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EFFECTS OF LONG-TERM VITAMIN D DEFICIENCY AND RESPONSE TO VITAMIN D REPLETION IN THE MATURE AND AGING MALE AND FEMALE RAT

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SUMMARY

This study investigated the sex- and age-related alterations in calcium homeostasis in 39- to 82-week-old rats raised from weaning on a vitamin D deficient (–D) diet. It was found that vitamin D deprivation decreased the life span of male, but not female, rats. Female –D animals exhibited a steady increase in serum calcium with age from 39 to 82 weeks, although circulating calcium of –D animals never reached normocalcemic levels. There was no attenuation of the secondary hyperparathyroidism. Serum calcium of –D males was significantly lower than that of age-matched females at all ages when sufficient males were alive to make the comparison. Serum parathyroid hormone levels were decreased in –D females when serum clacium was elevated to hypercalcemic levels by calcium injection. Similarly, administration of vitamin D_3 or 1,25-dihydroxyvitamin D_3 elevated serum calcium and depressed parathyroid hormone in 14- and 22-month-old –D females. These animals also exhibited increased intestinal calcium binding protein content. Administration of vitamin D_3 or dihydroxyvitamin D_3 repaired renal adenylate cyclase refractoriness to parathyroid hormone. The sex- and diet-related alterations in serum phosphorus that were found at earlier ages disappeared by 67 weeks of age. Serum calcitonin was elevated in mature and aging +D males and females and –D females relative to younger animals. In –D males, calcitonin levels were less markedly elevated. The results of this study indicate that there are several important sex differences related to the regulation of calcium homeostasis in mature and aging rats. In addition, it was found that mature (14 month) and old (22 month) chronically –D female rats were able to respond to repletion with dihydroxyvitamin D_3 or vitamin D within 10 days.

Key words: Vitamin D repletion; Serum calcium; Serum iPTH; Renal adenylate cyclase; Aging rat

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INTRODUCTION

Much of the information gained about the physiologic role of vitamin D and its metabolites in the regulation of mammalian calcium homeostasis has been obtained using 8- to 10-week old male rats raised from weaning on vitamin D deficient (−D) diets [1–3], or older male rats deprived of vitamin D for relatively short periods of time [4]. The major exceptions are recent studies from this laboratory [5], and by Halloran *et al.* [6,7] and Boass *et al.* [8] on the effects of vitamin D deficiency during pregnancy and lactation. None of this work has addressed the possibility of sex differences in either the response to vitamin D deficiency or the requirement for vitamin D in maintenance of calcium homeostasis.

Information of this nature is necessary if we are to develop a complete picture of regulation of calcium homeostasis in both sexes. Similarly, it is unknown whether animals chronically deprived of vitamin D are able to sustain a normal life-span, or what alterations occur in calcium-regulatory processes during long-term vitamin D deficiency. As an initial approach to these questions, in the present study serum calcium, phosphorus, immunoreactive (i) parathyroid hormone (PTH), calcitonin (CT), and survivability data have been obtained from male and female rats from 39 to 82 weeks of age. During this period of time, maturation is complete, and rats are undergoing senescence and death. The −D animals in this study were raised from weaning on a vitamin D deficient diet and maintained for up to 22 months.

Since one of the characteristics of aging is the loss of hormone responsiveness, we have also studied the ability of −D females 14 and 22 months of age to respond to repletion with vitamin D_3 and/or 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. In the repletion studies, serum calcium, iPTH and iCT were measured. In addition, renal PTH-dependent adenylate cyclase and intestinal calcium-binding protein were measured, because renal adenylate cyclase is known to become refractory to activation by PTH in vitamin D deficient animals [3], and intestinal calcium-binding protein content is decreased in vitamin D deficiency as well as with age [9]. Therefore, measurement of these two parameters was undertaken to determine the ability of kidney and intestine of aging, chronically D deficient animals to respond to vitamin D_3.

MATERIALS AND METHODS

Animals

Weanling rats obtained from the Holtzman Company, Madison, WI, were placed on a diet containing no vitamin D, 0.47% calcium, 0.35% phosphorus and 0.04% magnesium. This diet, containing 45% corn starch, 25% dextrose, and 18% casein, was formulated in our laboratory. The animals were divided into four groups: −D females, +D females, −D males and +D males. The +D groups were given 70 IU vitamin D (cholecalciferol) in 50 μl of propylene glycol orally, twice each week. All animals were housed individually in stainless steel cages with a 12-h light/dark cycle free of ultraviolet light and allowed free access to the diet and deionized water.

Vitamin D repletion of long-term −D animals was carried out by administration of either 0.15 μg/kg $1,25(OH)_2D_3$ or 0.75 μg/kg vitamin D_3 daily for 10 days by subcutaneous injection. The goal of the repletion studies was to determine whether vitamin D_3 and $1,25(OH)_2D_3$ would repair the effects of vitamin D deficiency in aging animals. This repletion protocol of daily administration of relatively low doses was designed to eliminate some of the problems resulting from differences in metabolism of the two compounds. It does not permit comparison of the relative potency, or time course of response.

Serum determination

Blood samples were obtained under light ether anesthesia from the orbital sinus. All blood samples were obtained between 0900 and 1100 h. Serum calcium and magnesium concentrations were determined by flame spectrophotometry (Perkin-Elmer, Norwalk, CT). The serum was diluted in LaCl to eliminate interference by phosphate. Serum phosphorus was measured by colorimetric assay (Rapid-State Kit, Pierce Chemical Co., Rockford, IL). Serum iPTH was measured using a non-equilibrium radioimmunoassay modified as described from the method Conaway and Anast [10]. The standard curve used in these experiments was made by dilution of pooled sera from vitamin D deficient rats containing a high level of iPTH. Multiple dilutions of individual samples gave standard curves parallel to that of the standard pooled serum. The lower limit of detectability of this assay was 1.4 μl equivalents per ml of serum. The antiserum was chicken anti-bovine PTH (CH977) which was raised in this laboratory. Each sample was assayed in duplicate 100 μl aliquots. A third 100 μl aliquot was assayed in the absence of antiserum to correct for nonspecific binding. Bound and free hormone were separated with Dextran-coated charcoal. Serum iCT was measured by radioimmunoassay [11] using a goat antibody to human CT, and human CT standards. The lower limit of detectability of this assay was 100 pg/ml.

Renal adenylate cyclase activity

Kidneys were removed under ether anesthesia. The tissue from 3 or 4 animals in each group was pooled and homogenized in 3 ml of 0.25 M sucrose, 10 mM Tris–HCl, pH 7.4, and 1 mM EDTA per g of kidney using a Teflon pestle and glass tube. The homogenate was processed according to the method of Fitzpatrick *et al.* [12] for preparation of rat kidney plasma membranes. Two different membrane preparations were prepared from each group of animals. Adenylate cyclase activity of these membrane preparations was assayed in the absence (basal) or presence of bovine PTH(1–34) as previously described [3] using the two-step chromatographic procedure of Salomon *et al.* [13] to separate the product ^{32}P-labeled cyclic AMP from other ^{32}P-labeled compounds in the reaction mixture. The data are expressed as specific enzyme activity in nmoles cyclic AMP formed per mg membrane protein after 15 min incubation at 30°C in a Dubnoff shaking incubator. Under these conditions, the formation of cyclic AMP continued at a linear rate for up to 20 min using 50–100 μg of protein. A maximally activating PTH concentration (10^{-6} M) was used. Membrane protein was determined using the Lowry [14] method. PTH-dependent adenylate cyclase activity was calculated by subtracting the basal activity from the total PTH-stimulated activity of the same membrane preparation.

Intestinal calcium-binding protein

Calcium-binding protein was assayed by single radial immunodiffusion using antiserum provided by Dr. Elizabeth Bruns (Department of Pathology, University of Virginia Medical Center, Charlottesville, VA). To prepare the sample, approximately 10 cm of intestine were removed from ether-anesthetized rats starting from the pylorus. The segments were rinsed in cold saline, everted and trimmed so that the first 5 cm distal to the pylorus were used. The mucosa was scraped from this segment with two glass slides, the scrapings washed into 2 ml of buffer (10 mM Tris–HCl, 5 mM benzamidine, 0.04% mercaptoethanol, 0.5% Triton X-100, pH 7.2) and homogenized by 20 strokes of a Teflon pestle. The homogenate was centrifuged at 24 000 *g* for 30 min, and the supernatant removed and frozen at −70°C until assayed for protein and calcium-binding protein. The radial immunodiffusion assay for calcium-binding protein with this preparation has been previously described [15]. In brief, diffusion was carried out in 0.8% agar containing diluted antiserum at room temperature, and equilibrium was attained by 18 h. A linear relationship was obtained between the area of the circular immunoprecipitate minus the area of the well and the calcium-binding protein concentration (5–37 μg/ml). Interassay variability of identical samples is 12.6% using this method [9,15].

Statistical significance of differences between means were calculated using Student's *t* test. The Mann-Whitney test was used to determined the level of significance of differences between hormone concentrations of different groups [16]. Undetectable points were assigned the value of the limit of detectability of the assay.

RESULTS

Effects of aging

Longevity. The incidence of mortality in the male −D group of rats was higher than in the corresponding −D females, and this trend was particularly evident in animals older than 45 weeks of age (Fig. 1). By 80 weeks, all of the −D males (original 20 −D females and 20 −D males) had died, while 55% of the −D females were still alive. In an earlier study, it was found that −D males between 12 and 18 weeks of age also had a high death rate [17] accounting for the fact that only 50% of these rats were alive at 30 weeks in the present study. The data in Fig. 1 indicate that survivability with age is similar for +D males and females and −D females. By 80 weeks of age, approximately 50% of the animals in each of these groups were still living, while all of the −D males were dead. Necropsy was performed by the Department of Veterinary Pathology at the University of Missouri School of Veterinary Medicine on some of the animals from each group that died at various ages throughout the study. Although this was not done on a systematic basis, the cause of death was never clear in any of the necropsied animals. There was no correlation between growth rate, size, or serum calcium and mortality. As we reported earlier [17], the −D male animals under our protocol grew much more slowly than their +D counterparts. Growth in −D females was not severely reduced.

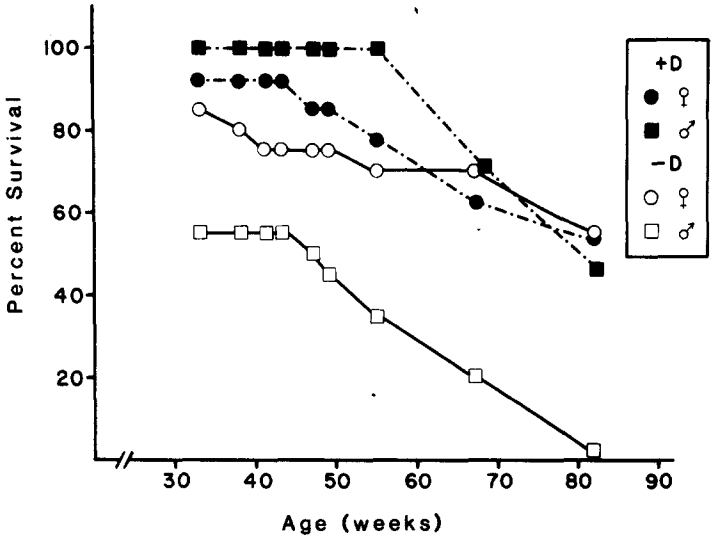

Fig. 1. Survival of male and female +D and −D rats from 33 to 82 weeks of age. There were 20 animals in each group when the dietary protocol was initiated at weaning (3 weeks of age).

Therefore, unlike +D rats there was not a major sex difference in the size of −D animals at any given age.

Serum calcium. Serum calcium increased with age in −D female rats from a mean of 5.35 ± 0.05 mg/dl at 39 weeks of age to 6.98 ± 0.53 mg/dl at 82 weeks (Fig. 2). As

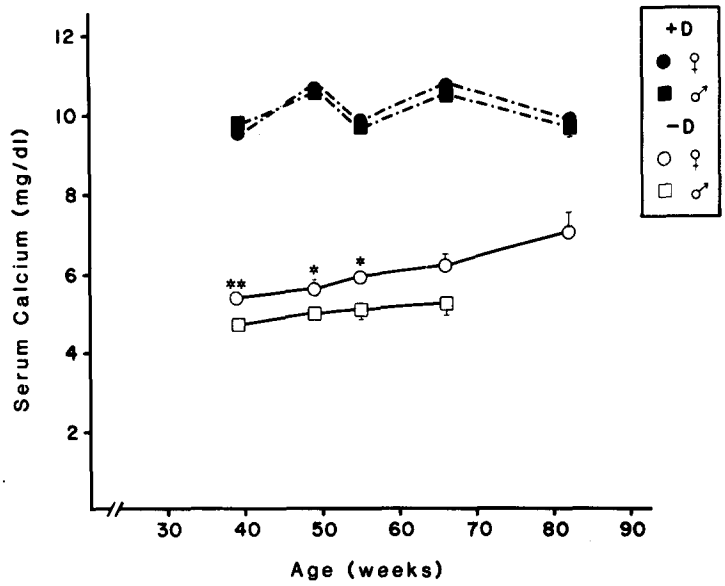

Fig. 2. Serum calcium of +D and −D male and female rats from 39 to 82 weeks of age. Each point represents the mean of 4–10 animals. At points where the S.E.M. extended beyond the symbol, it is indicated by vertical line. $*p < 0.05$, $**p < 0.01$ for the difference between male and female −D animals.

reported earlier [17], the serum calcium nadir of 4.7 mg/dl was reached at 7 weeks of age for both −D males and females, and the slow, steady increase in serum calcium shown by −D female rats in this study appeared to have begun between 19 and 23 weeks of age [17]. Serum calcium did not change with age in +D animals of either sex. Due to the high mortality of −D males, it was difficult to determine whether serum calcium increased with age. However, serum calcium was significantly higher in −D females than males at 40 and 55 weeks of age ($p < 0.01$ and $p < 0.05$, respectively). When serum calcium of −D females was compared with age, there was a positive correlation ($r = 0.58$, $p < 0.001$).

Serum phosphorus. With increasing age, the differences in circulating levels of phosphorus which were reported to occur between sexes and between +D and −D diet groups [17] disappeared (Fig. 3). By 67 weeks there was no significant difference in serum phosphorus between any of the groups.

Serum iPTH. Serum iPTH did not change with age in either the +D or the −D animals (Fig. 4). The circulating iPTH of −D animals remained elevated to the same extent achieved by 7 weeks of age (4 weeks of vitamin D deprivation) [17]. In order to determine whether the hypersecreting parathyroid glands from long-term −D rats would still respond to hypercalcemia, 55-week-old −D males and females were injected with 12 mg

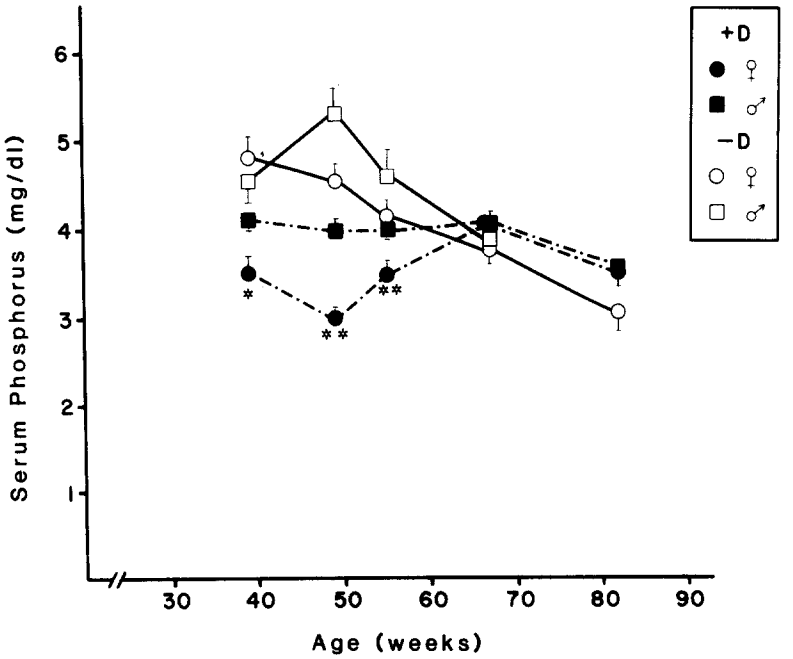

Fig. 3. Serum phosphorus of +D and −D male and female rats from 39 to 82 weeks of age. Each point represents the mean 4–10 values. At points where the S.E.M. extended beyond the symbol, it is indicated by a vertical line. $*p < 0.05$, $**p < 0.01$ for sex differences in +D animals at each age. There were no significant sex differences in phosphorus of −D animals from 39 to 67 weeks.

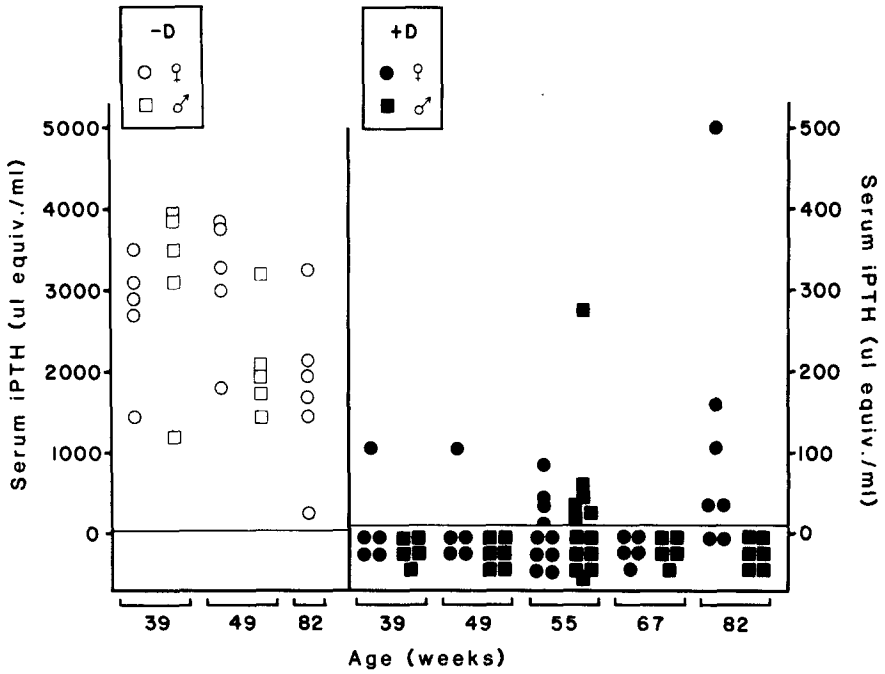

Fig. 4. Serum iPTH of male and female +D and −D animals from 39 to 82 weeks of age. The difference between iPTH of male and female +D animals at 82 weeks ($p < 0.01$) was the only statistically significance sex difference at any age in either group.

of calcium (as $CaCl_2$) via the tail vein. Blood was collected from each animal prior to the injection as well as 10 and 60 min following, and calcium and iPTH were measured (Fig. 5). By 10 min following injection, serum calcium increased to hypercalcemic levels, and iPTH decreased to near control (+D) levels. By 60 min following the injection of calcium, serum iPTH had returned to preinjection levels, and serum calcium had declined, but remained elevated relative to preinjection levels. Calcium injection also elevated iCT above the measurement range of our assay in +D and −D animals of both sexes (data not shown).

Serum iCT. Serum iCT levels were high relative to younger animals [17] in male and female +D and −D rats (Fig. 6). Female −D rats had higher iCT than male −D animals, but this sex difference did not occur in +D rats older than 39 weeks.

Repletion studies

Three groups of female rats were placed on the vitamin D deficient diet at weaning. At the time of the experiment there was one group at each of 22, 14, and 2 months of age. The ability to respond to vitamin D_3 and $1,25(OH)_2D_3$ was tested in the 22-month-old −D animals. Due to lack of 14-month-old −D animals, repletion with $1,25(OH)_2D_3$, but not D_3, was carried out in this group.

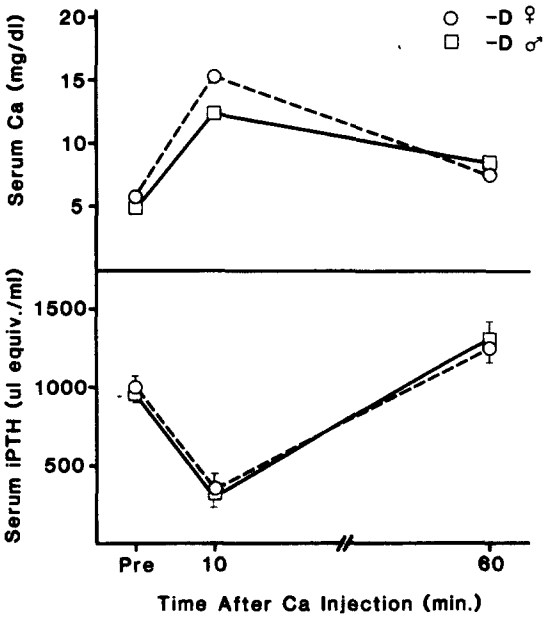

Fig. 5. Serum calcium and iPTH following intravenous injection of $CaCl_2$ (12 mg calcium per rat) in male and female —D rats 55 weeks old. Where the S.E.M. extended beyond the symbol, it is indicated by a vertical line. Each point represents the mean of 4 values.

Serum calcium. The data for serum calcium in the three —D age groups shown in Table I re-emphasize the greater than 2 mg/dl increase in serum calcium with age in —D female rats which was found in the longitudinal aspect of the study. However, in this experiment there was no difference in serum calcium between the two different groups of —D rats 14 and 22 months of age. Repletion with $1,25(OH)_2D_3$ produced hypercalcemia in 14-month-old and, to a lesser extent, 22-month-old —D females. Repletion with vitamin D_3 produced normocalcemia in the 22-month-old animals.

Intestinal calcium-binding protein. Intestinal calcium-binding protein was measured as an index of intestinal responsiveness to $1,25(OH)_2D_3$ (Table II). As previously reported for male rats [18], intestinal calcium-binding protein levels in +D females decreased between 2 and 14 months of age and remained at the low level at 22 months. The content was much lower in intestine from —D than +D animals at 2 and 14 months and declined further by 22 months. Repletion with $1,25(OH)_2D_3$ produced an approximately 10-fold increase in intestinal calcium-binding protein of both 14- and 22-month-old animals. The intestinal content of $1,25(OH)_2D_3$-repleted 14-month-old rats was 16.7 μg/mg protein compared to 4 μg/mg in 22-month-old animals. Repletion of 22-month-old animals with vitamin D_3 increased calcium-binding protein to the same extent as $1,25(OH)_2D_3$ repletion.

Renal adenylate cyclase activity. Renal PTH-dependent adenylate cyclase activity was similar in +D animals at all three ages tested (Table III). The renal adenylate cyclase

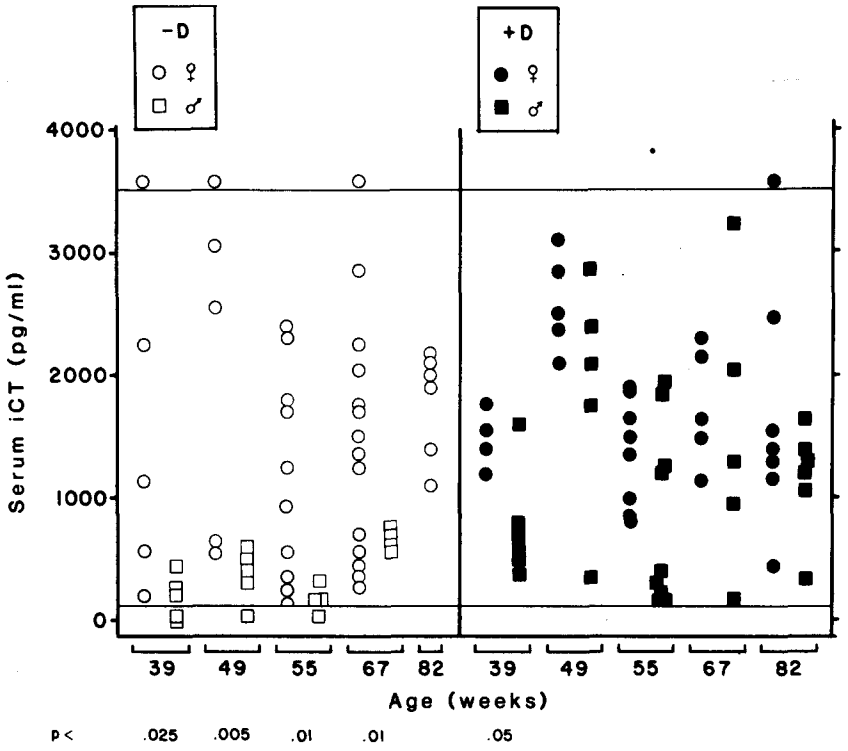

Fig. 6. Serum iCT of +D and –D male and female rats from 39 to 82 weeks of age. Levels of statistical significance for sex differences are indicated below each age.

TABLE I

SERUM CALCIUM: EFFECTS OF REPLETION WITH VITAMIN D_3 AND $1,25(OH)_2D_3$ IN 14- AND 22-MONTH-OLD –D FEMALES

Female rats were placed on the –D diet at weaning. Repletion was carried out by daily subcutaneous injections for 10 days of either 0.15 μg/kg $1,25(OH)_2D_3$ or 0.75 μg/kg vitamin D_3. Each mean ± S.E.M. was obtained from 4–6 animals.

	Serum calcium (mg/dl)		
	2-month-old	*14-month-old*	*22-month-old*
+D (mean ± S.E.M.)	9.8 ± 0.13	9.7 ± 0.06	9.8 ± 0.2
–D	4.7 ± 0.18	6.9 ± 0.77*	7.0 ± 0.53*
–D + $1,25(OH)_2D_3$	–	12.4 ± 0.42**	11.0 ± 0.53**
–D + D_3	–	–	9.5 ± 0.11

*$p < 0.01$ vs. 2-month-old –D.
**$p < 0.05$ vs. age-matched +D.

TABLE II

INTESTINAL CALCIUM-BINDING PROTEIN CONTENT OF +D, −D, AND −D REPLETED FEMALE RATS

Intestinal supernate preparations were pooled from the groups of animals described in Table I. Calcium-binding protein content of each pooled supernate was determined by radial immunodiffusion.

	Calcium-binding protein (μg/mg protein)		
	2-month-old	*14-month-old*	*22-month-old*
+D	8.3	2.96	2.43
−D	1.5	1.49	0.43
−D + $1,25(OH)_2D_3$	–	16.7	3.99
−D + D_3	–	–	4.42

response to PTH was markedly diminished in −D animals of all three ages. Repletion with $1,25(OH)_2D_3$ also increased PTH-dependent adenylate cyclase toward +D levels in 14- and 22-month-old animals.

Serum iPTH and iCT. Circulating levels of iPTH were elevated in −D animals in each age group. Repletion with vitamin D_3 or $1,25(OH)_2D_3$ returned PTH to +D levels (Fig. 7).

As seen in the prospective study (Fig. 5), iCT levels also increased with age in this experiment (Fig. 8). The 14-month-old −D animals had lower iCT than age-matched +D animals, but there was no difference in iCT of +D and −D animals 2 or 22 months old. When −D animals 14 or 22 months old were repleted with vitamin D_3 (22-month) or $1,25(OH)_2D_3$, iCT levels became elevated relative to the age-matched −D groups.

TABLE III

PTH-DPENDENT RENAL ADENYLATE CYCLASE ACTIVITY FOLLOWING VITAMIN D-REPLETION IN 14- AND 22-MONTH-OLD −D FEMALE RATS

From each group of rats described in Table I, two different renal particulate fractions were prepared and each was assayed in triplicate. Basal activity for each pool was subtracted from the activity measured in the presence of 10^{-6} M PTH to yield PTH-dependent activity. S.E.M. cannot be calculated for these values. Within each group, S.E.M. (calculated from $n = 6$; three determination from each of two samples of pooled kidneys) of basal and PTH-stimulated adenylate cyclase activities was always less than 10% of the corresponding mean.

	PTH-Dependent adenylate cyclase activity (pmol cyclic AMP per mg protein per 15 min)		
	2-month-old	*14-month-old*	*22-month-old*
+D	1180	1006	1378
−D	198	345	452
−D + $1,25(OH)_2D_3$	–	864	728
−D + D_3	–	–	1083

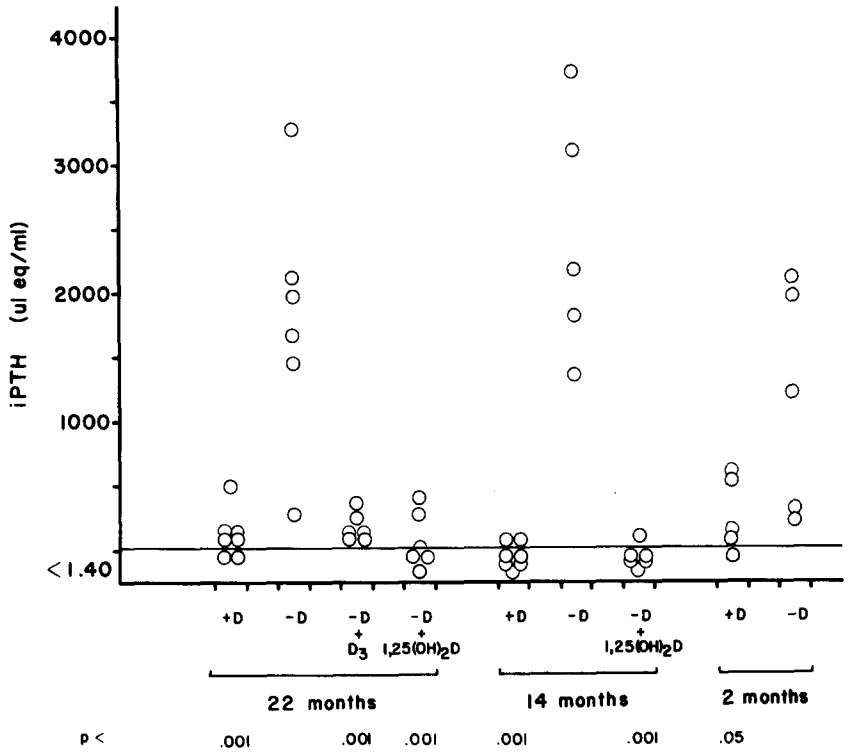

Fig. 7. Serum iPTH in female rats 22, 14, or 2 months' old. See Table I. Below the graph, p values are indicated for the difference from age-matched −D animals.

DISCUSSION

Several sex differences were exhibited during the course of this experiment. Vitamin D deficiency decreased the life span of male, but not female, rats. Serum calcium was higher in −D females than −D males at all ages in this study. In addition, −D females had higher circulating iCT levels than age-matched males. However, the sex difference in serum phosphorus which has been reported in younger animals [17] was found in the present study to disappear with age in both diet groups.

The decreased life span of −D male rats was quite striking. By 82 weeks of age, all of the male rats raised from weaning on the −D diet had died. However, there was no apparent difference in the mortality rate of −D females, +D males, and +D females up to 82 weeks of age. We reported earlier that male −D rats underwent a period of high mortality between 10 and 18 weeks of age [17]. In the present study, we have found that there was a second period of high death rate in −D male rats beginning after 40 weeks of age. This second period of high mortality corresponds to an increased death rate among −D females as well as +D animals of both sexes. The decreased life span of −D males is therefore probably due to the earlier period (10 to 18 weeks) of high

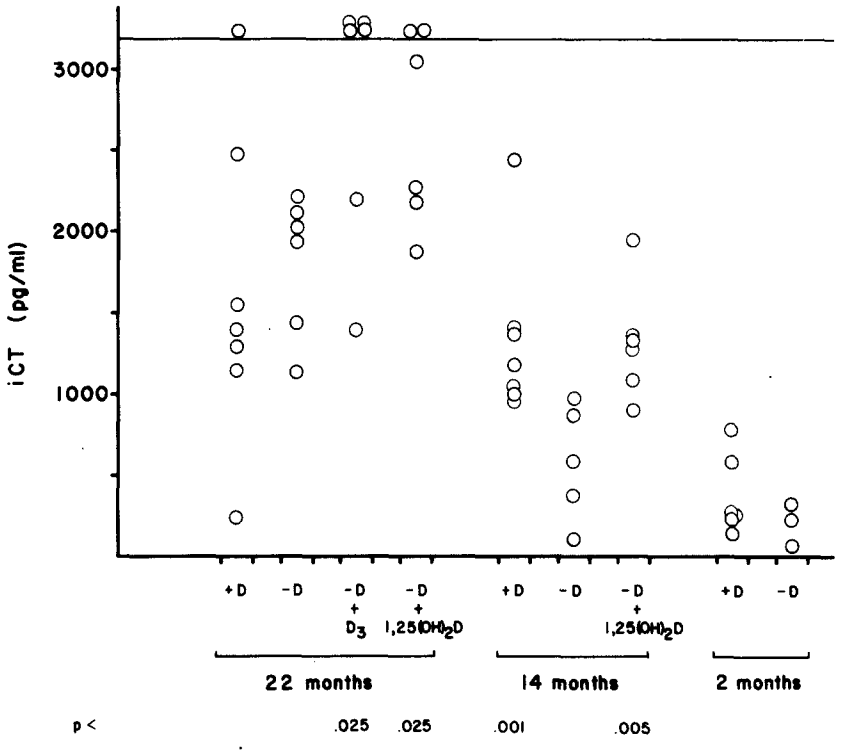

Fig. 8. Serum iCT in female rats 22, 14, or 2 months old. Horizontal line indicates the upper limits of the assay. See legend for Fig. 7.

mortality. Throughout this study, care was taken to insure that all of the animals were equally exposed to pathogens, environmental stress, potential dietary contaminants, *etc.* All of the animals were obtained from Holtzman as weanlings at the same time, continuously housed in the same room, fed the same diet and underwent the same amount of handling. Therefore, the two variables most likely to influence mortality in this study were vitamin D status and sex. However, since no cause of death was clear at necropsy, we have no data to indicate how sex and vitamin D status were interrelated to decreased life span in the −D male.

The steady increase in serum calcium of −D females with age suggests that the relative requirement for vitamin D in maintenance of serum calcium may decrease with sexual maturity in the female rat. We have found that serum calcium increased during pregnancy in this same vitamin D deficient rat model [5]. Vitamin D independent mechanisms to increase calcium mobilization from bone during lactation [6] and from intestine during pregnancy [7] and lactation [8] have been reported by others. The data from the present study in conjunction with these findings in pregnant and lactating rats suggest the possibility that female endocrine factors present in the cycling rat and increased during pregnancy and lactation function to elevate serum calcium without working through vitamin

D mediated processes. Pahuja and DeLuca [19] have shown that prolactin has a direct effect to increase active intestinal transport of calcium in vitamin D deficient male rats which results in elevated serum calcium. The role of endogenous prolactin in regulating calcium homeostasis in the normal and −D female remains to be explored.

It was not surprising that the secondary hyperparathyroidism of −D females was not alleviated as serum calcium rose with age since the calcium levels never reached the range of normocalcemia. Injection of $CaCl_2$ into −D rats elevated serum calcium markedly and suppressed iPTH levels, indicating that, in spite of hypersecretion for nearly a year, the parathyroid glands of −D animals could be acutely suppressed by circulating calcium. In addition, repletion of −D females for 10 days with either $1,25(OH)_2D_3$ or vitamin D_3 reduced iPTH to control (+D) levels. The $1,25(OH)_2D_3$-treated animals became slightly hypercalcemic relative to controls while the vitamin D treated rats became normocalcemic. Therefore, the hormone secretion of parathyroid glands in long-term −D animals appeared to be suppressed by normal or elevated levels of serum calcium. In humans, calcium infusion has been found to lower serum iPTH in patients with primary hyperparathyroidism [20]. However, hyperparathyroidism secondary to chronic renal failure is often not suppressed when normal renal function returns following renal allograft [21].

Serum iCT levels were high in male and female +D rats and female −D rats compared to younger ages [17]. This elevation was not sustained in −D males to the degree found in −D females. Elevation of serum calcium in 22-month-old −D females to normal levels in response to vitamin D_3 caused further elevation in iCT as did the slightly hypercalcemic levels resulting from repletion with $1,25(OH)_2D_3$. In spite of the high "basal" secretion of CT in old rats, the thyroid C cells of females appeared to be responsive to elevation of serum calcium, even between the hypo- and normocalcemic range in vitamin D_3 repleted animals.

Results of the repletion showed that in spite of prolonged hypocalcemia and hyperparathyroidism, kidney and intestine of aging female −D rats retained the ability to respond to vitamin D metabolites. Under the treatment regimen used in this study, $1,25(OH)_2D_3$ elevated serum calcium to a greater extent than did vitamin D_3, while both agents had a similar effect to decrease circulating iPTH and increase intestinal calcium-binding protein. Both $1,25(OH)_2D_3$ and vitamin D_3 also returned renal adenylate cyclase activity toward +D levels. The data in Table III indicate that vitamin D_3 may have been more effective than $1,25(OH)_2D_3$ in repairing the renal refractoriness to PTH. More detailed experiments designed to address this question specifically will be required to determine whether $1,25(OH)_2D_3$ can effect full restoration of PTH-dependent adenylate cyclase in D deficient animals. However, it has been previously reported that in acutely vitamin D deficient male rats raised from weaning for 4 weeks on a −D diet, $25(OH)D_3$, but not $1,25(OH)_2D_3$, was able to return renal cyclic AMP responsiveness to PTH to normal levels [22].

Armbrecht *et al.* [4] have shown that renal formation of $1,25(OH)_2D_3$ decreases with age in the male rat, which corresponds to the finding that circulating levels of

$1,25(OH)_2D_3$ decrease with age in humans [23]. The present finding that the symptoms of vitamin D deficiency were repaired as well or better by vitamin D_3 as by $1,25(OH)_2D_3$ indicates that, although the ability to metabolize vitamin D_3 to its active metabolites may be impaired with age, in cases of severe vitamin D depletion in the female rat, metabolism is still adequate so that repletion with the parent compound is effective.

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