Regulation of Duodenal Insulin-Like Growth Factor I and Active Calcium Transport by Ovariectomy in Female Rats[1] (43466)

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Abstract. **There is a significant body of data that supports the concept that reproductive hormones in females have effects on duodenal calcium transport that are not mediated via altered circulating concentrations of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Previously, we have shown parallel alterations in duodenal Ca transport and longitudinal bone growth rate in sexually maturing female rats in response to ovariectomy and estradiol (E) treatment of ovariectomized (OVX) rats (OVX+E) without any change in circulating levels of $1,25(OH)_2D$ or parathyroid hormone. Results are presented here from experiments designed to: (i) further explore the relationship between $1,25(OH)_2D$ and ovarian status in the regulation of duodenal calcium transport, and (ii) determine whether OVX and E replacement alter circulating and duodenal levels of insulin-like growth factor I (IGF-I) that might be related to effects on Ca transport. Growth hormone, which has been shown to affect intestinal Ca absorption and vitamin D metabolism, is thought to act indirectly by stimulating IGF-I.**

Six-week-old female rats were OVX, given estradiol implants (OVX+E), and fed a diet containing either 0.5% or 0.1% Ca for 3 weeks. In both diet groups, the OVX animals exhibited a higher level of Ca transport, as measured by the everted gut sac method, than either the intact controls or the OVX+E group; there was no difference in calcium transport between the different diet groups. Although there was no difference in circulating levels of $1,25(OH)_2D$ among the intact, OVX, and OVX+E groups fed either diet, animals fed the 0.1% Ca diet had higher circulating levels of $1,25(OH)_2D$ than those fed the 0.5% Ca diet. There was no difference in duodenal levels of $calbindin_{9K}$ among intact, OVX, and OVX+E animals in either diet group, although the animals fed the 0.1% Ca diet had higher levels of $calbindin_{9K}$ than the animals fed the 0.5%Ca diet. In animals fed the 0.5% Ca diet, OVX resulted in elevated serum and duodenal levels of IGF-1, as compared with intact and OVX+E animals on the same diet. In animals fed the 0.1% Ca diet, there was no elevation of IGF-I in the OVX group relative to intact and OVX+E animals. These results lend additional support to the concept that alterations in duodenal active calcium transport that occur with alterations in ovarian hormones are not mediated by changes in serum levels of $1,25(OH)_2D$, but may be related to some factor related to growth, possibly IGF-I.

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A significant body of data now supports the concept that reproductive hormones in females have effects on duodenal calcium transport that are not mediated via altered circulating concentrations of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (1–5). We have shown previously that during sexual maturation, duodenal Ca transport in the female rat decreases in parallel to the decrease in the long bone growth rate (5); ovariectomy prior to sexual maturation prevents the age-related decreases in both Ca transport and growth rate from occurring (6). These alterations in calcium transport occurred without any change in circulating levels of $1,25(OH)_2D$ or parathyroid hormone. One possible explanation for the observed effect is that ovarian hormones somehow modulate the sensitivity

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of target tissues to the effects of $1,25(OH)_2D$. Another possibility is that the regulation of duodenal active calcium transport is not solely dependent upon $1,25(OH)_2D$. Our working hypothesis has been that both duodenal calcium transport and bone growth are coordinately regulated, either by an endocrine factor that stimulates both calcium transport and bone growth, or by a factor produced by growing bone that stimulates intestinal calcium transport.

Growth and growth hormone have been shown to have effects on the regulation of intestinal calcium transport. Although there are conflicting reports concerning the effects of growth hormone on $1,25(OH)_2D$ production (7–9), growth hormone has been shown to stimulate intestinal calcium transport in hypophysectomized male rats (10). Growth hormone administration has been shown to have no effect on receptor number or affinity of intestinal $1,25(OH)_2D$ receptors (11). Additionally, duodenal content of the vitamin D-dependent calcium-binding protein, $calbindin_{9K}$, has been correlated with growth rate and has been shown to be stimulated by growth hormone administration to hypophysectomized rats (12). Present evidence suggests that, in many cases, growth hormone acts indirectly by stimulating the production of insulin-like growth factor I ([IGF-I] somatomedin C), which appears to be the direct mediator of growth (13, 14). IGF-I is produced at multiple sites and is thought to act locally as a paracrine factor, as well as circulate in the blood to act as a classical endocrine factor (15, 16). IGF-I has been implicated as the direct mediator of increases in serum $1,25(OH)_2D$ in response to a low phosphorus diet (17, 18). One goal of the present work was to determine whether ovariectomy and estradiol replacement might result in alterations in circulating and duodenal concentrations of IGF-I, which could be playing a role in the sex hormone effects on duodenal Ca transport.

A second goal of these experiments was to gain more insight into the interaction between $1,25(OH)_2D$ and ovarian function in the regulation of duodenal Ca transport. We have shown previously that consumption of a diet that is only marginally sufficient in Ca (0.1%) allows young female rats to grow at a normal rate and maintain normocalcemia, while circulating parathyroid hormone and $1,25(OH)_2D$ are elevated and bone mineralization is much lower than in animals fed a higher Ca (0.5%) diet (19). We hypothesized that animals fed the 0.1% Ca diet might exhibit enhanced effects of ovariectomy to increase Ca transport, because circulating $1,25(OH)_2D$ would be elevated in response to the low Ca diet. To address these two questions, we compared the effects of 0.1% and 0.5% Ca diets in intact ovariectomized (OVX), and estradiol-replaced OVX animals to learn more about the interaction of $1,25(OH)_2D$, as altered by dietary Ca consumption, and ovarian hormone status in regulating duodenal Ca transport in sexually maturing female rats.

Materials and Methods

Experimental Design and Animal Manipulation. Rats were obtained from Holtzman Co. (Madison, WI) at approximately 38–40 days of age and were acclimatized to the animal facility for 3 to 5 days before experimental manipulations were performed. At 6 weeks of age (160 g), female rats were ovariectomized under ether anesthesia. At the time of ovariectomy, silastic capsules were placed subcutaneously in the scapular region of appropriate animals. Sham surgery was not performed because it had been determined previously that there was no significant difference between intact animals and sham-operated animals (data not shown). The rats were given free access to deionized water and a diet containing either 0.5% calcium and 0.4% phosphorus (TD83028; Teklad Test Diets, Madison, WI) or 0.1% calcium and 0.4% phosphorus (TD84093) for a period 3 weeks after surgery. The animals were maintained on a 12:12-hr light:dark cycle. At 9 weeks of age, the rats were sacrificed by ether overdose, at which time blood was collected from the abdominal vena cava for serum assays and tissues were collected.

Steroid hormones were administered by way of Silastic (polydimethylsiloxane) capsules, as described by Smith *et al.* (20). Silastic tubing (i.d. 0.078 in, o.d. 0.125 in; Dow Corning Corp., Midland, MI) was packed with crystalline hormone. Wooden applicator sticks were used as plugs and the capsules were sealed with silicone sealant, type A (Down Corning). The dose of the hormone delivered is determined by the surface area of the implant. The length of the implant capsule (4 mm) was designed to produce a hormone level that would approximate a circulating level of estradiol equivalent to the estrus level in the adult female rats (21).

The present study consisted of two separate experiments. In the first experiment, 45 6-week-old female rats were randomly assigned to an intact control group, an ovariectomized group (OVX), and an OVX group given 4-mm estradiol implants (OVX+E), as described above. Each of these groups were divided into two diet groups: 0.5% calcium diet and 0.1% calcium diet. Serum concentrations of Ca, P, estradiol, $1,25(OH)_2D$, and IGF-I were determined from blood collected from these animals. A 6-cm section of duodenum, 0–6 cm from the pyloric sphincter, was used to measure active calcium transport by the everted gut sac method and IGF-I was extracted from the mucosal scraping from a 3-cm section of intestine, 6–9 cm from the pyloric sphincter (see below). IGF-I was also extracted from kidneys taken from these animals. A second experiment was run in which 35 animals were prepared in a manner identical to those used in the first experiment. At sacrifice, a 6-cm section of intestine, 0–6 cm from the pyloric sphincter, was collected from each animal for

the measurement of intestinal calbindin$_{9K}$, as described below.

Duodenal Active Calcium Transport. Duodenal active calcium transport was measured using a modification of the everted gut sac method of Wilson and Wiseman (22) as described previously (6). The proximal duodenum was removed from each rat at the time of sacrifice. A 6-cm section of the duodenum was cleaned, everted, and ligated on one end. Ths sac was then filled with a transport buffer containing 0.25 m*M* Ca, 154 m*M* NaCl, 10 m*M* fructose, and 30 m*M* Tris-HCl (pH 7.4). The sac was ligated on the other end and placed in an Erlenmeyer flask containing the same calcium transport buffer. The Erlenmeyer flask was incubated for 90 min in a shaking water bath, during which time the flask was gassed at 30-min intervals with 95% O_2 and 5% CO_2. After 90 min, the calcium concentration of samples taken from the flask (mucosal compartment) and from the inside of the everted gut sac (serosal compartment) was determined by atomic absorption spectrophotometry. This laboratory has shown previously that an equilibrium is established between the serosal and mucosal calcium concentrations by 60 min and that this equilibrium remains stable for an additional 60 min (6). The ratio of the calcium concentrations found in the serosal and mucosal compartments after 90 min is used as an index of active calcium transport.

Serum Assays. Serum calcium concentrations were determined by atomic absorption spectrophotometry in serum that had been diluted with a 0.5% lanthanum solution in order to eliminate interference by serum phosphates. Serum phosphorus was determined by a colorimetric assay of phosphorus, as described by Daly and Ertingshausen (23), using a commercially available reagent and standards (Stanbio, San Antonio, TX).

Serum estradiol was measured by radioimmunoassay using a commercially available kit (Diagnostic Products Corp., Los Angeles, CA). The limit of detection supplied by the manufacturer was listed as 1.4 pg/ml. We used 2 pg/ml as the lowest concentration in our standard curve, and any sample that displaced fewer counts than the 2-pg/ml standard was considered to contain undetectable amounts of 17β-estradiol.

Immunoreactive IGF-I was measured using a commercially available radioimmunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). The kit employs an antibody from rabbit raised against human IGF-I and uses human IGF-I as a standard. The kit has been validated for use on rat samples (17) and dilutions of rat samples are parallel to the standard curve (data not shown). Although the kit is designed to be used directly on plasma samples, all samples were extracted from serum by acid-ethanol extraction, as described by Daughaday *et al.* (24). This is done in order to reduce interferences from specific IGF-binding proteins in blood (25) and serum proteases that release IGF-I from its binding proteins and affect the reproducibility of the assay (26).

Serum, 1,25-dihydroxyvitamin D was measured using a commercially available radioreceptor assay kit (INCSTAR, Stillwater, MN). This assay is based on the method of Reinhardt *et al.* (27). Sample preparation involves the extraction of 1,25$(OH)_2$D from serum using C_{18} and Silica Sep-Paks (Water's Associates, Milford, MA). Recovery, calculated for each sample, was in the range of 65–75%. The coefficient variation for this assay is 6.3% within an assay and 12.2% between assays, as reported previously (5).

Tissue Content Assays. Tissue was collected from each animal in the first experiment at the time of sacrifice and was placed in ice-cold saline. Adherent fat and fascia was first cleaned from the tissues. The kidney was cut into four to six pieces, placed in glycine paper, frozen in liquid nitrogen, and stored at −70°C until extraction. The intestinal mucosa was scraped from a 3-cm section of duodenum into a plastic petri dish and transferred to a 12 × 75-mm polypropylene test tube with a minimum of deionized water. The sample was frozen immediately and stored at −20°C until the time of extraction. Tissue content of IGF-I was measured in acetic acid extracts of tissue homogenates using the method described by D'Ercole *et al.* (16). Although recovery was not determined, a recovery of 91–128% of added IGF-I was reported for this method. IGF-I was measured by radioimmunoassay as described above for serum assays.

Sections of intestine were collected from the animals in the second experiment at the time of sacrifice and placed into ice-cold saline. Adherent fat and fascia were rapidly cleaned from the tissue. The lumen of the intestine was then rinsed with 5 ml of saline containing aprotinin (100 kIU/ml), placed in glycine paper, and frozen in liquid nitrogen. The tissue samples were stored at −70°C until shipped on dry ice to the laboratory of Dr. M. E. Bruns (Charlottesville, VA) for the purpose of assaying the intestinal levels of calbindin$_{9K}$. Calbindin$_{9K}$ was measured by radial immunodiffusion by Bruns *et al.* (28). Immunoreactive calbindin$_{9K}$ is expressed as μg calbindin/mg protein.

Statistical Analysis. All data are expressed as the mean and SE values for each group. Experimental data were evaluated by means of a two-way analysis of variance, using the general linear model procedure with modifications for an unbalanced design and missing values in a statistical package (SAS Institute, Cary, NC). When the F-value indicated a significant effect ($P < 0.05$), post hoc *t* tests were used to determine significant differences among groups. In the case in which serum estradiol levels were undetectable in some samples by the assay method used, all serum estradiol levels were given rank values such that nonparametric statistics could be performed. In each diet group, the Kruskal-

Wallis test was used to determine the effect of surgery and Silastic implants on serum estradiol levels while the Mann-Whitney *U* test was used to compare the individual groups. Possible differences within the same treatment group fed the different diets were tested by the Mann-Whitney *U* test.

Results

Duodenal calcium transport, as measured by the everted gut sac method, is displayed in Figure 1. As in previous experiments, there was a significant effect of sex hormone status on calcium transport. Within each diet group, the ovariectomized group had a higher level of calcium transport than either the intact control group or the estradiol-treated group. There were no significant differences in the level of calcium transport between intact and OVX+E animals in either diet group. There was no significant effect of diet on the level of calcium transport.

Table I shows the serum levels of calcium, phosphorus, estradiol, and $1,25(OH)_2D$ from these animals. There were no significant differences among any of the groups for serum calcium or phosphorus. Ovariectomy resulted in undetectable levels of circulating estradiol in both diet groups, which were significantly lower than in animals in either the intact group or the OVX+E group. There was no significant difference in circulating estradiol levels between the intact and OVX+E groups. There was also no significant difference between serum concentrations of estradiol in intact animals fed the two different diets or in the OVX+E animals fed the two different diets.

There was a significant effect of the marginal calcium diet, but not of sex hormone status, on serum levels of $1,25(OH)_2D$ (Table I). Each of the groups fed the marginal calcium diet had a circulating level of $1,25(OH)_2D$ that was higher than the same group fed the normal calcium diet.

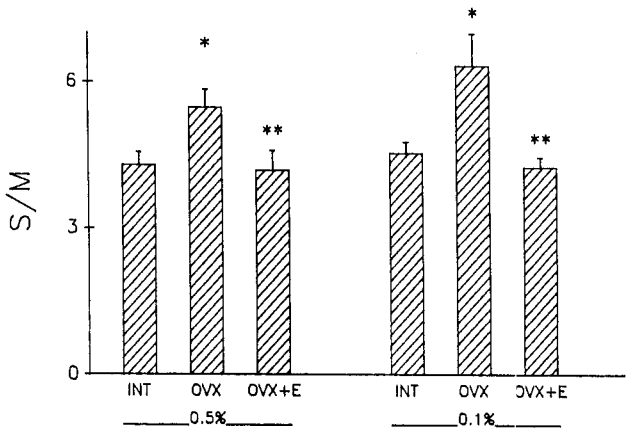

Figure 1. Level of duodenal active calcium transport (serosal to mucosal [S/M]) as measured by the everted gut sac method in 9-week-old female rats fed either a 0.5% Ca diet or a diet containing 0.1% Ca. *$P < 0.05$ versus the intact control group (INT) fed the same diet. **$P < 0.05$ versus OVX animals fed the same diet.

There was a significant effect of sex hormone status on body weight (Table II). In both diet groups, rats were heavier 3 weeks after ovariectomy than the corresponding intact controls. Three weeks of estradiol administration to ovariectomized rats resulted in a decrease in weight gained during the 3-week period. There were no differences in body weight between animals of the same gonadal status fed the different diets. Three weeks after ovariectomy and estradiol implantation, OVX+E animals weighed less than intact animals fed the same diet. There were no effects of diet or gonadal status on kidney weight. However, the 0.1% Ca diet resulted in an increased duodenal weight in the OVX and OVX+E animals relative to the same groups fed the 0.5% Ca diet (Table II).

The relative organ weights: body weight for the kidney and duodenum are shown in Figure 2. There was a significant effect of sex hormone status, but not diet, on the kidney weight to body weight ratio. There was a significant effect of both sex hormone status and diet on duodenum sac weight to body weight ratio (DW:BW). Because there was no effect of sex hormone status on duodenum sac weight (Table II), the effect observed in animals fed the normal calcium diet (0.5%) was due to the effects of sex hormone status on body weight. In animals fed the marginal calcium diet (0.1%), there was a significant increase in DW:BW in intact and OVX animals relative to the same groups fed the normal calcium diet, probably due to the significant effect of the marginal calcium diet on the duodenum weight. In contrast to the pattern seen in animals fed the normal calcium diet, OVX animals fed the marginal calcium diet did not have a lower DW:BW. The OVX+E animals fed the marginal calcium diet had a significantly higher DW:BW than the OVX animals fed the same diet; however, there was no difference between the OVX+E groups fed the two different diets.

Concentrations of insulin-like growth factor I extracted from serum, kidney, and duodenal mucosa are shown in Table III. There was a significant effect of both sex hormone status and marginal calcium diet on total circulating levels of IGF-I. In the animals fed the normal calcium diet, serum IGF-I was increased in OVX animals relative to intact control group animals, and OVX+E animals had a lower circulating level of IGF-I than OVX animals in a manner parallel to the effect of sex hormone status on body weight. However, OVX animals fed the marginal calcium diet did not exhibit an increase in serum IGF-I, although OVX+E animals fed the marginal calcium diet did exhibit a lower circulating level of IGF-I than the OVX animals fed the same marginal calcium diet. There was no significant effect of sex hormone status or marginal calcium diet on kidney levels of IGF-I, which is consistent with the lack of effect of these treatments on kidney weight.

There was a significant effect of sex hormone status

Table I. Serum Ca, P, Estradiol, and $1,25(OH)_2D$ in Female Rats 3 Weeks after Ovariectomy or OVX + E_2 Treatment

Diet (%Ca)	Group	Ca (mg/dl)	P (mg/dl)	Estradiol (pg/ml)	$1,25(OH)_2D$ (pg/ml)
0.5%	Intact	9.7 ± 0.3 ($n = 7$)	8.5 ± 0.4 ($n = 7$)	20.5 ± 7.8 ($n = 6$)	70.4 ± 15.2 ($n = 7$)
	OVX	10.1 ± 0.2 ($n = 5$)	8.2 ± 0.1 ($n = 5$)	<2.0[a] ($n = 5$)	103.4 ± 15.5 ($n = 5$)
	OVX + E	10.0 ± 0.2 ($n = 6$)	8.4 ± 0.4 ($n = 6$)	42.6 ± 7.9[b] ($n = 6$)	83.0 ± 9.7 ($n = 6$)
0.1%	Intact	9.6 ± 0.3 ($n = 6$)	8.1 ± 0.2 ($n = 6$)	47.4 ± 11.6 ($n = 6$)	194.6 ± 20.4[c] ($n = 6$)
	OVX	9.6 ± 0.3 ($n = 4$)	8.9 ± 0.1 ($n = 4$)	<2.0[a] ($n = 4$)	185.6 ± 5.3[c] ($n = 4$)
	OVX + E	10.2 ± 0.4 ($n = 7$	8.3 ± 0.4 ($n = 7$)	65.9 ± 11.1[b] ($n = 7$)	187.1 ± 14.7[c] ($n = 7$)

[a] $P < 0.05$ versus intact, same diet.
[b] $P < 0.05$ versus OVX, same diet.
[c] $P < 0.05$ versus same group on 0.5% Ca diet.

Table II. Average Body, Kidney, and Duodenum Sac Weights from Female Rats 3 Weeks after Ovariectomy or OVX + E Treatment

Diet (%Ca)	Group	Body (g)	Kidney (g)	Duodenum (g)
0.5%	Intact	247 ± 8.6 ($n = 7$)	1.77 ± 0.08 ($n = 7$)	0.53 ± 0.01 ($n = 7$)
	OVX	296 ± 4.7[a] ($n = 5$)	1.71 ± 0.09 ($n = 5$)	0.49 ± 0.01 ($n = 5$)
	OVX + E	216 ± 5.4[a,b] ($n = 6$)	1.82 ± 0.12 ($n = 6$)	0.58 ± 0.02 ($n = 6$)
0.1%	Intact	241 ± 7.1 ($n = 7$)	1.82 ± 0.08 ($n = 7$)	0.58 ± 0.03 ($n = 7$)
	OVX	279 ± 4.8[a] ($n = 4$)	1.70 ± 0.09 ($n = 4$)	0.60 ± 0.01[c] ($n = 4$)
	OVX + E	217 ± 5.4[a,b] ($n = 7$)	1.82 ± 0.08 ($n = 7$)	0.60 ± 0.02[c] ($n = 7$)

[a] $P < 0.05$ versus intact, same diet.
[b] $P < 0.05$ versus OVX, same diet.
[c] $P < 0.05$ versus same group on 0.5% Ca diet.

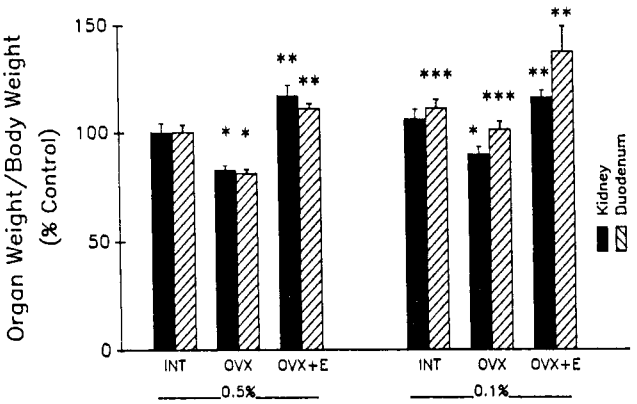

Figure 2. Relative kidney weight and duodenal sac weight to body weight ratios of 9-week-old female rats fed either a 0.5% Ca diet or a diet containing 0.1% Ca. The value shown is the percentage of the value for the intact control group (INT) fed the 0.5% Ca diet. *$P <$ 0.05 versus INT group fed the same diet. **$P < 0.05$ versus OVX animals fed the same diet. ***$P < 0.05$ versus animals of the same sex hormone status fed the normal calcium diet.

and a significant interaction between sex hormone status and dietary calcium on the level of IGF-I in duodenal mucosa from these animals (Table III). Ovariectomized animals fed the normal calcium diet exhibited an increased level of duodenal IGF-I relative to the intact animals fed the same diet, whereas OVX+E animals had a decreased level of mucosal IGF-I relative to the OVX animals. In animals fed the marginal calcium diet, there was no difference in duodenal levels of IGF-I between intact, OVX, and OVX+E animals. OVX animals fed the marginal calcium diet exhibited a lower level of duodenal IGF-I than OVX animals fed the normal calcium diet.

The data in Figure 3 show that there was a significant effect of marginal calcium diet, but not sex hormone status, on duodenal $calbindin_{9K}$. Each group fed the marginal calcium diet had more duodenal $calbindin_{9K}$ than the corresponding group fed the normal calcium diet. Neither diet group exhibited an effect of gonadal status on duodenal $calbindin_{9K}$.

Discussion

The results of these experiments show that the increased Ca transport resulting from ovariectomy of female rats occurs in the absence of change in duodenal $calbindin_{9K}$ content. In confirmation of previous findings (5), the increased Ca transport also occurs in the absence of alterations in circulating concentrations of $1,25(OH)_2D$. Increased $1,25(OH)_2D$ in response to low Ca diet failed to alter the effect of ovariectomy on Ca transport, although it did result in increased duodenal $calbindin_{9K}$ regardless of the gonadal status of the animal. Assuming that duodenal $calbindin_{9K}$ content reflects cellular responsiveness to $1,25(OH)_2D$ (29), these

Table III. Levels of IGF-I in Female Rats 3 Weeks after Ovariectomy or OVX + E

Diet (%Ca)	Group	Serum (units/ml)	Kidney (units/g pro)	Duodenum (units/g pro)
0.5%	Intact	14.1 ± 1.0 ($n = 7$)	25.8 ± 6.4 ($n = 7$)	2.6 ± 0.3 ($n = 7$)
	OVX	21.8 ± 1.4[a] ($n = 5$)	33.7 ± 8.5 ($n = 5$)	4.4 ± 0.6[a] ($n = 5$)
	OVX + E	10.0 ± 1.1[b] ($n = 6$)	19.8 ± 6.5 ($n = 6$)	2.1 ± 0.5[b] ($n = 6$)
0.1%	Intact	13.6 ± 2.3 ($n = 7$)	30.3 ± 9.3 ($n = 7$)	1.9 ± 0.3 ($n = 7$
	OVX	14.7 ± 0.6[c] ($n = 4$)	38.5 ± 9.1 ($n = 4$)	2.4 ± 0.3[c] ($n = 4$)
	OVX + E	9.2 ± 1.3[b] ($n = 7$)	27.8 ± 10.4 ($n = 7$)	3.1 ± 0.4 ($n = 7$)

[a] $P < 0.05$ versus intact, same diet.
[b] $P < 0.05$ versus OVX, same diet.
[c] $P < 0.05$ versus same group on 0.5% Ca diet.

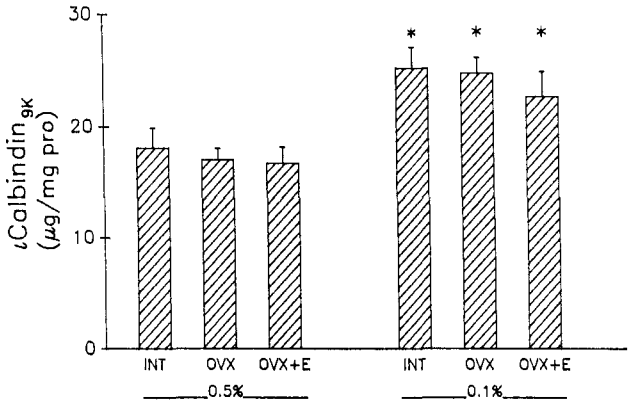

Figure 3. Duodenal levels of calbindin$_{9K}$, as measured by radial immunodiffusion, of 9-week-old female rats fed either a 0.5% Ca diet or a diet containing 0.1% Ca. *$P < 0.05$ versus animals of the same sex hormone status fed the normal calcium diet.

results suggest that sex hormone status does not alter the sensitivity of the intestine to 1,25(OH)$_2$D because there were no significant differences in duodenal calbindin$_{9K}$ among intact, OVX, and OVX+E animals within either diet group.

Experiments by Favus *et al.* (30) have demonstrated that although low amounts of dietary calcium can stimulate 1,25(OH)$_2$D production and calcium transport in duodenal tissue in the Ussing preparation, there is no alteration in the activity of basolateral membrane vesicles, prepared from the same animals, to accumulate calcium by the high affinity, basolateral Ca-ATPase. This apparent vitamin D-independence is consistent with earlier findings by Van Corven *et al* (31) that vitamin D deficiency does not alter the activity of the basolateral Ca-ATPase. Since the everted gut sac preparation is a measure of the ability of the intestine to establish and maintain a calcium gradient, we feel that this preparation primarily reflects the activity of the calcium extrusion pump. Therefore, the apparent lack of dependence on circulating levels of 1,25(OH)$_2$D of the effects of sex hormone status on calcium transport in the present report is consistent with the lack of dependence of basolateral ATPase activity on 1,25(OH)$_2$D as reported by others.

In the present study, we have shown that sex hormone status not only alters the level of calcium transport but also alters serum and duodenal levels of IGF-I. There have been previous reports of intestinal localization of both IGF-I (15) and its messenger RNA (32–34). Not only has its production been localized to the intestine, but also receptors for IGF-I have been identified and characterized (34–36). These receptors have been shown to be distributed in a gradient along the villus, with more receptor expressed in the crypt cells and less expressed in the villus tip cells (35). IGF-I may play a role in speeding the differentiation of immature low villus cells such that they have the function of cells more mature with regard to calcium absorption. Alternatively, IGF-I could have some direct effect on the enterocyte to increase calcium transport, possibly at the level of the calcium extrusion pump, which has been shown previously for the high affinity (Ca^{2+}-Mg^{2+})-ATPase in dog kidney basolateral membranes (37).

The alterations in growth rate with sex hormone status were not uniform for all organs, as seen by the differences in kidney and duodenum to body weight ratios. The alterations in kidney weight to body weight ratio with sex hormone status were dependent upon the body weight and not the kidney weight, which was not significantly affected by sex hormone status. Kidney levels of IGF-I were likewise not altered by sex hormone status. The kidney level of IGF-I has been shown previously to be responsive to physiologic stimuli (38). In the present study, it appears that alterations in sex hormone status did not stimulate differences in kidney growth. However, DW:BW was dependent upon both alterations in body weight and an effect on the weight of the duodenal sac. There was apparently intestinal hypertrophy related to decreased dietary calcium intake in this study, although it was not related to alterations in the rate of intestinal calcium transport nor to intestinal levels of IGF-I. The intestinal hypertrophy may have been mediated by a transient rise in IGF-I, as is the case in the physiologic stimulation of renal hypertrophy (38), which could have been missed by measurement at only one point 3 weeks after ovariectomy and/or hormone replacement, as was done in the present study.

If IGF-I plays a role in the alterations in growth and intestinal calcium transport seen with ovariectomy and estradiol treatment, it is unknown why alterations in IGF-I do not parallel the alterations in somatic growth and intestinal calcium transport in the animals fed the 0.1% calcium diet. Although the calcium in the test diets was different, the phosphorus content for both diets was 0.4%. Since dietary phosphorus restriction is

known to stimulate increases in circulating levels of IGF-I, it may be that the higher phosphorus to calcium ratio in the marginal calcium diet suppressed serum and duodenal levels of IGF-I. The observed alterations in intestinal growth and calcium transport in animals fed the 0.1% calcium diet may be due to an alteration of the responsiveness of the intestine to IGF-I or to the decrease of some inhibitory influence that is normally present.

In conclusion, the present study indicates that the alterations in duodenal active calcium transport which occur with alterations in ovarian hormone status in sexually maturing female rats are not correlated with alterations in circulating levels of $1,25(OH)_2D$ or altered end organ sensitivity to $1,25(OH)_2D$, but may be mediated by some factor related to growth, possibly IGF-I. In addition, these data show that in the young female rat, intestinal Ca transport, as measured by the everted sac, is more responsive to ovariectomy than to a 2.5-fold increase in circulating levels of $1,25(OH)_2D$ induced by mild dietary Ca restriction. Furthermore, there was no apparent interaction between the effects of ovariectomy and elevated circulating $1,25(OH)_2D$ to increase duodenal Ca transport.

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