Acute Effect of Prolactin on Bone \( ^{45}\text{Ca} \) Accumulation in Rats

NATEETIP KRISHNAMRA, JARUGOOL SEEMOUNG, AND LIANGCHAI LIMLOMWONGSE

Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Abstract. In an attempt to evaluate the acute effect of PRL on bone turnover, experiments were performed in sexually mature female Wistar rats which were given an intraperitoneal (ip) administration of 1.25 mM CaCl\(_2\) solution containing 6 \( \mu \)Ci \( ^{45}\text{Ca} \). Dose response study showed a two fold higher rate of \( ^{45}\text{Ca} \) uptake in 30 min by control trabecular bones (sternum and vertebrae 5–6) compared with control compact bones (femur and tibia). Femur exhibited a dose dependent increase in \( ^{45}\text{Ca} \) uptake in response to pharmacological doses of 0.01 and 0.02 mg PRL/100 g body weight but was less responsive to 0.04 mg PRL/100 g body weight. Tibia only responded with increased \( ^{45}\text{Ca} \) uptake to 0.02 and 0.04 mg PRL/100 g body weight while trabecular bones were unresponsive. By varying the intervals between administration of \( ^{45}\text{Ca} \) (0 min) and PRL (0, 30 or 60 min) and bone harvesting (30, 60 or 90 min), it was found that 0.01 mg PRL/100 g body weight had a biphasic action on \( ^{45}\text{Ca} \) movement. It initially enhanced \( ^{45}\text{Ca} \) influx into femur by 30 min followed by an accelerated \( ^{45}\text{Ca} \) efflux from femur back to the circulation. It could be concluded that PRL acutely stimulated calcium turnover in compact bone.

Key words: Bone, Bone calcium turnover, \( ^{45}\text{Ca} \), Calcium uptake, Compact bone, PRL, Trabecular bone

PREGNANCY and lactation place great stress on maternal calcium metabolism as a result of calcium loss to fetus and pups. The increase in intestinal calcium transport seen during pregnancy [1] has been attributed to the hypertrophy of mucosal tissue and to the elevated plasma level of 1,25(OH)\(_2\)D [2, 3]. However, increased duodenal calcium transport in vitamin D deficient pregnant [1] and lactating [4] rats suggested an involvement of other factor(s). PRL is one of the likely candidates because of its elevated plasma level during these periods. Chronic administration of PRL has been reported to indirectly enhance the in vitro duodenal [5] and jejunal [6] active calcium absorption by stimulating renal production of 1,25(OH)\(_2\)D [7]. The possibility of PRL having an acute and direct effect on calcium absorption was first indicated by our earlier observations that a pharmacological dose of 0.02 mg PRL/100 g body weight enhanced calcium absorption as early as 60 min [8, 9]. Since enhanced calcium absorption did not always lead to calcium gain, we further investigated both acute and long term effects of PRL on the distribution of absorbed calcium. We were unable to detect any change in urinary calcium excretion or alteration in \( ^{45}\text{Ca} \) accumulation in gastrocnemius muscle or tibia 3 h after PRL administration, despite a significant increase in calcium absorption [10]. This may be either due to inappropriate time intervals between PRL and \( ^{45}\text{Ca} \) administration and the sample collection or the unresponsiveness of tibia to PRL. On the other hand, 13 day treatment with PRL increased tibial calcium content in young rats and enhanced bone formation in weaned rats [10].

Thus, the present study aimed to evaluate the acute effect of PRL on \( ^{45}\text{Ca} \) accumulation in compact and trabecular bones of rats and to elaborate on possible mechanism of action of PRL.

Received: April 19, 1996
Accepted: November 11, 1996
Correspondence to: Dr. Nateetip KRISHNAMRA, Mahidol University, Faculty of Science, Department of Physiology, Rama VI Road, Bangkok 10400, Thailand
**Materials and Methods**

**Animals**

The experiments were performed on sexually mature 8 weeks old female Wistar rats. The animals, purchased from the Animal Centre of Thailand, Salaya Campus, Mahidol University, were kept in hanging stainless steel cages under a 12 h light: 12 h dark cycle and maintained on laboratory rat chow (F. E. Zuellig Ltd., Bangkok, Thailand) and tap water ad libitum at least 7 days before the start of the experiment.

**Experimental procedure**

After being anaesthetized with sodium pentobarbital (Nembutal, 50 mg/kg body weight) femur, tibia, sternum and lumbar vertebrae 5–6 were harvested and cleaned of adhering tissues. Each bone was rinsed in warm saline, blotted dry and cut in half. Bone cavity was flushed with saline before bones were extracted in 1:1 mixture of absolute alcohol and diethyl ether for 48 h and with diethyl ether for 24 h to remove fat. Then they were dried at 80 °C for 48 h to obtain constant dry weight. Dry defatted and dehydrated bones were then ashed in the muffle furnace at 600 °C for 16 h. The ash was weighed and later dissolved in 3N HCl and the supernatant was determined for 45Ca contents.

**Experimental protocol**

Dose response study in femur, tibia, sternum and lumbar vertebrae 5–6 of sexually mature rats: After an overnight fast with access to water, the rats were weighed and intraperitoneally injected with 0.9% NaCl or pharmacological doses of 0.01, 0.02 or 0.04 mg PRL/100 g body weight; this was regarded as time 0 min. Thirty min later, 0.5 mL/100 g body weight of 1.25 mM calcium chloride solution containing approximately 6 μCi 45Ca (initial specific activity 2 mCi/mL, 1 Ci=37 Gbq, Radio-chemical Centre, Amersham, U.K.) was administered intraperitoneally. Bones were harvested at 60 min.

Acute effect of PRL on 45Ca turnover in femur of sexually mature rats: In this series of experiments, sexually mature rats were divided into 7 groups. Each group was subdivided into control and PRL treated group, the latter of which received 0.01 mg PRL/100 g body weight. The time intervals between administration of PRL and 45Ca and the harvest of femur were varied in an attempt to evaluate the acute effect of PRL on 45Ca movement into and out of the femur.

**Analyses**

The 45Ca radioactivity was measured by standard liquid scintillation technique (LKB liquid scintillation counter model 1219) with quench corrected by the external standard ratio method.

Ovine PRL (Sigma, St. Louise, Mo) was dissolved in isotonic saline adjusted to pH9 with 2 M NaOH.

**Statistical analyses**

All data were presented as mean ± SEM. Two sets of data were compared using student’s t-test. Multiple comparisons were made by one way analysis of variance (ANOVA); and the significance of difference between groups was determined by Neuman-Keuls test. The level of significance for all statistical tests was 0.05. The calculation was performed with the Primer Stat Program, McGraw-Hill Inc., version 10.

**Results**

**Dose response study in femur, tibia, sternum and lumbar vertebrae 5–6 of sexually mature rats**

Table 1 shows the total calcium contents in mmole/g dry weight of femur, tibia, sternum and lumbar vertebrae of control and PRL treated sexually mature rats. Calcium contents in the four bones despite some variations were comparable when expressed per dry weight. PRL regardless of the dose used had no effect on total calcium contents at 60 min. Total 45Ca contents (% administered dose/g dry weight) of femur, tibia, sternum, and lumbar vertebrae 5–6 in sexually mature rats, were depicted in Fig. 1. In contrast to the total calcium contents, compact bones, namely femur and tibia accumulated less 45Ca than trabecular bone. Results showed that femur demonstrated responses to 0.01 and 0.02 mg PRL/
PRL AND BONE $^{45}$Ca UPTAKE

100 g body weight in a dose dependent manner. $^{45}$Ca contents significantly increased from a control value of $2.49 \pm 0.13\%$ dose/g dry weight to $3.84 \pm 0.41$ and $8.27 \pm 0.41\%$ dose/g dry weight in 0.01 and 0.02 mg PRL/100 g body weight groups, respectively. Peak response in femoral and tibial $^{45}$Ca contents were seen with 0.02 mg PRL/100 g body weight. A higher dose of 0.04 mg/100 g body weight, although still significantly elevated the $^{45}$Ca content above control value, was less effective than 0.02 mg PRL/100 g body weight. In contrast, trabecular bone did not respond to 0.01, 0.2 mg or 0.04 mg PRL/100 g body weight.

Acute effect of PRL on $^{45}$Ca turnover in femur of sexually mature rats

Table 2 shows femoral $^{45}$Ca contents in rats which received $^{45}$Ca administration prior to or concurrently with PRL administration. Concurrent administration of $^{45}$Ca and PRL resulted in a significant decrease in $^{45}$Ca content from $8.25 \pm 0.40$ to $6.44 \pm 0.37\%$ dose/g dry weight ($P<0.01$) and from $5.79 \pm 0.82$ to $2.45 \pm 0.67\%$ dose/g dry weight ($P<0.01$) if bones were harvested at 60 and 90 min, respectively. On the other hand, femoral $^{45}$Ca content was significantly increased from $5.57 \pm 0.26$ to $6.62 \pm 0.40\%$ dose/g dry weight ($P<0.05$) if PRL was administered at 30 min and bone harvest was at 60 min.

**Discussion**

More than 99% of the body calcium is in the skeleton which, besides being the major storage site for calcium, also plays an important role in regulating extracellular fluid calcium concentration. This calcium homeostasis is regulated by the magnitude of bone turnover as well as the calcium content of the bones. Measurements of bone formation and resorption made by morphometric methods at the tissue level and by biochemical markers have revealed the metabolic heterogeneity of the skeletal system between different types of bone. However, to study bone calcium turnover which can be detected in the time scale of min, the
technique involving calcium isotopes was used. 45Ca has proved to be valuable tool for physiological and clinical studies of bone as it mixes rapidly after administration with part of the exchangeable calcium of the body [11].

In the present study, 45Ca was used as an extrinsic label for calcium solution which was intraperitoneally administered. 45Ca was absorbed into the mesenteric vein and subsequently into portal vein to the liver then returned to the heart to be distributed to all parts of the body. The intraperitoneal route of administration was selected for 45Ca injection in order to avoid variation in the absorption rate of 45Ca which normally accompanies oral or intragastric route, and to delay the rapid disappearance rate from serum seen with an intravenous administration. This experimental design allowed one to follow the passage of calcium as represented by 45Ca into the rapidly exchangeable calcium compartment in bone. Once there, some 45Ca could almost immediately be returned to the circulation without being incorporated into the mineralized bone [12]. On the other hand, if 45Ca was incorporated into bone, it could be released reversibly through the processes of cell-mediated resorption and non-cell-mediated physicochemical exchange [13, 14].

The response of sexually mature rats to PRL did depend on individual bone and the dose of PRL. As shown in Fig. 1, a dose dependent increase in bone 45Ca uptake was observed in femur. The response of tibia was similar to that of femur but an increase in tibial 45Ca content in response to 0.01 mg PRL/100 g body weight was not significant. The higher dose (0.04 mg PRL/100 g body weight) on the other hand was not as effective. In contrast, trabecular bones were not responsive to PRL. The different responses shown by cortical and trabecular bone have been observed before. For instance, hypophysectomy results in a decrease in trabecular bone volume [15]. In cortical bone, on the other hand, hypophysectomy suppressed periosteal bone formation activity and thereby, a cessation of the cortical bone growth in radius without changing the cortical mass [16]. It is not known why cortical and trabecular bones respond differently to pituitary hormone deficiency in previous investigations or to PRL in the present study. Besides differences in the structure and developmental pattern of these bone types, availability of hormone receptors are obviously important determinant of the response. The different responses of cortical and trabecular bones should be reflected by different rates of 45Ca accumulation in the shaft and in the head of femur which, unfortunately, were not measured in the present investigation.

The present results showed that the femoral 45Ca content increased and then decreased in response to PRL injection 60 and 90 min, respectively, before bone harvesting (Table 2). Furthermore, when the time interval between PRL administration and bone harvesting was fixed at 90 min, while the labeling period was prolonged from 30 min to 60 min, the reducing effect of PRL on the femoral 45Ca content was not observed.

In an attempt to explain the data, we use the kinetic model proposed by Talmage and Grubb [12] for the role of bone in moment to moment serum calcium regulation, to postulate the acute action of PRL on calcium uptake by bone. The labeling time of 30 min allowed 45Ca to enter, according to the model, through the intercellular space between the surface osteoblasts and accumulate in the bone fluid compartment (the first compartment), which constitutes a rapidly exchangeable pool of calcium. Based on the

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Femoral 45Ca contents (% dose/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) 45Ca (0) PRL (0) Bone (30)</td>
<td>4.30 ± 0.24</td>
</tr>
<tr>
<td>- Control (n=7)</td>
<td>4.30 ± 0.24</td>
</tr>
<tr>
<td>- PRL (n=6)</td>
<td>5.51 ± 0.68</td>
</tr>
<tr>
<td>(ii) 45Ca (0) PRL (0) Bone (60)</td>
<td>8.25 ± 0.40</td>
</tr>
<tr>
<td>- Control (n=7)</td>
<td>8.25 ± 0.40</td>
</tr>
<tr>
<td>- PRL (n=7)</td>
<td>6.44 ± 0.37**</td>
</tr>
<tr>
<td>(iii) 45Ca (0) PRL (0) Bone (90)</td>
<td>5.79 ± 0.82</td>
</tr>
<tr>
<td>- Control (n=7)</td>
<td>5.79 ± 0.82</td>
</tr>
<tr>
<td>- PRL (n=6)</td>
<td>2.45 ± 0.67</td>
</tr>
<tr>
<td>(iv) 45Ca (0) PRL (30) Bone (60)</td>
<td>5.57 ± 0.26</td>
</tr>
<tr>
<td>- Control (n=10)</td>
<td>5.57 ± 0.26</td>
</tr>
<tr>
<td>- PRL (n=9)</td>
<td>6.62 ± 0.40*</td>
</tr>
<tr>
<td>(ii) 45Ca (0) PRL (60) Bone (90)</td>
<td>5.53 ± 1.23</td>
</tr>
<tr>
<td>- Control (n=7)</td>
<td>5.53 ± 1.23</td>
</tr>
<tr>
<td>- PRL (n=7)</td>
<td>4.42 ± 1.09</td>
</tr>
</tbody>
</table>

n, number of rats; minutes are shown in brackets; *P<0.05, **P<0.01 compared with control.
rapidity by which the skeleton can correct for deviations in serum calcium, it is believed that the instantaneous moment to moment regulation of serum calcium is handled by movement of calcium across this large quiescent surface area and not by the active remodeling mechanisms [17, 18]. Several lines of evidence suggest that bone fluid is compartmentalized [19–23]. The first compartment probably constitutes the fluid in bone canaliculi and the water bound to the mineral and the osteoid [24]. \(^{45}\)Ca was then returned to the circulation from this compartment by the intracellular transport system involving the osteocyte-surface osteoblast complex [12]. At the same time, some \(^{45}\)Ca in this compartment moved into other compartments i.e., the crystal surface and crystal lattice interior [24] away from the surface osteoblasts or the transport system, becoming less readily exchangeable. The depth of penetration thus determined the magnitude of ion exchangeability between bone and the ECF. The present data showed that the earlier action of PRL involved an enhancement of \(^{45}\)Ca entering bone, resulting in elevated bone \(^{45}\)Ca content 30 min after PRL administration (experiment iv, Table 2). The mechanism involved could not be defined from the present data of total bone \(^{45}\)Ca content.

Results in Table 2 showed that concurrent intraperitoneal administration of PRL and \(^{45}\)Ca 30 min before bone harvest (experiment i) led to an insignificant increase in femoral \(^{45}\)Ca uptake. PRL action could be observed if \(^{45}\)Ca prelabeling took place 30 min prior to PRL and bone was harvested 30 min after PRL as seen in experiment iv. Apparently \(^{45}\)Ca distribution required about 30 min to attain sufficient concentration in the extracellular fluid before reflecting the first action of PRL on femoral calcium uptake. On the other hand, if bone harvest was delayed to 60 or 90 min after PRL administration (as in experiments ii and iii), a reduction in femoral \(^{45}\)Ca content reflected the second action of PRL i.e., acceleration of \(^{45}\)Ca efflux from bone fluid compartment. The appropriate timing of \(^{45}\)Ca, PRL and bone harvest was crucial as seen in experiment v whereby the first action of PRL was not seen if PRL administration and bone harvest were performed at 60 and 90 min, respectively, after \(^{45}\)Ca injection. The first action of PRL i.e., enhancement of \(^{45}\)Ca uptake, which should have been detected in femur harvested 30 min after PRL was not observed here because by 90 min \(^{45}\)Ca had already equilibrated beyond the first compartment.

Interestingly, the appropriate 30 min labeling time was consistent with half time of the femur \(^{45}\)Ca uptake previously reported [25]. Concerning the readily exchangeable pool of calcium, it has recently been proposed [26] that the calcium binding sites on the quiescent bone surface may be involved in the acute regulation of serum calcium. These sites could also be the target of PRL action. Expression of PRL receptor mRNA has been reported in a number of tissues [27–30] but not yet in bone of mature rats. A recent study demonstrated PRL receptor gene expression in the proliferating and maturing chondrocytes of developing bones among other tissues of fetal rats [31]. On the other hand, GH has been shown to stimulate production of insulin like growth factor I (IGF-I) which together with other cytokines, stimulate osteoblast proliferation and differentiation [32]. Since GH and PRL are closely related and their receptors belong to the same family [33], a direct action of PRL on bone is a likely possibility.

Regardless of the mechanism of action, we proposed that PRL had a biphasic action, first acutely enhancing the uptake of serum calcium into the rapidly exchangeable compartment of calcium in the femur and later on accelerating the efflux of calcium back to the circulation. The biphasic response, together with the finding that 0.04 mg PRL/100 g body weight produced a smaller response than 0.02 mg PRL/100 g body weight (Fig. 1), suggested that there may be more than one type of PRL receptors of different affinity and capacity. This is consistent with the report of two major PRL receptor isoforms which can coexist in the same tissue but may have different biological functions [34]. Although there were no significant change in the total calcium content of bone after acute PRL administration, it was not known whether this acute action of PRL on bone calcium turnover had any bearing on the long term effect on bone. Interestingly, pituitary hormone deficiency has been reported to suppress both bone formation and resorption [15,16] but there has been no direct evidence as to which pituitary hormone is the prime regulator of bone metabolism. Nevertheless, since the bone surfaces covered by the surface osteoblasts occupy about 80% of the
skeleton, although these quiescent surfaces do not participate in active bone formation and resorption [35], they play a crucial role in moment to moment regulation of serum calcium through the rapid process of calcium movement across this large surface area [18]. If PRL had influence on this process as suggested by the present data, it could have a substantial effect on serum calcium regulation, especially with calcium homeostasis beyond weanling age being controlled mainly by long bones [36]. Reports on the interaction between PRL and the calciotrophic hormones suggest a possibility of PRL action on bone being mediated by parathyroid hormone or calcitonin. Evidence of calcitonin inhibitory effect on PRL secretion is numerous [37–39] but reports on the influence of PRL on calcitonin secretion are contradictory. Patients with active acromegaly with elevated serum levels of GH and PRL were reported to have normal basic secretion of calciotrophic hormones [40]. Administration of dopamine agonists and antagonists significantly decreased and increased PRL secretion, respectively, without modifying secretion of parathyroid hormone or calcitonin [41]. On the other hand, serum calcitonin levels were slightly reduced in hyperprolactinemic women with PRL-producing pituitary tumour [42] and in hyperprolactinemic rats with pituitary transplant [43]. Even if hyperprolactinemia results in reduction of calcitonin secretion, since calcitonin plays minimum role in the regulation of bone metabolism under normal condition, lower serum calcitonin levels should cause very little change in bone calcium turnover. There has been no evidence of PRL effect on parathyroid hormone secretion. However, production of parathyroid hormone-related peptide (PTHrP) is stimulated by PRL [44–47]. Thus, a possibility of increased bone turnover seen after exogenous PRL administration being secondary to induced secretion of PTHrP cannot be excluded.

Sex hormones exhibit very characteristic changes during the menstrual and estrous cycles. Whether these cyclic changes have any impact on bone turnover is not known since data on cyclic variations in the biochemical parameters of bone turnover is scant [48, 49]. A recent report showed slight cyclic changes in serum pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (sICTP), a marker of bone resorption, but there were changes neither in other markers of bone resorption nor markers of bone formation [50]. Nevertheless, sex hormones may well be responsible for the variation in $^{45}$Ca uptake and the total bone calcium contents in control animals in the present study. However, this should not interfere with a much larger changes observed under the influence of exogenous PRL.

Decreased bone mineral in hyperprolactinemic human has been assumed to be related to sex hormone deficiency [51–53]. However, bone mineral content in hyperprolactinemic, amenorrheic women was lower than that in both amenorrheic women with normal serum PRL and women with normal gonad [54]. In some cases restoration of gonadal function was not associated with normalization of bone mineral and bone loss in hyperprolactinemia [55]. Thus, besides causing bone loss via suppressing gonadal production of sex hormones, it was likely that PRL could exert direct effect on bone metabolism. The present results supported an hypothesis that PRL accelerated bone turnover of calcium which could be beneficial during lactating period, but could also lead to decreased bone mineral content under hyperprolactinemic condition or in aging adults who already exhibit a negative bone balance.

**Acknowledgements**

The authors would like to thank Wassana Saengumnart and Nittaya Phonethong for technical assistance and Puckjira Gatebute and Chonlada Sapeeya for typing the manuscript.

**References**


