Changes in the regulation of calcium metabolism and bone calcium content during growth in the absence of endogenous prolactin and during hyperprolactinemia: A longitudinal study in male and female Wistar rats

Pritsana Piyabhan, Nateetip Krishnamra, and Liangchai Limlomwongse

Abstract: Since endogenous prolactin has been shown to enhance food consumption, calcium absorption, and bone calcium turnover in the pregnant rat, the role of endogenous prolactin in the regulation of calcium metabolism was investigated in 3-day balance studies of female Wistar rats from the age of 3 to 11 weeks. The study was divided into two parts. In part I, calcium metabolism in males and females was compared. In part II, 3-week old female rats were divided into 5 groups: (*i*) control animals receiving 0.9% NaCl; (*ii*) animals receiving 6 mg bromocriptine/kg/day (– PRL_{endo} group); (*iii*) animals receiving 2.5 mg ovine prolactin/kg/day (+PRL_{exo}); (*iv*) sham-operated animals receiving 0.9% NaCl, and (*v*) animals with two extra pituitaries implanted under the renal capsule, receiving 0.9% NaCl (AP group). Results showed that rapid growth occurred between 3 and 6 weeks with maximum fractional calcium absorption and calcium retention at 5 weeks of age in both sexes. The data also showed a physiological significance of endogenous prolactin in enhancing calcium absorption and retention in 5 week old rats. In an absence of prolactin, peak calcium absorption was delayed in 7-week old animals, and vertebral calcium content of 11-week old animals was reduced by 18%. Hyperprolactinemia in the AP group was found to enhance fractional calcium absorption and calcium retention at 7, 9, and 11 weeks and increased the femoral calcium content by 16%. It could be concluded that a physiological role of prolactin is the stimulation of calcium absorption and maintainance of bone calcium content during growth and development.

Key words: bone calcium content, calcium absorption, calcium balance, hyperprolactinemia, prolactin.

Résumé : Des travaux antérieurs ont montré que la prolactine endogène stimule la consommation alimentaire. Dans le présente étude, on a examiné l'absorption du calcium et le renouvellement du calcium osseux chez des rats femelles gravides, ainsi que le rôle de la prolactine endogène dans la régulation du métabolisme calcique en effectuant le bilan calcique, période de 3 jours, de rats Wistar femelles entre les âges de 3 et 11 semaines. L'étude a comporté deux volets : I, comparaison du métabolisme calcique entre mâles et femelles; II, répartition des femelles âgées de 3 semaines dans les groupes suivants : (*i*) témoin, recevant du NaCl à 0,9 %; (*ii*) –PRL_{endo}, recevant 6 mg de bromocriptine/kg/jour; (*iii*) +PRL_{exo}, recevant 2,5 mg prolactine ovine/kg/jour; (*iv*) opéré de manière fictive, recevant du NaCl à 0,9 %; et (*v*) groupe AH, auquel deux hypophyses ont été implantées sous la capsule rénale, recevant du NaCl à 0,9 %. Les résultats ont montré une croissance rapide entre les semaines 3 et 6 ainsi qu'une rétention et une absorption fractionnaires maximales de calcium à 5 semaines chez les deux sexes. Les résultats ont aussi montré que la prolactine endogène a joué un rôle physiologique en stimulant la rétention et l'absorption du calcium chez les rats de 5 semaines. En l'absence de prolactine, l'absorption maximale de calcium a été retardée à 7 semaines, et la teneur en calcium vertébral des rats de 11 semaines a été réduite de 18 %. Chez le groupe AH, l'hyperprolactinémie a stimulé la rétention et l'absorption fractionnaires de calcium à 7, 9 et 11 semaines, et augmenté la teneur en calcium fémoral de 16 %. On a pu conclure que la prolactine stimule l'absorption de calcium et maintient la teneur en calcium osseux durant la croissance.

Mots clés : teneur en calcium osseux, absorption de calcium, bilan calcique, hyperprolactinémie, prolactine.

[Traduit par la Rédaction]

Received December 31, 1999. Published on the NRC Research Press web site on September 14, 2000.

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Introduction

During pregnancy and lactation, the intestinal absorption of calcium and bone resorption are increased to meet fetal and neonatal demand for calcium (Toverud et al. 1976; Atkinson and West 1970). Halloran and DeLuca (1980) reported enhanced calcium absorption in vitamin D deficient pregnant and lactating rats, suggesting that $1,25(OH)_2D_3$ was not the only hormone responsible for such adaptation.

With its elevated plasma levels during pregnancy and lactation (Amenomori et al. 1970; Simpson et al. 1973) and its reported action on electrolyte transport (Horrobin et al. 1971; Mainoya 1972, 1978), prolactin was regarded as a likely candidate for regulating intestinal calcium absorption. Although prolactin has been shown to stimulate the activity of 1-α-hydroxylase to convert 25-OH-D_3 to $1,25(OH)_2D_3$ (Spanos et al. 1976; Baksi et al. 1978), which stimulates active calcium absorption, prolactin was also reported to increase calcium absorption in vitamin D deficient rats (Pahuja and DeLuca 1981). Our laboratory reconfirmed this possibly direct action of prolactin on the intestine by demonstrating an acute stimulatory effect of a single pharmacological dose of prolactin on calcium absorption within 60 min after intraperitoneal administration (Krishnamra et al. 1990). We further demonstrated that prolactin action on passive calcium absorption was luminal sodium dependent (Wangdee et al. 1991; Krishnamra et al. 1993), while its action on active calcium transport in the duodenum was independent of luminal sodium (Krishnamra and Taweerathitam 1995).

After having established the acute effects of prolactin, we turned our attention to a prolonged effect of a pharmacologic dose of prolactin. Daily subcutanous administration of 2.5 mg prolactin/kg body weight resulted in a significant retention of absorbed calcium (Krishnamra and Cheeveewattana 1994) and enhanced calcium deposition in the bones of young growing rats while increasing bone turnover in sexually mature animals (Krishnamra and Seemoung 1996). Furthermore, we have demonstrated a physiological significance of endogenous prolactin in the regulation of calcium metabolism. Endogenous prolactin was found to stimulate food intake in pregnant and lactating rats, and increase the fractional calcium absorption in pregnant rats, resulting in increased calcium retention (Lotinun et al. 1997). On the other hand, a high dose of exogenous prolactin suppressed the fractional calcium absorption in pregnant animals, confirming the previously reported biphasic action of prolactin in bone (Krishnamra et al. 1997*a*, *b*).

So it seems that endogenous prolactin secreted at considerable rates during pregnancy and lactation does increase calcium intake, calcium delivery to the circulation, and bone calcium turnover, presumably to increase calcium availability in the plasma compartment. This was evident particularly during lactation, when milk production and milk calcium concentration were related to prolactin levels (Lotinun et al. 1997).

Physiological significance of endogenous prolactin has never been addressed in nonmated rats, although there were reports of prolactin influence on early phases of fetal bone development (Migliaccio et al. 1995; Clement-Lacroix et al. 1999). Since prolactin receptors have been found in a large number of tissues including bone, gastrointestinal tract, and kidney (Bole-Feysot et al. 1998), the three main target organs of the calcium regulating hormones, it was interesting to investigate the possible involvement of endogenous prolactin in the regulation of calcium metabolism during growth and development.

The aims of the present investigation were to demonstrate changes in calcium balance and bone calcium contents in male and female rats from weanling age up to sexual maturation; second, to evaluate the physiological significance of endogenous prolactin in calcium regulation in bromocriptine-treated weaned female rats; and third, to study the effect of hyperprolactinemia on calcium balance in anterior pituitary-implanted rats and in rats injected with pharmacological dosage of ovine prolactin. The longitudinal studies began in 3-week old rats and were terminated when the animals reached the age of 11 wks.

Materials and methods

Animals

In the present investigation, 3-week old male and female Wistar rats were purchased from the Animal Centre of Thailand, Salaya Campus, Mahidol University. After arrival at the Faculty of Science, the animals were kept in stainless steel cages in a temperature controlled room for 4 d with access to food containing 1.4% calcium, 0.9% phosphorus, and 4,000 IU/kg vitamin D and tap water before the start of the balance study. They were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Experimental procedure

Calcium balance study

Each calcium balance study was performed over 3 days, during which the animals were placed in metabolic cages. Each cage had a urine funnel, and beneath the cage floor there were two more pieces of wire mesh of different pore sizes to separate feces from food and food from urine. Body weight and food consumption were recorded on a daily basis. Drinking water contained a low calcium concentraion, therefore calcium intake from drinking water was considered negligible. Fecal and urine samples were collected over the 3 d. Fecal pellets were dried to a constant weight and ashed at 650°C in a muffle furnace. On the last day of each 3-day calcium balance study, blood sample was collected from the tail vein for measurement of the plasma calcium concentration.

Each animal underwent the 3-day calcium balance study every two weeks, i.e., at the age of 3, 5, 7, 9, and 11 weeks. After the last balance study was performed at 11 weeks of age, the animals were sacrificed using ether and the left femur and lumbar vertebrae $L_{5–6}$ were removed. The femur was cut in half and its marrow cavity was flushed with 0.9% NaCl. After blotting dry, the bones were defatted in acetone for 3 days then dried at 80°C for 48 h to record the dry weight before they were ashed in a muffle furnace at 650°C for 16 h. The bone and fecal ashes were later dissolved in 3M HCl and the total calcium concentration was determined.

Calculations

[1] Fractional calcium absorption (% intake) =

$$\frac{\left[I_{ca} - F_{ca}\right]}{I_{ca}} \times 100$$

[2] calcium retention (% intake) =

$$\frac{\left[I_{ca}-\left(U_{ca}+F_{ca}\right)\right]}{I_{ca}}\times 100$$

where I_{Ca} = calcium intake (mmol/kg body weight/day); U_{Ca} = urinary calcium excretion (mmol/kg body weight/day); and F_{Ca} = fecal calcium excretion (mmol/kg body weight/day).

Anterior pituitary implantation

To study the effect of hyperprolactinemia on the handling of calcium in female Wistar rats, hyperprolactinemia was induced by implantation of two extra pituitary glands removed from two sexually mature female donor rats and place under the left renal capsule of weaned recipient rat (Chen et al. 1970; Aguado et al. 1977, 1981). Three days after the operation, the anterior pituitary-grafted rats were subjected to the first calcium balance experiment.

In pituitary implantation, the distance between the hypothalamus and the implanted pituitary glands under the renal capsule removes the inhibitory and stimulatory regulation from the hypothalamus (Harris and Jacobsohn 1952). Since prolactin is the only pituitary hormone that is normally under the dopaminergic inhibitory regulation from the hypothalmus (the other hormones are under stimulatory control), the implanted glands continued to secrete prolactin into the circulation. This was confirmed by the plasma levels of prolactin. Sham-operated animals were subjected to the same surgical procedure but they received implantation of pituitary-sized pieces of the cerebral hemisphere under the left renal capsule.

Experimental protocols

Protocol 1: Longitudinal study of calcium balance in normal male and female Wistar rats

To perform a longitudinal study the calcium handling in male and female rats, the 3-week old normal male (n = 6) and female rats (n = 6) were repeatedly subjected to a three day calcium balance experiment at two weeks intervals until they were 11 weeks old.

Protocol 2: To evaluate a physiological significance of endogenous prolactin and the effects of hyperprolactinemia induced by pituitary implantation or by exogenous prolactin on the handling of calcium in female Wistar rats

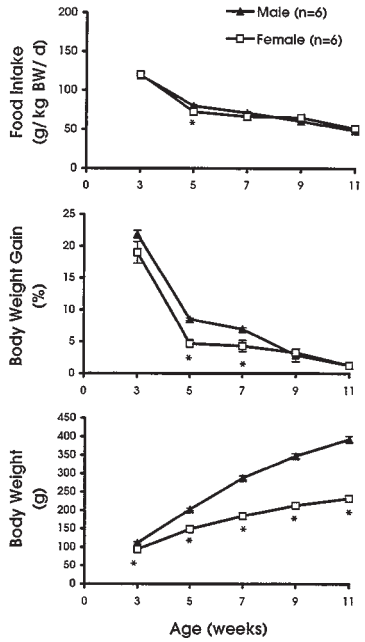
In this series of experiments only female Wistar rats were used. The significance of endogenous prolactin was evaluated from changes in the calcium balance and bone calcium contents in its absence in bromocriptine-treated animals ($-PRL_{endo}$). Hyperprolactinemia, on the other hand, was induced either by anterior pituitary implantation (AP) or daily injection of exogenous prolactin ($+PRL_{exo}$).

In Protocol 2, female weaned rats were divided into 5 groups as follows: (*i*) a control group receiving 0.9% NaCl; (*ii*) a $-PRL_{endo}$ group receiving intraperitoneal injection of 3 mg bromocriptine myselate/kg body weight, twice a day on daily basis; (*iii*) a $+PRL_{exo}$ group receiving daily subcutaneous injection of 2.5 mg ovine prolactin/kg body weight; (*iv*) a sham group receiving 0.9% NaCl; and *v*) an AP group with anterior pituitary implantation receiving 0.9% NaCl.

Analyses

The total calcium concentration was determined by atomic absorption spectrophotometry (Spectr AA-300, Varian Techtron Pty.Ltd., Springvale, Australia). Plasma prolactin levels were determined using a commercial kit (Amersham International Plc., Buckinghamshire, England).

Fig. 1. Food intake (g/kg/day), body weight gain (%), and bodyweight (g) over the 3-day balance study in the control male and female rats at the age of 3, 5, 7, 9, and 11 wks. *P < 0.05 compared with the male group.



Statistical analyses

Results were presented as mean ± SEM. Data were analyzed using Student's t-test for comparison between two sets and one-way ANOVA and the Newman-Keuls test at the 95% confidence level for multiple comparisons.

Results

Longitudinal study of the calcium balance in normal male and female Wistar rats

To investigate the gender difference in calcium metabolism between male and female rats during growth and development, a 3-day calcium balance study was performed, beginning at 3 wks of age. As shown in Fig. 1, the body weight of both sexes increased with age throughout the 8-week study. Comparison between sexes clearly showed that males grew faster after the age of 3 wks and attained 59% greater body weight than females despite similar food intake. The percent body weight gain decreased with age in both sexes. Plasma calcium concentrations were not different in the two genders (data not shown).

Figure 2 shows that as urinary calcium excretion was very small, total calcium excretion actually reflected the fecal excretion. Compared with male rats, total calcium excretion was lower in female weaned rats, but the opposite was true for the sexually mature animals. However, the total calcium excretion markedly decreased at 5 wks of age in both males and females, corresponding to an increase to peak values in both fractional calcium absorption and calcium retention (Fig. 3). At this age, calcium absorption and retention were slightly, but significantly, lower in the female rats. The gender difference was also observed in 9- and 11-week old rats but the differences were not significant. Table 1 shows a sig-

Fig. 2. Urinary, fecal and total calcium excretion (mmol/kg/day) over the 3-day balance study in the control male and female rats at the age of 3, 5, 7, 9, and 11 wks. *$P < 0.05$ compared with the male group.

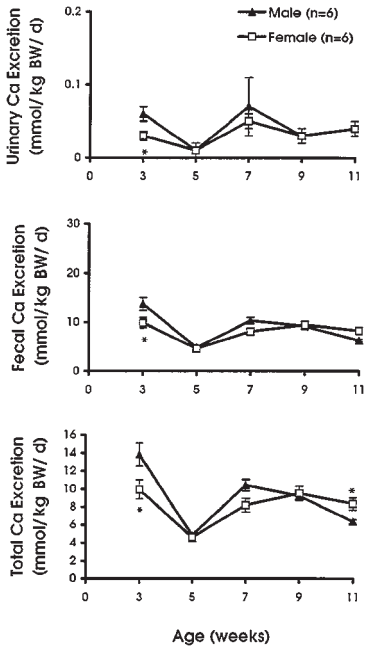
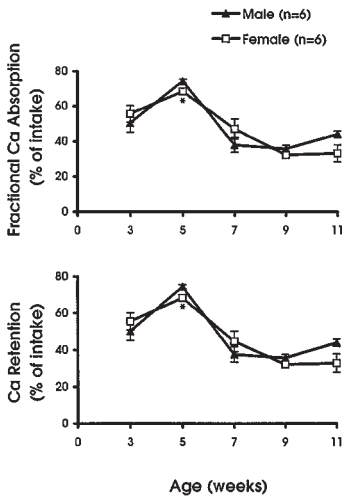


Fig. 3. Fractional calcium absorption (% of intake) and the calcium retention (% of intake) over the three day balance study in the control male and female rats at the age of 3, 5, 7, 9, and 11 wks. *$P < 0.05$ compared with the male group. (Note: the fractional calcium absorption and calcium retention were nearly identical because most of the total calcium excretion was of fecal origin).



nificantly higher calcium content in femur and lumbar vertebrae of male rats.

Physiological significance of endogenous prolactin and the effects of hyperprolactinemia on the handling of calcium in female Wistar rats

Table 2 shows that the daily administration of bromocriptine in the regimen used here reduced plasma prolactin to undetectable levels. On the other hand, both daily administration of exogenous prolactin and the anterior pituitary implantation resulted in significant hyperprolactinemia with the plasma prolactin levels being more than 10 times the levels in control rats.

As seen in Fig. 4, hyperprolactinemia induced by exogenous prolactin did not have any effect on food intake, body weight, or body weight gain. However, bromocriptine-treated rats exhibited a lower body weight gain at 3 weeks of age (i.e., 6.82 ± 1.58 vs. 19.03 ± 1.67% intake, $P < 0.05$, in $-PRL_{endo}$ and control goups, respectively) and 9 weeks of age (i.e., 0.20 ± 0.67 vs. 3.29 ± 0.51% intake, $P < 0.05$, in $-PRL_{endo}$ and control goups, respectively).

Figure 5 depicts the excretion of calcium which was mainly by fecal excretion. Control rats as discussed before showed a marked decrease in fecal and thus total calcium excretion at the age of 5 wks after which calcium excretion rose to a stable level. The excretion patterns in the $-PRL_{endo}$ group and $+PRL_{exo}$ group appeared to be out of phase from that of the control group. In the $-PRL_{endo}$ group, the reduction in fecal and total calcium excretion was observed later at 7 wks of age. Since the total calcium excretion was mainly fecal excretion (Fig. 5), the body calcium retention actually appeared the same as the fractional calcium absorption (Fig. 6) although the values were not identical. As shown in Fig. 6, the peak in fractional calcium absorption and calcium retention shifted from 5 wks to 7 wks of age. As for the $+PRL_{exo}$ group, acute exposure to a high dose of prolactin for a few days in 3-week old rats led to a marked increase in the fractional calcium absorption, i.e., 69.53 ± 2.16% of intake as compared with the control value of 55.61 ± 4.71% of intake. Continued exposure to high dose of exogenous prolactin decreased the fractional calcium absorption to a nadir of 26.51 ± 3.01% of intake at 7 wks of age.

Similar to the exogenous prolactin-induced hyperprolactinemia, hyperprolactinemia induced by anterior pituitary implants (AP group) did not affect the food intake or body weight of the animals (results not shown). The urinary excretion of calcium of the AP group was also very small and was not different from that of the sham control (results not shown). However, after the 5th week, the fractional calcium absorption increased to levels consistantly higher than that of the sham control by 58, 33, and 54% of intake at 7, 9, and 11 wks of age respectively. The calcium retention of the AP group was similarly higher than that of the sham control. The calcium retention of the AP group was similarly higher than that of the sham group (Fig. 7).

Table 1 presents the weight, length, and total calcium content per gram dry weight of femur and lumbar vertebrae 5–6 in the various groups. The data showed no differences in the weight and length of femurs or weight of the lumbar vertebrae from control and experimental groups. The only difference was in the total calcium content. In the bromocriptine-treated group, an absence of endogenous prolactin resulted in a lower calcium content in the lumbar vertebrae (3.24 ± 0.17 mmol/g dry weight) as compared with controls (3.94 ± 0.20 mmol/g dry weight). The AP-hyperprolactinemic group, in contrast, exhibited a 16% increase in femoral calcium content in the sham group (4.56 ± 0.23 mmol/g dry weight) compared with the AP group (5.28 ± 0.18 mmol/g dry weight, $P < 0.05$).

Table 1. Weight of femur and lumbar vertebrae (g/kg body weight), length of femur (cm), and calcium content in femur and lumbar vertebrae (mmol/g dry weight) of control males and females, and bromocriptine-treated ($-PRL_{endo}$), ovine prolactin treated ($+PRL_{exo}$), sham and pituitary implanted (AP) female rats at 11 weeks of age.

Group	Bone Femur			Lumbar vertebrae 5-6	
	Weight (g/kg BW)	Length (cm)	Total Ca (mmol/g dry weight)	Weight (g/kg BW)	Total Ca (mmol/g dry weight)
Control male (n = 6)	1.52 ± 0.03	3.60 ± 0.03	6.23 ± 0.60	1.16 ± 0.22	6.12 ± 0.46
Control female (n = 6)	1.81 ± 0.06	3.25 ± 0.02	3.70 ± 0.38†	1.21 ± 0.04	3.94 ± 0.20*
Female:					
$-PRL_{endo}$ (n = 12)	1.91 ± 0.06	3.24 ± 0.03	3.71 ± 0.22	1.15 ± 0.04	3.24 ± 0.17**
$+PRL_{exo}$ (n = 7)	1.95 ± 0.03	3.36 ± 0.05	4.54 ± 0.29	1.19 ± 0.01	3.63 ± 0.28
Sham (n = 7)	1.88 ± 0.05	3.35 ± 0.03	4.56 ± 0.23	1.21 ± 0.05	4.36 ± 0.22
AP (n = 12)	1.79 ± 0.02	3.23 ± 0.02	5.28 ± 0.18†	1.15 ± 0.02	4.38 ± 0.14

*P < 0.05 compared with male control group.
**P < 0.05 compared with the corresponding values of female control group.
†P < 0.05 compared with the corresponding values of female sham group.

Table 2. Prolactin levels in plasma (ng/mL) of control males and females, and bromocriptine treated ($-PRL_{endo}$), exogenous prolactin-treated ($+PRL_{exo}$), sham, and AP-implanted (AP) 7–8 week-old female rats.

Group	Plasma PRL (ng/mL)
Control male (n=6)	7.99 ± 1.10
Control female (n=6)	7.05 ± 1.12
Female:	
$-PRL_{endo}$ (n=12)	–0.10 ± 0.07*
$+PRL_{exo}$ (n=7)	75.88 ± 6.56*
Sham (n=7)	5.34 ± 0.70
AP (n=12)	91.69 ± 2.88†

*P < 0.05, compared with the corresponding values of female control group.
†P < 0.05, compared with the corresponding values of female sham group.

Discussion

This study, to our knowledge, was the first to undertake a longitudinal study of the calcium balance in rats to demonstrate change with age in calcium absorption, retention, and excretion from weaning age through development up to 11 weeks of age when the animals became fully mature and bone turnover became steady (Ijiri et al. 1995; King et al. 1995). In mammals, both males and females exhibit the maximum growth rate, especially bone growth, during adolescence and puberty (Peacock 1991), then bone growth decelerates until longitudinal growth is complete. Data on body weight gain confirmed the previous report (Thomas and Ibarra 1987) that growth was most rapid between 3 and 6 weeks of age, the period between weaning and the onset of sexual maturation. At the stage of young adulthood, i.e., 9 weeks old, body proportions have been achieved, with males as a group significantly larger than females. Bone was no longer growing but the cycle of bone resorption and formation continued, although at a slower rate than during adolescence. Food consumption when expressed per body weight was greatest in weaned rats of both genders, before decreasing with increasing age. Although males had a greater body weight and denser bone than females, the efficiency of their calcium absorption and renal calcium handling did not differ. Calcium absorption peaked at 5 wks of age for both sexes and then decreased with increasing age (Ireland and Fordtran 1973; Armbrecht et al. 1979; Favus et al. 1983; Recker et al. 1988). This has been attributed to decreased capacity of the parathyroid hormone to stimulate the

Fig. 4. Food intake (g/kg/day), body weight gain (%), and the body weight (g) over the 3-day balance study in the control female rats, females treated with 6 mg bromocriptine/kg/day to inhibit endogenous prolactin secretion ($-PRL_{endo}$) and in females given 2.5 mg prolactin/kg/day ($+PRL_{exo}$) at the age of 3, 5, 7, 9, and 11 wks. *P < 0.01 when $-PRL_{endo}$ and control groups are compared.

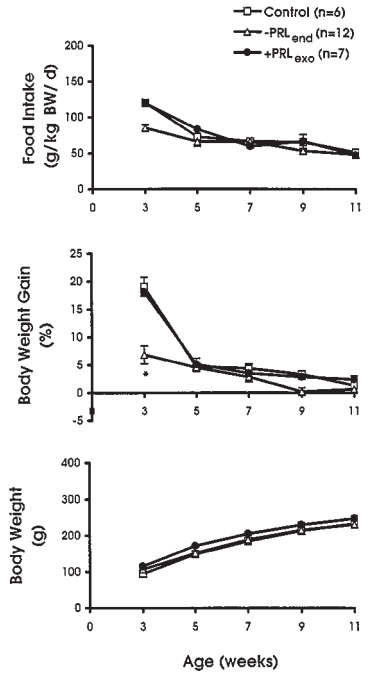


Fig. 5. Urinary, fecal and total calcium excretion (mmol/kg/day) over the 3-day balance study in the control female rats, females treated with 6 mg bromocriptine/kg/day to inhibit endogenous prolactin secretion ($-PRL_{endo}$) and in females given 2.5 mg prolactin/kg/day ($+PRL_{exo}$) at the age of 3, 5,7, 9, and 11 wks. $^{*}P < 0.01$ when $-PRL_{endo}$ and control groups are compared; $^{\dagger}P < 0.05$ when $+PRL_{exo}$ and control groups are compared.

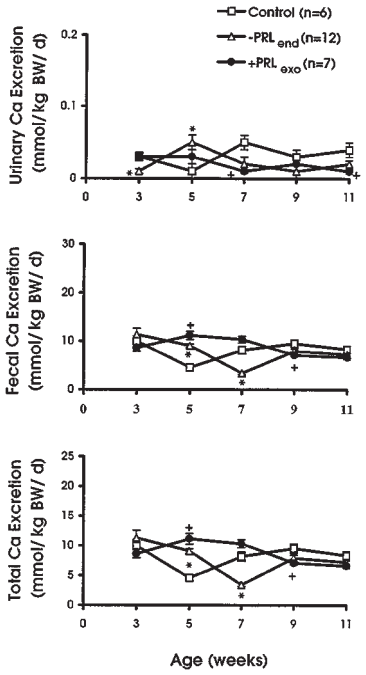
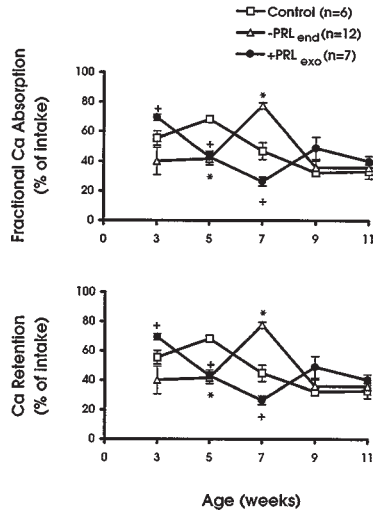


Fig. 6. Fractional calcium absorption (% of intake) and calcium retention (% of intake) over the 3-day balance study in the control female rats, female treated with 6 mg bromocriptine/kg/day ($-PRL_{endo}$), and female given 2.5 mg prolactin/kg/day ($+PRL_{exo}$) at the age of 3, 5, 7, 9 and 11 wks. $^{*}P < 0.05$ when $-PRL_{endo}$ and control groups are compared; $^{\dagger}P < 0.05$ when $+PRL_{exo}$ and control groups are compared.

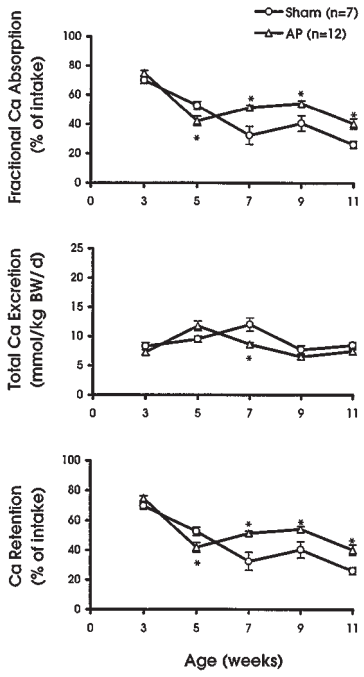


renal production of $1,25(OH)_2D$ (Armbrecht et al. 1986), and a decline in the vitamin D-dependent calcium binding protein content in the intestine (Armbrecht et al. 1979). During the intense growth periods, young animals were retaining most of the calcium they absorbed rather than excreting it in the urine (Nordin et al. 1967; Matkovic et al. 1990). Morphological studies of the intestine of Wistar rat have shown little change in villus height, crypt depth, or crypt : villus ratio between 6 wks and 1 y of age (Clarke 1977; Armbrecht et al. 1979) so the changes were mostly functional. In females, duodenal calcium transport decreased with sexual maturation (Thomas et al. 1987). It was interesting to note that the abrupt drop in calcium excretion coincided with sudden increases in calcium absorption and retention at 5 wks and may be related to the most active bone formation phase.

The second part of the study demonstrated, for the first time, the physiological significance of endogenous prolactin in the regulation of calcium metabolism in young nonmated rats. A lower body weight gain and a tendency for a lower food intake in the $-PRL_{endo}$ group were consistent with the action of endogenous prolactin in increasing food consumption reported in pregnant (Lotinun et al. 1997) and lactating rats (Gerardo-Gettens et al. 1989; Lotinun et al. 1997). However, since bromocriptine has been reported to cause nausea (Zarate et al. 1978; Thorner et al. 1980) and loss of appetite (Eiler et al. 1995) in human subjects; the reduction in food intake and body weight gain in this experimental group may also be partly due to the effect of bromocriptine per se. In control female rats maximum bone growth, and possibly calcium utilization, took place around 5 wks of age when total calcium excretion was at its nadir and calcium absorption efficiency and total calcium retention reached their peak values. However, in the absence of prolactin, maximum absorption and calcium retention were delayed from 5 to 7 wks, suggesting that endogenous prolactin was essential for initiating or maintaining the high efficiency of intestinal calcium absorption and retention in the young growing female rat. Moreover, measurement of bone calcium content of the 11-week old rats showed that the presence of endogenous prolactin prevented a net loss of calcium from the trabecular bone. How prolactin affected bone metabolism was not known at present. Studies on prolactin receptor expression in human, mouse, and rat fetal tissues have shown expression of prolactin receptor messenger RNA in bone tissues (Royster et al. 1995; Freemark et al. 1997; Tzeng and Linzer 1997). Recently, a direct demonstration of prolactin receptor expression in bone cells was reported (Clement-Lacroix et al. 1999). But the putative role of prolactin in bone remained speculative. The different effect of endogenous prolactin on bone calcium content in young growing animals and pregnant rats may have resulted from the interplay among many factors namely prolactin levels, age of the animals, and the hormonal environment.

In the present studies, two models of hyperprolactinemia were used, i.e., hyperprolactinemia induced by daily administration of a high dose of ovine prolactin and that induced by rat pituitary implantation. Ovine prolactin shares 61% amino acid sequence homology with rat prolactin and is able to bind to the rat prolactin receptor (Hooper et al. 1993) with similar potency (Stier et al. 1984; Sinha 1995). The marked stimulatory effect of ovine prolactin in the 3-week old rats confirmed our previous reports of the acute action of

Fig. 7. Fractional calcium absorption (% of intake), total calcium excretion (mmol/kg/day), and the calcium retention (% of intake) over the 3 d balance study in sham-operated female rats (sham) and females with implanted pituitaries (AP) at the age of 3, 5, 7, 9, and 11 wks. *$P < 0.05$ when compared with the sham group.



prolactin in sexually mature rats (Krishnamra et al. 1990; Wangdee et al. 1991; Krishnamra et al. 1993). On the other hand, we could not explain the decrease in fractional calcium absorption in 5- and 7-week old rats. It was speculated that long term treatment with ovine prolactin may have induced an immune response to neutralize the bioactivity of the ovine prolactin. Unfortunately, the presence of prolactin antibodies in treated animals was not evaluated.

Another interesting aspect of prolactin action, i.e., a biphasic action, was demonstrated in the 5-week old rats. While low plasma levels (7.05 ± 0.12 ng/mL) of endogenous prolactin increased the calcium absorption and retention, high plasma levels (91.69 ± 2.88 ng/mL) of prolactin from implanted pituitaries decreased the same parameter. Biphasic action of prolactin has also been reported in Leydig cell steroidogenesis (Weissmesser et al. 1996) and lymphocyte proliferation (Reber 1993). This phenomenon could be explained based on the sequential dimerization of prolactin receptors (Bignon et al. 1994). At high concentrations, more one receptor–one hormone complexes are formed, reducing the cell response and the hormone thus acts like an antagonist (Kuo et al. 1998). On the other hand, the decreased response may be due to the down regulation of the receptors through internalization (Lane and Chen 1991). Moreover, variation in cell response may depend on the type of receptor and its density (Boutin et al. 1998; Shirota et al. 1990; Jahn et al. 1991). For instance, the short form of prolactin receptor in the mammary gland is inhibitory (Berlanga et al. 1997). Thus, the type of receptors, their distribution, homo- or dimerization, and duration of exposure to prolactin may all contribute to the differential responses of the tissues to prolactin.

The overall effects of the two models of hyperprolactinemia were different. Ovine prolactin-induced hyperprolactinemia suppressed the fractional calcium absorption and retention in 5- and 7-week old rats and had no effect on adult rats. Pituitary implantation-induced hyperprolactinemia, on the other hand, consistently increased the absorption and retention of calcium in 7-, 9-, and 11-week old rats. Only the pituitary implanted rats exhibited a significant 16% increase in femoral calcium content. Thus, both low circulating levels of endogenous prolactin and high levels in AP group had effect on bone calcium content. Endogenous prolactin appeared to be required for the maintenance of calcium content in the vertebrae while hyperprolactinemia over the growing period further increased the calcium content in femur.

Very little is known about the putative role of prolactin with respect to bone remodelling. A number of studies have suggested a possible role for prolactin in bone during pregnancy, lactation (Cross et al. 1995; Sowers et al. 1996), and hyperprolactinemia (Schlechte 1995) but no definite conclusion has been drawn. Possible direct action of prolactin on bone cells is supported by a recent report of prolactin receptors on murine osteoblasts, but not osteoclasts, suggesting that prolatin may be required for normal bone formation and maintenance of bone mass (Clement-Lacroix et al. 1999).

Hypogonadism is often associated with hyperprolactinemia and has been evoked to explain the associated osteoporosis or decreased bone mineral content (Biller et al. 1992; Schlechte et al. 1992; Rosen and Adler 1987; Fujimaki et al. 1994; Schlechte 1995). Unfortuately, estrogen levels were not monitored in the present study. However, the fact that femoral calcium content was actually increased in the pituitary implanted group and was not decreased in the $+PRL_{exo}$ – hyperprolactinemic group suggests that mild hyperprolactinemia did not suppress the secretion of gonadal sex hormones. In fact, pituitary-implanted rats had prolactin levels lower than those in pregnant and lactating rats. With the characteristic biphasic action of prolactin in mind, severe hyperprolactinemia (as in prolactinomas) would increase the risk for osteoporosis due either to the associated hypogonadism or a direct effect of prolactin on bone.

In summary, the present longitudinal study of weaned male and female Wistar rats through adoolescence and sexual maturation revealed the most rapid growth occuring between 3 and 6 wks of age. Calcium absorption and retention peaked at 5 wks of age in both genders and appeared to coincide with the most active bone formation period.

The present study also illustrates for the first time a physiological significance of the relatively low circulating levels of endogenous prolactin in enhancing calcium absorption and calcium retention in the 5-week old female rat. An absence of endogenous prolactin shifted peak calcium absorption from 5 wks to 7 wks of age and resulted in a lower vertebral calcium content. Hyperprolactinemia induced by pituitary implantation, on the other hand, consistently enhanced the calcium absorption efficiency and calcium retention from week 7 to week 11. In conclusion, prolactin has been shown to have a role in the regulation of calcium me-

tabolism during growth and development. However, how prolactin acts on its target tissue and whether it exerts action in concert with other hormones remains to be investigated.

Acknowledgements

The authors would like to thank Professor Surat Komindr for his valuable suggestions. We also thank Ms. Kosoom Vongthai for typing the manuscript. This study was supported by research grants from the Thailand Research Fund and the National Science and Technology Development Agency.

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