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Effects of Ovariectomy on Duodenal Calcium Transport in the Rat: Altered Ability to Adapt to Low-Calcium Diet[1] (42521)

MARY L. THOMAS[2] AND MICHAEL J. IBARRA

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550

Abstract. Studies were undertaken to determine whether ovariectomy (ovx) would alter the ability of female rats to adapt to low dietary Ca intake by exhibiting an increase in duodenal active Ca transport. Intact and ovx female rats were fed diets containing 1.5, 0.50, or 0.02% Ca prior to measuring active Ca transport using everted duodenal sacs *in vitro*. In some experiments, ovx animals were pair-fed to intact animals of the same age consuming the same diet. When ovx animals were allowed to eat *ad lib*, we found that both growth rate and duodenal active Ca transport increased relative to age-matched, intact controls. However, when growth of ovx animals was maintained at the control rate by pair-feeding, ovx per se did not affect intestinal active Ca transport. Ovx did not alter circulating levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). We found that intact females responded to the low-Ca (0.02%) diet with increased circulating $1,25(OH)_2D$ levels and increased intestinal active Ca transport. Ovx animals exhibited the same increase in circulating concentrations of $1,25(OH)_2D$ in response to low-Ca diet, but did not demonstrate increased duodenal active Ca transport. When ovx animals consumed the diet *ad lib*, they became larger and exhibited higher Ca transport rates than intact animals fed the high-Ca diet, but there was no difference in Ca transport between ovx animals fed diets containing different Ca contents. The results of these experiments demonstrate that in female rats, the ability to adapt to altered dietary Ca intake is dependent on intact ovarian function and is not necessarily directly related to circulating concentrations of $1,25(OH)_2D$.

When animals are fed a diet containing high amounts of Ca, such as is contained in most standard laboratory animal chows, adequate amounts of Ca appear to be absorbed by passive transport processes. It has been shown, using male rats, that young animals are able to adapt to low-Ca diets by increasing their duodenal active Ca transport processes (1), but that the ability to adapt to low-Ca diets declines with age (2, 3).

Recently, work from several laboratories using vitamin D-deficient rats demonstrated that during pregnancy and lactation, duodenal Ca transport is markedly increased even in the absence of vitamin D or its metabolites (4, 5). These findings suggest that some vitamin D-independent mechanism exists for increasing intestinal Ca transport. Furthermore, the fact that these alterations occur at a time when there are marked changes in sex hormone status suggests that there may be an effect of one or more female reproductive hormones on intestinal active Ca transport. The current experiments were carried out to test the hypothesis that sex hormone status might affect the ability of female rats to adapt to low-Ca diets. In the course of these studies, we found that a complication exists in studying effects of ovarian hormones on duodenal Ca transport in female rats: the increase in growth rate of female rats following ovx is accompanied by an increase in duodenal Ca transport. We are, therefore, also reporting this effect, which is an important factor to be considered in designing future studies using the ovx rat to study the role of ovarian hormones in the regulation of Ca metabolism.

Methods. *Animals.* Female rats (weanlings or 200–225 g) were obtained from Holtzman Co. (Madison, WI). All animals were fed test diets obtained from Teklad Test Diets (Madison, WI) containing either 1.5, 0.50, or 0.02% Ca. All diets contained 0.4% phosphorus and 2.2 IU vitamin D_3 per kilogram. Ovariectomy

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(ovx) was performed under ether anesthesia via a dorsal incision. Completeness of ovx was verified by examination of the uterus at sacrifice. In some studies, ovx rats were pair-fed to age-matched intact controls. In these cases, intact animals were allowed to eat *ad lib.* The diet was weighed daily to assess consumption, and ovx animals were given the weight of diet consumed on the previous day by intact control animals eating the same diet. All animals received free access to deionized water and were maintained on a 12-hr-light/dark cycle. Animals were killed between 0930 and 1200 hr. They were not fasted prior to carrying out the experiments.

Intestinal active Ca transport. Active Ca transport was measured in everted duodenal sacs *in vitro,* using a modification of the method of Wilson and Wiseman (6). The proximal duodenum was removed from ether-anesthetized rats and placed in cold saline. The intestine was cleaned of adherent fat, rinsed with cold saline, and everted using polyethylene tubing (1.67 mm, o.d.). Sacs were formed from the proximal 6 cm of duodenum by ligating with 3-O silk suture 0.5 cm from each end. Each sac was filled with 0.35 ml of transport buffer (125 m*M* NaCl, 10 m*M* fructose, 30 m*M* Tris, 0.25 m*M* $CaCl_2$, pH 7.4), placed in a 25-ml Erlenmeyer flask containing the same buffer, and gassed with O_2. The flasks were placed in a 37°C shaking water bath for 90 min and regassed every 30 min. At the end of the incubation period, the medium was collected from the flasks (M, the mucosal side of the sac) and from inside of the sacs (S, serosal side). The total Ca concentration of each fraction was determined by atomic absorption spectrophotometry. The active transport capability of each sac is expressed as the serosal-to-mucosal ratio (S/M) of Ca. If there were no active Ca transport, the S/M would be 1.0. Using this procedure, we have demonstrated that equilibrium is established by 30 min and is maintained throughout at least 120 min of incubation (data not shown).

Serum Ca was measured using atomic absorption spectrophotometry. Serum samples were diluted in lanthanum–HCl to eliminate interference by serum PO_4. Circulating $1,25(OH)_2D$ levels were determined in serum using radioreceptor assay kits obtained from Immuno Nuclear Corp. (Stillwater, MN). The coefficient of variation of this assay was 12.2% between assays and 6.3% within assay.

The statistical significance of the differences among mean values was determined using Student's *t* tests. Two-way analysis of variance was also used in the analysis of the studies of dietary Ca effects.

Results. The experiment depicted in Fig. 1 was designed to determine the relationship between three dietary Ca content levels (0.02, 0.5, and 1.5% Ca) and duodenal active Ca transport in intact and ovx female rats. At 3 months of age, 21 females were ovx. Following surgery, these animals and 21 age-matched intact animals were placed on the 0.5% Ca diet and allowed to eat *ad lib* for 2 weeks. Each group was then subdivided into three diet groups: 0.02, 0.5, and 1.5% Ca. These diets were fed for 2 additional weeks prior to measurement of duodenal active Ca transport. At the time when Ca transport studies were carried out, animals had been ovx for a total of

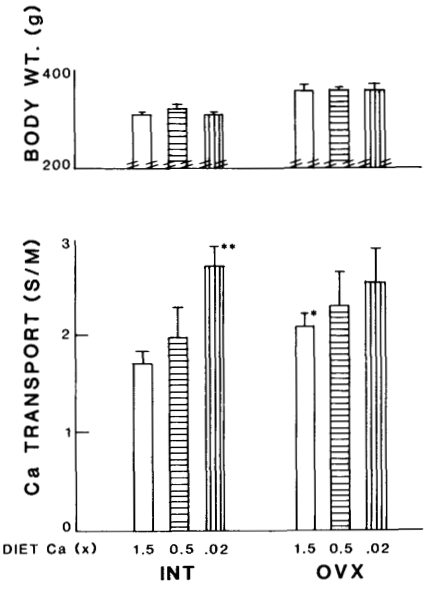

FIG. 1. Active Ca transport (lower panel) and body weight (upper panel) in intact and ovx rats fed high- (1.5%), normal- (0.5%), or low- (0.02%) Ca diet. Animals were ovx 2 weeks prior to beginning the test diets for 2 additional weeks. All animals were allowed to eat *ad lib* throughout the experiment. Each bar represents the mean + SE for five to seven animals. In each diet group, the ovx animals were significantly heavier ($P < 0.01$) than the intact animals. * $P < 0.05$, ** $P < 0.03$ vs intact 1.5% Ca.

4 weeks and has been fed the 1.5 or 0.02% Ca diets for 2 weeks.

The data in Fig. 1 show that the intact animals exhibited increased active Ca transport in response to the low-Ca diet. Although there was no statistically significant difference in Ca transport between the 1.5 and 0.5% Ca groups, the data suggest a concentration–response relationship between dietary Ca content and duodenal active Ca transport in intact animals. The ovx animals all exhibited higher transport levels than the intact 1.5% Ca group, but between the ovx animals, there was no significant effect of dietary Ca concentration on Ca transport. The ovx animals were significantly heavier than the intact rats were. There were no significant differences in serum Ca among any of the groups in this experiment or any of the following experiments (data not shown).

In order to clarify the relationship between the effects of ovx on weight gain and Ca transport, animals were ovx at 9 weeks of age and allowed to eat the 1.5% Ca diet *ad lib* for 3 weeks. Age-matched intact animals were maintained under the same conditions. Ovx resulted in a significant increase in both weight and duodenal active Ca transport relative to intact animals (Fig. 2a). In order to determine whether ovx, per se, or the associated growth was responsible for the increase in duodenal Ca transport, a similar experiment was carried out in which 9-week-old females were ovx and then pair-fed to intact, age-matched animals for the subsequent 3 weeks. In this experiment there was no difference in weight or Ca transport between the two groups (Fig. 2b). In other studies we have further explored the relationship between body weight gain and duodenal Ca transport (data not shown). We have found that *ad-lib*-fed ovx rats always exhibit an increase in body weight which is accompanied by an increase in duodenal active Ca transport. Pair-feeding is able to maintain ovx animals at a normal rate of body weight gain for periods of 3–8 weeks, depending on the age of the animals. We have consistently found that as long as body weight is maintained at control levels by pair-feeding, active Ca transport also remains at control rates.

The following studies were undertaken to determine the ability of ovx animals pair-fed to intact controls to adapt to low dietary Ca intake. Ovx was performed at $3\frac{1}{2}$ weeks of age, pair-feeding of ovx to intact animals was begun immediately following ovx, and animals were raised to maturity on the 1.5% Ca diet. At 10 weeks of age, after the intact animals had reached sexual maturity, half of each group was placed on the 0.02% Ca diet. For 2 additional weeks, the ovx animals in each diet group were pair-fed to the intact animals eating the same diet. As can be seen in Table I, cir-

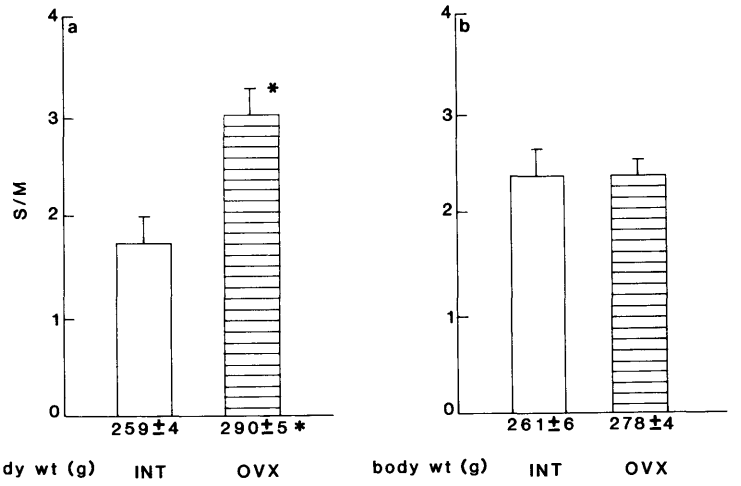

FIG. 2. Effect of ovx on weight gain and intestinal active Ca transport in *ad lib*-fed animals (a) and pair-fed animals (b). Ovx was performed at 9 weeks of age. Measurements were made 3 weeks later. Each bar represents the mean + SE from five or six animals. There was no significant difference in weight or Ca transport between intact and pair-fed animals (b). For free-fed animals, * $P < 0.01$ vs intact.

TABLE I. SERUM $1,25(OH)_2D$

	Serum $1,25(OH)_2D$ (pg/ml)	
Dietary Ca (%)	1.5	0.02
Intact	55 ± 5.5	127 ± 10*
Ovx	60 ± 4.2	123 ± 10*

Note. See Fig. 3 legend for experimental details.
* $P < 0.001$ vs 1.5% Ca diet, same ovarian status.

culating $1,25(OH)_2D$ levels increased in response to the low-Ca diet to the same extent in both intact and ovx animals. The data in Fig. 3 show that match-feeding was successful in keeping the growth of the ovx females the same as that in intact females. As in Fig. 1, the intact animals exhibited increased Ca transport in response to the low-Ca diet. Ca transport of ovx animals on the 1.5% Ca diet was not significantly different from intact animals consuming the same diet. However, the ovx animals failed to exhibit increased duodenal Ca transport in response to the low-Ca diet in spite of the fact that serum $1,25(OH)_2D$ was elevated relative to ovx animals fed the 1.5% Ca diet. By two-way analysis of variance, the interaction of ovx and dietary Ca content was significant at the $P < 0.05$ level. These findings have been confirmed in other, similar experiments.

Discussion. We are reporting two new findings concerning the relationship between ovarian hormone status and duodenal active Ca transport in the rat. The possibility that ovarian hormones may, either directly or indirectly, affect intestinal Ca absorption is of interest with regard to several periods of life including skeletal growth during adolescence and mineral and bone metabolism during reproduction and aging. The finding reported here, that increased growth following ovx in rats is accompanied by increased duodenal Ca transport, is of interest both in terms of understanding the underlying mechanism and in terms of the possible complications when using the ovx rat as a model for studying the effects of ovarian hormones on Ca metabolism. In addition to the data presented here, we have measured Ca transport in *ad lib*- and pair-fed ovx animals in a variety of other studies. In every case, an increase in body weight gain in ovx animals relative to intact animals has been accompanied by an increase in duodenal active Ca transport.

We used the everted duodenal sac *in vitro* for measurement of active Ca transport. This technique was chosen because it permitted sampling from large numbers of animals in each experiment, and also because it has been used to demonstrate the vitamin D-independent effects of pregnancy (4) and lactation (4, 5) on intestinal Ca transport.

Ovx is known to increase growth rate in both immature and mature rats. Jansson *et al.* (7) have shown that estrogen can decrease long bone growth in the presence or absence of GH, and that in oxv rats, long bone growth is increased. We have shown that ovx-induced increase in growth rate is accompanied by increased duodenal active Ca transport, and that limiting growth by limiting food intake is sufficient to prevent the increase in Ca transport. To our knowledge, this effect of ovx to increase Ca transport in the rat has not been previously reported. The ovx rat has been of interest as a model for the study of postmenopausal osteoporosis. The present data indicate that pair-feeding ovx animals to intact controls would be useful in this model to prevent the increase in Ca transport which accompanies increased growth.

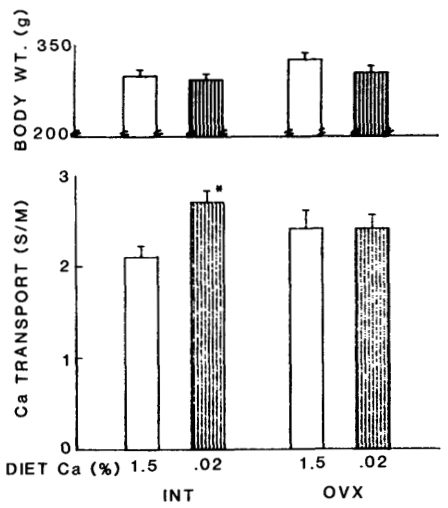

FIG. 3. Active Ca transport (lower panel) and body weight (upper panel) of intact and ovx animals fed high- (1.5%) or low- (0.02%) Ca diets. Ovx was performed at $3\frac{1}{2}$ weeks of age. Ovx animals were match-fed to intact animals throughout the experiment. The low-Ca diet was begun at 10 weeks of age, 2 weeks prior to obtaining these data. Each bar represents the mean + SE for five to eight animals. There were no significant differences in weight among any of the groups. * $P < 0.01$ vs intact 1.5% Ca.

The results of the present studies indicate that intact ovarian function is required for adult female rats to exhibit a normal relationship between dietary Ca intake and duodenal Ca transport. We have shown that, as has been previously reported in male rats (1, 2), intact female rats exhibit an increase in circulating $1,25(OH)_2D$ and an increase in duodenal active Ca transport in response to low dietary Ca intake. However, ovx females fed a low-Ca diet did not exhibit an increase in active Ca transport, even though they had elevated circulating $1,25(OH)_2D$ levels.

Previous work using vitamin D-deficient female rats has indicated that there are vitamin D-independent mechanism(s) which can serve to increase duodenal Ca absorption during pregnancy and lactation (4, 5, 8). The studies reported here were carried out based on those reports in an attempt to define a situation in nonpregnant, nonlactating, vitamin D-replete rats in which alteration of sex hormone status is associated with altered duodenal Ca transport. To our knowledge, this is the first report to demonstrate alterations in duodenal Ca transport in response to ovx, which cannot be attributed to alterations in circulating $1,25(OH)_2D$. We should be able to use the finding that ovx female rats do not exhibit increased duodenal Ca transport in the same manner as intact females as a model system in which to determine the direct mediators of the sex hormone effects on duodenal Ca absorption.

The present finding that ovx did not alter serum concentrations of $1,25(OH)_2D$ is in agreement with results reported by Pavlovitch *et al.* (9), who found that ovx of female rats decreased neither circulating $1,25(OH)_2D$ nor the duodenal mucosal content of vitamin D-dependent calcium-binding protein. The fact that ovx does not alter the $1,25(OH)_2D$ increase in response to low-Ca diet indicates that the mechanism underlying the loss of duodenal adaptation is different from that which occurs in aging rats. Armbrecht and colleagues have presented data in a series of studies (10–12) indicating that the alteration which prevents older animals from adapting to low-Ca diets is a loss of renal response to PTH in terms of activation of the 25-hydroxyvitamin D-1-hydroxylase. In old animals that fail to adapt to low-Ca diets, intestinal Ca transport has been shown to be responsive to $1,25(OH)_2D$ (12). In contrast, the present data suggest that in ovx females, the duodenum may be unable to respond to increased circulating $1,25(OH)_2D$ with an increase in Ca transport. Further studies will be required to determine if this is the case.

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