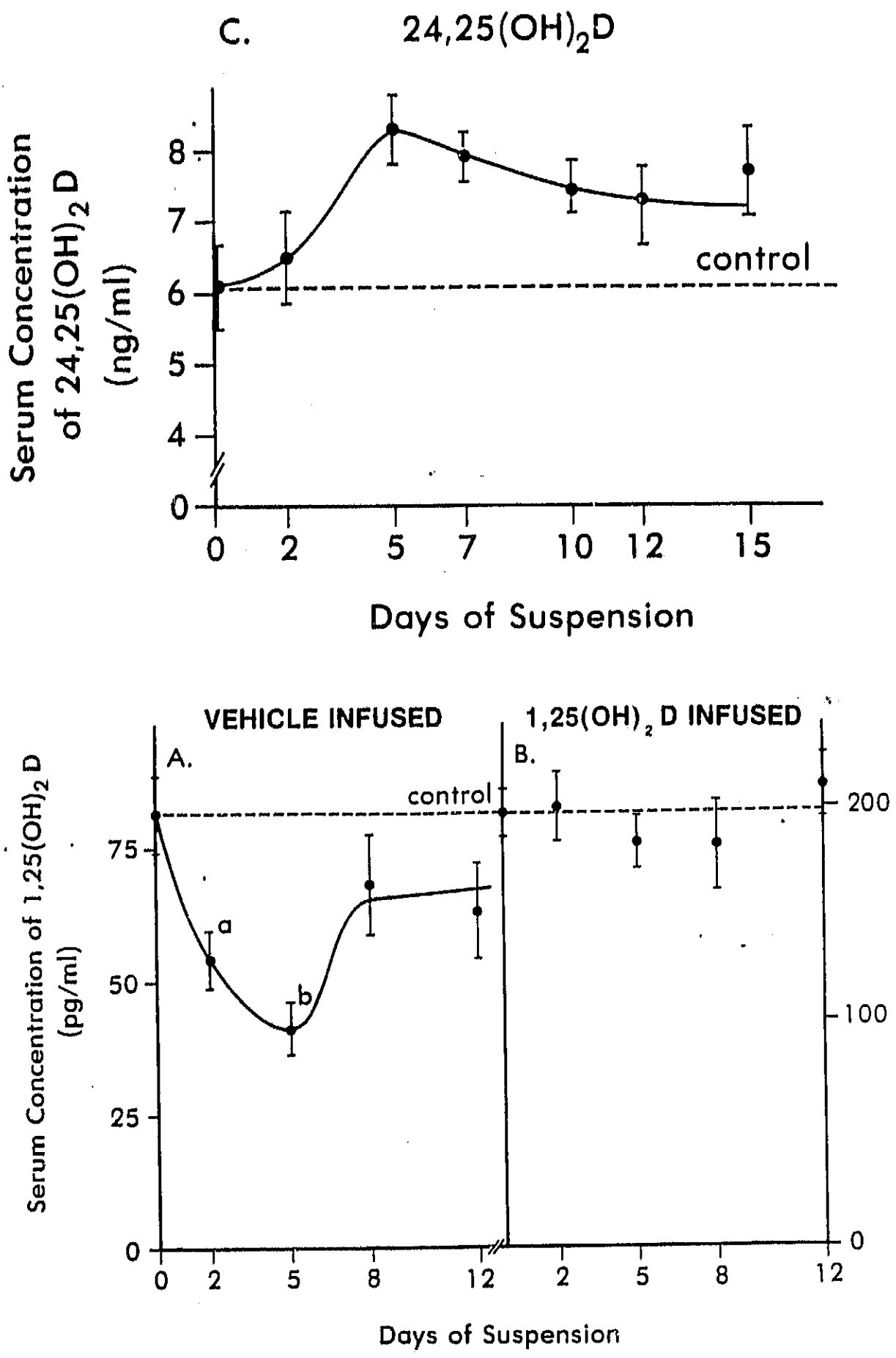
[e] Body weight, total bone Ca and bone Ca/body weight were significantly ($P < .001$) different in $1,25(OH)_2D_3$ infused animals when compared to vehicle infused animals (multivariate analysis).


A.
TIBIA
control
100
90
80
70
0
a
b
b
Ca Content of Tibia
(percent of control)
0
2
5
7
10
12
15
Days of Suspension


B.
LUMBAR VERTEBRAE (L-1)
control
100
90
80
70
0
a
b
b
b
Ca Content of Lumbar Vertebrae (L-1)
(percent of control)
0
2
5
7
10
12
15
Days of Suspension


A.
CALCIUM
Serum Concentration of Ca
(mg/dl)
10
9
0
a
control
0
2
5
7
10
12
15
Days of Suspension


B.
1,25(OH)2 D
Serum Concentration
of 1,25(OH)2 D3
(pg/ml)
150
100
50
0
control
a
a
b
0
2
5
7
12
15
Days of Suspension


C.
24,25(OH)2D
Serum Concentration of 24,25(OH)2D (ng/ml)
8
7
6
5
4
0
control
0
2
5
7
10
12
15
Days of Suspension
VEHICLE INFUSED
1,25(OH)2D INFUSED
A.
B.
control
75
50
25
0
a
b
200
100
0
Serum Concentration of 1,25(OH)2D (pg/ml)
0
2
5
8
12
2
5
8
12
Days of Suspension