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Laboratory Investigation

Effects of Age and Sex on the Regulation of Plasma 1,25-$(OH)_2$-D by Phosphorus in the Rat

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Summary. Dietary phosphate deprivation in women, but not men, is accompanied by a fall in plasma PO_4 and a rise in plasma 1,25-$(OH)_2$-vitamin D concentrations. In contrast, young male rats exhibit a fall in plasma PO_4 and a rise in plasma 1,25-$(OH)_2$-D concentrations in response to PO_4 deprivation. To evaluate whether age and sex influence basal plasma 1,25-$(OH)_2$-D levels and their regulation by PO_4 deprivation, plasma 1,25-$(OH)_2$-D, PO_4, and Ca levels were measured in male and female rats ranging in age from 6 weeks to 6 months while they were eating normal or low PO_4 diets for 1 to 16 days. Similar observations were also made in 6-week-old castrated male and female rats, males replaced with testosterone, and females replaced with estradiol. Basal plasma 1,25-$(OH)_2$-D levels were higher in 6-week-old males (228 ± 76 pmol/l) than in 6-week-old females (148 ± 62 pmol/l; $P < 0.01$) and declined by age 11 weeks to stable levels averaging about 100 pmol/l without sex difference. Dietary PO_4 deprivation resulted in a three- to fourfold increase in plasma 1,25-$(OH)_2$-D concentrations regardless of age and sex, accompanied by a correlated rise in serum Ca concentrations. Castration of 6-week-old males and females eliminated the sex difference in basal plasma 1,25-$(OH)_2$-D levels and appeared to enhance the elevation of plasma 1,25-$(OH)_2$-D concentrations in response to PO_4 deprivation in females. Although gonadal hormones may modify basal plasma 1,25-$(OH)_2$-D levels, they are not required for the augmentation of plasma 1,25-$(OH)_2$-D levels in response to PO_4 deprivation.

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Key words: 1,25-$(OH)_2$-D — Calcium — Phosphorus — Sex — Age.

Dietary phosphate deprivation in young male rats is accompanied by a fall in serum inorganic phosphate levels, increased conversion of radiolabeled 25-OH-vitamin D to 1,25-$(OH)_2$-D [1, 2], and increased plasma concentrations of 1,25-$(OH)_2$-D [3]. These observations indicate that renal synthesis of 1,25-$(OH)_2$-D is increased during dietary phosphate deprivation. This response is also observed in parathyroidectomized animals [3], indicating that PTH, a known stimulant of renal 1,25-$(OH)_2$-D synthesis [4, 5], does not mediate the response. Other experiments have shown that iPTH levels in rats fall during PO_4 deprivation while plasma 1,25-$(OH)_2$-D levels rise [6]. It has also been observed that hypophosphatemia in patients with calcium nephrolithiasis is associated with elevated plasma 1,25-$(OH)_2$-D levels in the absence of elevated PTH levels [7, 8]. Finally, it has been observed that dietary phosphate deprivation in healthy young women is accompanied by a fall in serum phosphate levels and a rise in plasma 1,25-$(OH)_2$-D concentrations, while in men, little change in serum PO_4 or plasma 1,25-$(OH)_2$-D levels was observed [7]. These observations suggested that there might be a sex difference in the activation of renal 1,25-$(OH)_2$-D synthesis in response to dietary PO_4 deprivation. In addition, the increase in plasma 1,25-$(OH)_2$-D levels in response to PO_4 deprivation in young male rats and the lack of such a response in adult men suggested the possibility that age might modify the response of renal 1,25-$(OH)_2$-D synthesis to phosphate deprivation. In the present studies, the response of plasma 1,25-$(OH)_2$-D levels to PO_4

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Table 1. Plasma 1,25-$(OH)_2$-D, 25-OH-D, Ca, and P concentrations in young and adult male and female rats eating normal diets

Age	Sex	Body weight g	1,25-$(OH)_2$-D pmol/l[2]	25-OH-D nmol/l	Ca nmol/l	P nmol/l
6 weeks	M	216 ± 10[a]	228 ± 76	42 ± 15	2.52 ± 0.20	2.67 ± 0.26
		(80)[b]	(40)[c]	(8)	(19)	(36)
	F	174 ± 7	146 ± 44	—	2.64 ± 0.12	2.41 ± 0.22
		(34)	(17)		(9)	(17)
P[d]			<0.001		NS	<0.001
11 weeks	M	283 ± 12	108 ± 45	—	2.28 ± 0.15	2.65 ± 0.29
		(20)	(10)		(10)	(10)
	F	198 ± 10	106 ± 63	57 ± 14	2.36 ± 0.19	2.46 ± 0.31
		(40)	(25)	(9)	(17)	(23)
P			NS		NS	NS
26 weeks	M	398 ± 26	105 ± 41	29 ± 5	2.48 ± 0.13	1.95 ± 0.12
		(16)	(9)	(9)	(11)	(11)
22 weeks	F	226 ± 12	101 ± 11	—	2.59 ± 0.08	2.10 ± 0.39
		(8)	(4)		(4)	(4)
P			NS		NS	NS

[a] Variance shown as SD
[b] Number of animals
[c] Number of measurements using pooled plasma from 2 rats per measurement
[d] P refers to comparison of age-matched males and females

deprivation was evaluated in male and female rats ranging in age from 6 weeks to 6 months. The effects of castration on the response have also been assessed.

Materials and Methods

Age-matched male and virgin female Sprague-Dawley rats aged 6 weeks, 11 weeks, and 22–26 weeks were obtained from King Animal Laboratories, Inc., Oregon, Wisconsin. Castrated 6-week-old males and females were also obtained from the same supplier. After delivery, the animals were placed on a control synthetic diet. Egg white, a low-phosphorus protein, served as the protein source in the diet. The diet was composed of 18% protein, 12% fat as corn oil, 60% carbohydrate as sucrose, 6% salt mixture, 2% fiber, 1% vitamin diet fortification mixture (ICN, Cleveland, Ohio), and 2% cod liver oil (USP). In this control diet, a mixture of sodium and potassium phosphates was used in the salt mixture to provide a normal phosphate intake. By analysis, this control diet contained 0.56% P and 0.63% Ca. The low-PO_4 diet was formulated by omitting sodium and potassium phosphates from the salt mixture and substituting equimolar quantities of NaCl. This diet provided only 0.02% P and the same amount of Ca as in the control diet. Both diets were calculated to provide 3 IU vitamin D_3 per g.

Two animals were housed per cage and all were initially fed the control diet for 2–4 days. Thereafter, control animals continued to eat the control diet while other animals were fed the low-PO_4 diet. After 1–16 days, groups of control and PO_4-deprived animals of each age and sex were weighed, anesthetized with ketamine (30 mg/kg intraperitoneally), and exsanguinated from the abdominal aorta into heparinized syringes.

Male and female rats castrated at age 4 weeks were received in the laboratory at 6 weeks of age together with age-matched intact males and females. Intact and castrated males and females, castrated males replaced with 0.5 mg testosterone propionate per kg subcutaneously in corn oil [9], and castrated females replaced with 10 μg 17β-estradiol subcutaneously in corn oil [10] were all then maintained on the control diet for 1 week. Half of each group was then deprived of PO_4 for 3 days while the other half continued to eat the control diet. Androgens and estrogens were continued during this experimental period in those castrated animals previously given replacement therapy.

Plasma 25-$(OH)_2$-D and 1,25-$(OH)_2$-D were measured by competitive protein binding assay as previously described using 1.5 to 5 ml samples [11]. For animals eating the control diet, plasma from 2 animals was pooled for analysis, whereas plasma from a single animal deprived of PO_4 was usually utilized for analysis. Inorganic PO_4 was measured spectrophotometrically and calcium was analyzed by atomic absorption spectrophotometry as previously described [7].

Results are presented as means ± standard deviation. Differences between groups were evaluated by Student's t test for unpaired data. Linear regression was calculated by least squares analysis [12].

Results

Effect of Age and Sex

Table 1 shows the measurements of plasma 1,25-$(OH)_2$-D for male and female rats ranging in age from 6 weeks to 26 weeks. Six-week-old males had significantly higher 1,25-$(OH)_2$-D levels than age-matched females. In rats 11 weeks of age or older, there was no difference in plasma 1,25-$(OH)_2$-D levels. Plasma 1,25-$(OH)_2$-D levels in both males and females aged 11 weeks were significantly lower than those in animals aged 6 weeks ($P < 0.001$) but did not fall further as the animals aged. Plasma 25-OH-D levels in those groups in whom measurements were made were slightly lower in 6-month-old

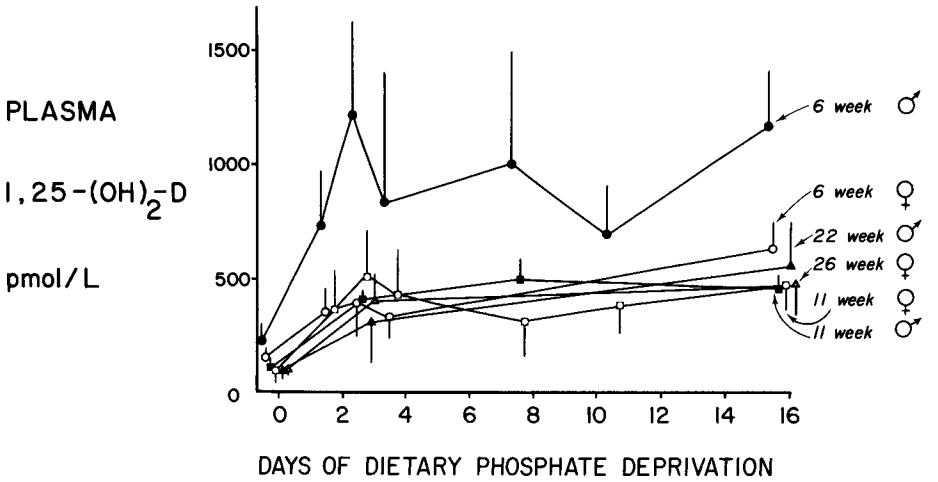

Fig. 1. Mean plasma 1,25-$(OH)_2$-D concentrations in male and in female rats aged 6, 11, and 22–26 weeks while eating the control diet and during 2–16 days of dietary phosphate deprivation. Vertical bars indicate SD

Table 2. Plasma 1,25-$(OH)_2$-D, 25-OH-D, Ca, and P concentrations in young and adult male and female rats during 2–16 days of dietary P deprivation

Age	Sex	Body weight g	1,25-$(OH)_2$-D pmol/l	25-OH-D nmol/l	Ca mmol/l	P mmol/l
6 weeks	M	215 ± 11[a] (67)[b]	965 ± 360 (67)[c]	39 ± 9 (9)	3.15 ± 0.38 (48)	1.48 ± 0.31 (59)
	F	164 ± 13 (46)	470 ± 200 (46)	—	2.85 ± 0.19 (21)	1.23 ± 0.30 (46)
P[d]			<0.001		<0.001	<0.001
11 weeks	M	287 ± 12 (42)	440 ± 80 (21)	—	2.66 ± 0.18 (21)	1.73 ± 0.40 (21)
	F	206 ± 13 (47)	400 ± 170 (47)	46 ± 13 (10)	2.90 ± 0.28 (47)	1.31 ± 0.36 (47)
P			NS		<0.001	<0.001
22 weeks	M	379 ± 28 (15)	440 ± 230 (15)	—	2.67 ± 0.14 (15)	1.45 ± 0.49 (15)
26 weeks	F	212 ± 10 (16)	450 ± 130 (12)	—	2.84 ± 0.14 (12)	1.30 ± 0.41 (12)
P			NS		<0.005	NS

[a] Variance shown as SD
[b] Number of animals
[c] Number of measurements using pooled plasma from 2 rats per measurement
[d] *P* refers to comparison of age-matched males and females

males eating the synthetic diet in comparison to 6-week-old males ($P < 0.05$) and in comparison to the mean of 41 ± 5 nmol/l in 5 other young male rats eating Purina Rat Chow (not shown in Table 1). Serum calcium concentrations were not different in male or female rats and did not change with age. Serum PO_4 concentrations were slightly, but significantly, higher in 6-week-old males than in 6-week-old females. At 11 weeks, a similar trend was also observed but the difference did not reach statistical significance because of greater variation between animals.

Phosphate Deprivation

Figure 1 shows the plasma 1,25-$(OH)_2$-D levels during control and after 2–16 days of dietary PO_4 deprivation in males and females at ages 6, 11, and 22–26 weeks. Since plasma 1,25-$(OH)_2$-D levels did not change in animals eating the control diet for 16 days, the control data in each age and sex group have been pooled. During PO_4 deprivation, plasma 1,25-$(OH)_2$-D levels in all groups were significantly elevated above control within 3 days and remained elevated as long as PO_4 deprivation was continued.

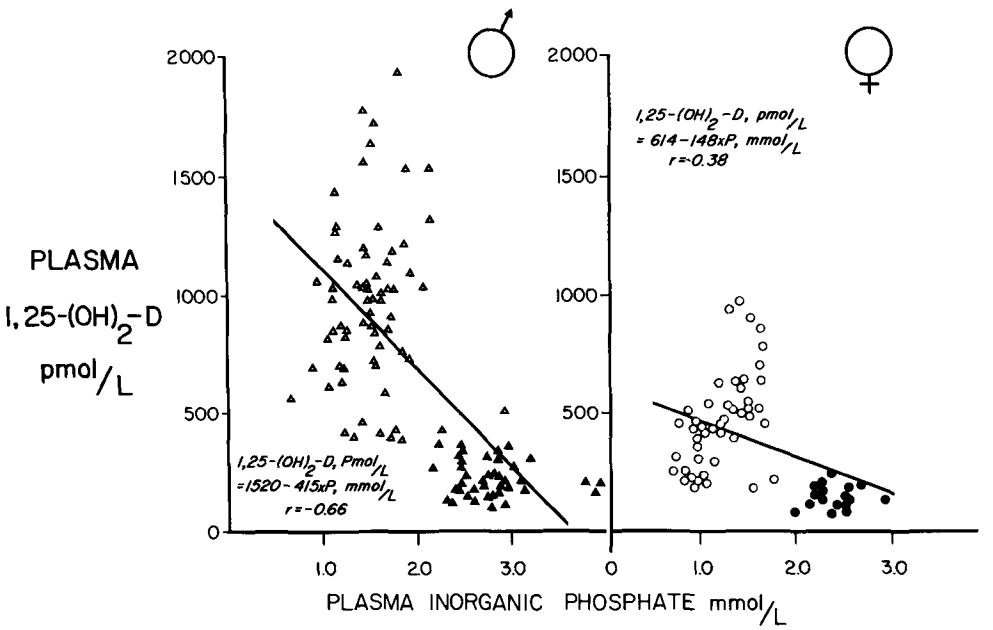

Fig. 2. Plasma 1,25-$(OH)_2$-D concentrations as a function of plasma inorganic phosphate concentrations during control (*closed symbols*) and 2–16 day of dietary phosphate deprivatic in 6-week-old male (*triangles*) and female (*circles*) rats

In 6-week-old male rats, plasma 1,25-$(OH)_2$-D levels in a single experiment rose promptly within 1 day: control 143 ± 42 pmol/l, $N = 7$; deprived of PO_4 for 1 day 313 ± 58 pmole/l, $N = 5$; $P < 0.001$ (not shown in Fig. 1). The 6-week-old male group also exhibited significantly higher plasma 1,25-$(OH)_2$-D levels throughout the period of PO_4 deprivation than older males or females of any age that did not differ.

Table 2 follows the format of Table 1 and summarizes body weight, and plasma 1,25-$(OH)_2$-D, 25-OH-D, calcium, and phosphorus concentrations for male and female rats aged 6, 11, and 22–26 weeks during 2–16 days of dietary PO_4 deprivation. Mean body weights were not significantly affected by 2–16 days of PO_4 deprivation. As has been repeatedly observed before in male rats [1, 3, 6], serum PO_4 levels were lower ($P < 0.01$ or less) and serum calcium levels were higher ($P < 0.01$ or less) during PO_4 deprivation than in control animals regardless of age or sex (compare Table 1). Plasma 25-OH-D levels for those groups in whom the measurements were made did not change from control in response to PO_4 deprivation. Among the 6-week-old male and female rats, plasma 1,25-$(OH)_2$-D levels rose strikingly to levels averaging 4–5 times mean control levels and, as in the control animals, were higher in the males. Among the rats 11 weeks of age or older, plasma 1,25-$(OH)_2$-D levels also increased four- to fivefold in comparison to the controls, but as in the older control animals, there was no sex difference in the plasma 1,25-$(OH)_2$-D levels during PO_4 deprivation.

Figure 2 shows plasma 1,25-$(OH)_2$-D concentrations as a function of the simultaneously measured serum PO_4 concentrations in 6-week-old male and in 6-week-old female rats during control and after 2–16 days of PO_4 deprivation. During control conditions, plasma 1,25-$(OH)_2$-D levels were higher in the males despite slightly but significantly higher plasma PO_4 levels (Table 1). During PO_4 deprivation, plasma 1,25-$(OH)_2$-D levels were also significantly higher among the males despite comparable reductions in plasma PO_4 levels in males and females in response to PO_4 deprivation and slightly but significantly higher mean plasma PO_4 concentrations during PO_4 deprivation in the 6-week-old males (Table 2).

Figure 3 is analogous to Fig. 2 and shows the relationship between plasma 1,25-$(OH)_2$-D levels and the plasma PO_4 concentrations during control and PO_4 deprivation for the 11-week-old and the 22–26-week-old males and females. A relationship similar to that observed in the 6-week-old females (Fig. 2) was found.

Figure 4 shows the plasma total calcium concentrations as a function of plasma 1,25-$(OH)_2$-D levels in 6-week-old male and female rats during control and dietary PO_4 deprivation. During control, plasma total calcium concentrations did not differ between males and females (Table 1). By contrast, during PO_4 deprivation, plasma calcium levels rose, on the average, to a greater extent among the youngest males than among the youngest females accompanying the higher plasma 1,25-$(OH)_2$-D levels observed in the males (Table 2).

Similarly, Fig. 5 shows plasma total calcium concentrations as a function of plasma 1,25-$(OH)_2$-D levels for the 11 and 22–26-week-old animals.

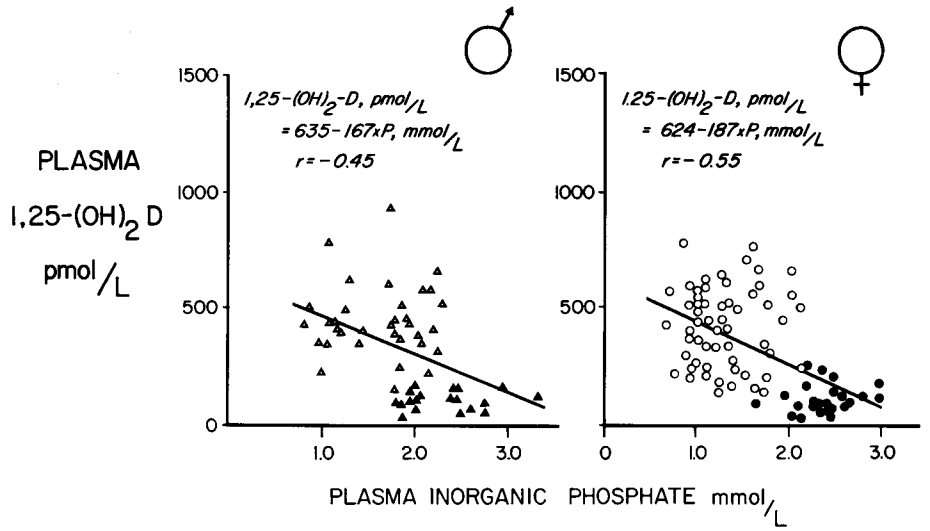

Fig. 3. Plasma 1,25-$(OH)_2$-D concentrations as a function of plasma inorganic phosphate concentrations in pooled data for 11- and 22–26-week-old male and female rats. Symbols as in Fig. 2

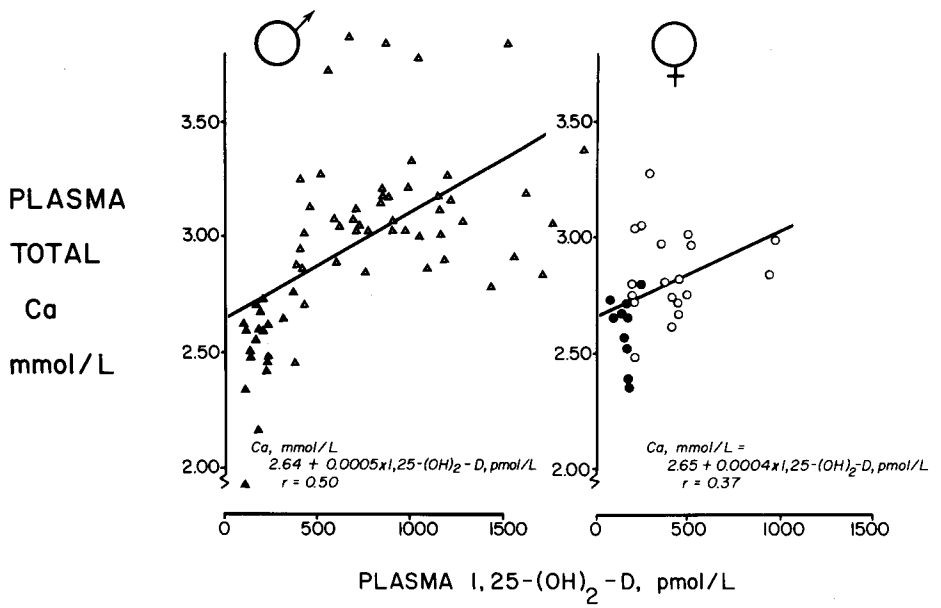

Fig. 4. Plasma total calcium concentrations as a function of plasma 1,25-$(OH)_2$-D concentrations in 6-week-old male and female rats. Symbols as in Fig. 2

During control conditions, plasma calcium and 1,25-$(OH)_2$-D levels did not differ in these groups (Table 1). During PO_4 deprivation, plasma calcium levels rose in all groups but to a slightly greater extent in females than in males (Table 2). The slope of the relationship between plasma calcium and plasma 1,25-$(OH)_2$-D was comparable in all groups (Figs. 4 and 5).

Effects of Castration

Figure 6 shows plasma 1,25-$(OH)_2$-D levels during control and after 3 days of PO_4 deprivation in intact and castrated 6-week-old males and females as well as in castrated males replaced with testosterone and castrated females replaced with estradiol. Control plasma PO_4 levels were not significantly altered by either castration or replacement therapy and, as in intact animals, fell to a comparable degree during PO_4 deprivation in these groups (data not shown). Among the males, neither castration nor castration with testosterone replacement altered plasma 1,25-$(OH)_2$-D levels during control conditions or the increments in plasma 1,25-$(OH)_2$-D in response to dietary PO_4 deprivation. Among the females, castration did not influence control plasma 1,25-$(OH)_2$-D levels, whereas the estradiol-replaced animals exhibited significantly lower plasma 1,25-$(OH)_2$-D levels (intact females 142 ± 47 pmol/l, $N = 17$; castrated females plus estradiol 76 ± 54 pmol/l, $N = 6$; $P < 0.01$). In response to PO_4 deprivation, the mean plasma 1,25-$(OH)_2$-D concentration was higher in castrated females than in intact

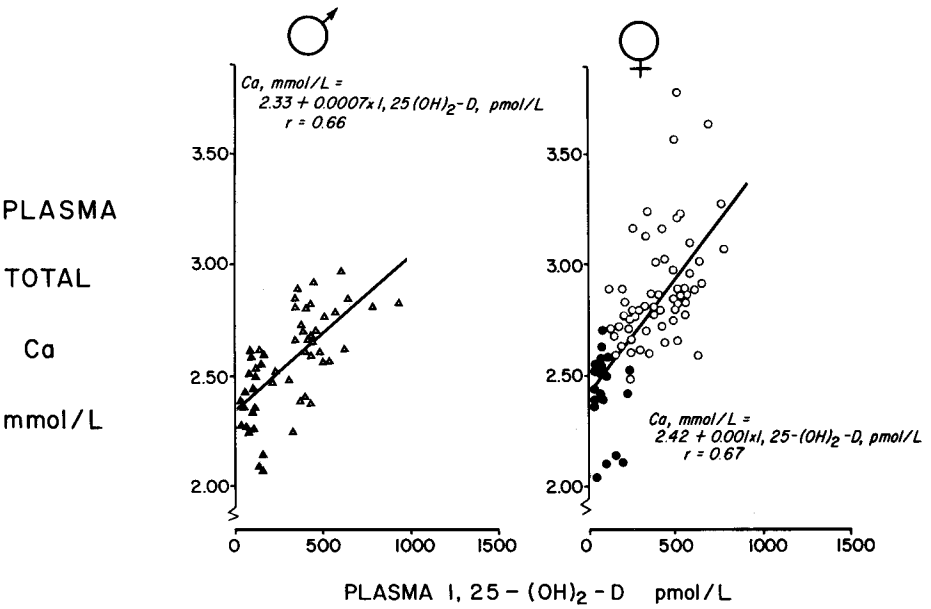

Fig. 5. Plasma total calcium concentrations as a function of plasma 1,25-$(OH)_2$-D concentrations in pooled data for 11- and 22–26-week-old male and female rats. Symbols as in Fig. 2

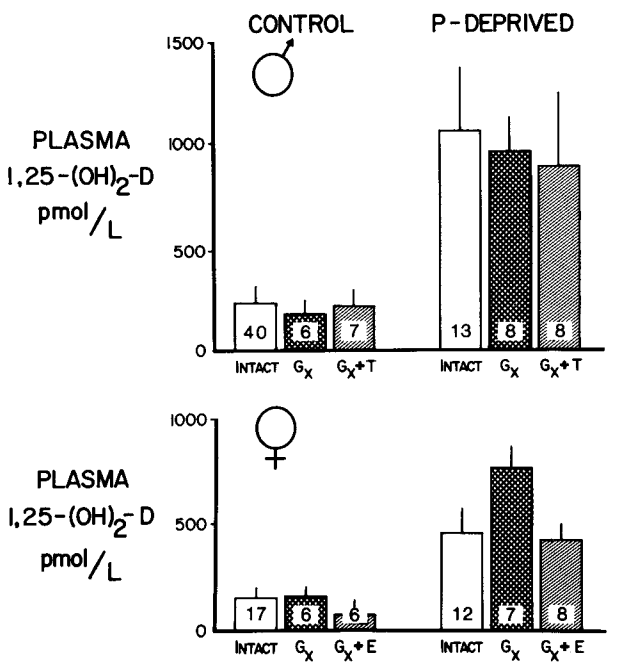

Fig. 6. Plasma 1,25-$(OH)_2$-D concentrations in intact (*open bars*) and castrated (G_x; *double cross-hatched bars*) male and female rats and in castrated males replaced with testosterone and castrated females replaced with estradiol (G_x + T and G_x + E; *single cross-hatched bars*) eating the control diet and during phosphate deprivation. The numbers within each bar refer to the number of observations in each group

females or castrated females replaced with estradiol (intact females 460 ± 115 pmol/l, N = 12; castrated females 769 ± 107 pmol/l, N = 7; $P < 0.01$; castrated females plus estradiol 424 ± 86 pmol/l, N = 8; $P < 0.001$ versus castrated females). The mean plasma total calcium concentration during PO_4 deprivation in the castrated females was also significantly higher than among either the intact females or the castrated females replaced with estradiol (intact females 2.85 ± 0.15 mM/l, N = 8; castrated females 3.28 ± 0.13 mM/l, N = 6; $P < 0.001$; castrated females plus estradiol 2.96 ± .30 mM/l, N = 6; $P < 0.02$ versus castrated females).

Discussion

The inverse relationship observed between plasma 1,25-$(OH)_2$-D levels and plasma PO_4 concentrations in the present studies documents hypophosphatemia in the PO_4-deprived animals and is in agreement with earlier observations in both the rat and man [3, 6, 7]. The slope of the relationship between plasma 1,25-$(OH)_2$-D levels and plasma PO_4 concentrations was similar in all groups of rats except the 6-week-old males, who showed higher absolute plasma 1,25-$(OH)_2$-D levels as plasma PO_4 fell during PO_4 deprivation. The slope for the relationship in the other groups that ranged from 148 to 187 pmol/mmol of PO_4 was similar to the value of 141 pmol/mmol reported previously in humans [7].

Hypercalcemia, in addition hypophosphatemia, is a hallmark of dietary PO_4 deprivation in rats, and was also documented in the present studies (Table 2). The direct relationship observed between plasma total calcium concentration and plasma 1,25-$(OH)_2$-D levels supports the hypothesis that the hypercalcemia of dietary PO_4 deprivation in rats is the result of increased calcium mobilization in response to 1,25-$(OH)_2$-D. Further evidence for this hypothesis is provided by the observation that,

during PO_4 deprivation, castrated females have higher plasma calcium concentrations in association with significantly higher plasma 1,25-$(OH)_2$-D levels than either intact females or castrated females replaced with estradiol whose plasma calcium concentrations and plasma 1,25-$(OH)_2$-D levels are not different from one another.

The present experiments also document for the rat that plasma 1,25-$(OH)_2$-D levels are higher in 6-week-old animals of both sexes that are not yet sexually mature than in older males and females. These observations are consistent with earlier studies in the rat [13] as well as studies in children who have higher plasma 1,25-$(OH)_2$-D levels than do adult men and women [14]. The mechanism responsible for the higher plasma 1,25-$(OH)_2$-D levels in the young animals is not clarified by the present studies.

During control conditions, plasma 1,25-$(OH)_2$-D concentrations in 6-week-old male rats averaged 228 pmol/l in agreement with previous observations in young male rats [3, 6], a value approximately 50% higher than in age-matched females. Speculatively, gonadal hormones in the male may play a role in this sex difference since plasma 1,25-$(OH)_2$-D levels tended to fall, although not significantly, in castrated males and were restored to control by testosterone therapy (Fig. 6).

During PO_4 deprivation, plasma 1,25-$(OH)_2$-D concentrations rose promptly in all groups and remained elevated as long as PO_4 deprivation was continued (Fig. 1). This confirms previous studies in young and old male rats [3, 6, 15] and extends these observations to show that dietary PO_4 is an important regulator of plasma 1,25-$(OH)_2$-D levels in young female rats as well as in older rats of both sexes. The higher plasma 1,25-$(OH)_2$-D levels observed in the 6-week-old male rats during PO_4 deprivation are probably a reflection of the higher control levels in this group since the percentage increase relative to control in plasma 1,25-$(OH)_2$-D levels during PO_4 deprivation was similar in all groups (Tables 1 and 2).

Castration of 6-week-old rats appears to have little effect on control plasma 1,25-$(OH)_2$-D levels, although it is interesting that the sex difference in plasma 1,25-$(OH)_2$-D levels in these animals is no longer apparent after the animals are castrated. Testosterone replacement had little effect on control plasma 1,25-$(OH)_2$-D levels in the castrated males, but estradiol replacement in castrated females significantly lowered control plasma 1,25-$(OH)_2$-D levels (Fig. 6). This was unexpected in view of an earlier report that estradiol stimulates in vivo production of 1,25-$(OH)_2$-D in 8-week-old female rats [16]. Since the dose of estradiol used in the earlier studies was about 300-fold greater than that used in the present study, it is possible that estradiol may have a biphasic effect on 1,25-$(OH)_2$-D synthesis in the rat.

Neither castration nor castration with testosterone replacement had any effect on the rise in plasma 1,25-$(OH)_2$-D concentrations during PO_4 deprivation in 6-week-old male rats. By contrast, castrated females had significantly higher plasma 1,25-$(OH)_2$-D levels during PO_4 deprivation than age-matched intact females. Evidence that this difference may be due to a lack of estrogen in the castrated females is provided by the observation that plasma 1,25-$(OH)_2$-D levels during PO_4 deprivation in castrated females replaced with estradiol were significantly lower than in identically treated castrated females not replaced with estrogen and were similar to levels found in PO_4-deprived age-matched females (Fig. 6).

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