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The Cecum Does Not Participate in the Stimulation of Intestinal Calcium Absorption by Calcitriol

Robert Brommage, Claudine Binacua and Anne-Lise Carrié*

Nestlé Research Centre, Vers-chez-les-Blanc, P.O. Box 44, 1000 Lausanne 26, Switzerland

Intestinal Ca absorption from the diet consumed during one night was measured in male rats fed a normal (0.5%) Ca, low fiber (3% cellulose) diet by determining the decrease in $^{47}Ca/^{47}Sc$ ratio between diet and feces. One-half of the rats had been cecectomized 9 weeks previously at 14 weeks of age. Calcitriol injections, given intraperitoneally the morning of the experiment, stimulated fractional intestinal Ca absorption 2.5-fold in intact rats (16.9 ± 2.0% to 42.2 ± 1.8%) and 2.3-fold in cecectomized rats (20.1 ± 1.4% to 46.8 ± 1.2%). Similar results were obtained when the data were calculated in terms of total Ca absorption expressed as mg/day. Thus, although the cecum can absorb Ca when diets contain large amounts of digestible fiber, cecectomy does not influence the stimulation of intestinal Ca absorption induced by calcitriol in vitamin D-replete rats fed a low fiber diet.

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INTRODUCTION

The distribution of Ca absorption along the various segments of both the small and large intestine continues to be a topic of study. Active transcellular Ca transport, stimulated by calcitriol (1,25-dihydroxyvitamin D_3, the active metabolite of vitamin D_3), has been studied extensively in the duodenum. The jejunum and ileum, considered as "leaky" epithelia, are thought to be capable of absorbing Ca paracellularly due to the fact that the transit time of the digesta through these segments is relatively slow. Certainly, the amount of Ca absorbed by each segment depends upon the circulating levels of calcitriol and the quantity of Ca in the meal. In one study [1] in which rats were previously fed a diet containing an unusually high Ca level of 1.9%, total Ca absorption was 19% from a meal containing ~33 mg of Ca with 88% of that absorption occurring in the ileum and the remaining 12% in the duodenum and jejunum.

The possibility that Ca absorption might also occur in the large intestine is supported by studies indicating that the cecum actively transports Ca both *in situ* [2] and *in vitro* [3–5]. These studies demonstrate the ability of the large intestine to transport Ca under idealized conditions when the tissue is in contact with ionized Ca present in physiological salt solutions. Although the actual concentrations and diffusability of ionized Ca within the cecum *in vivo* are not known, the limited availability of ionized Ca in the large intestine presumably restricts its accessibility to the Ca transport system present in the cecal mucosa and several reports [6–9] have minimized the role of the cecum in intestinal Ca absorption. However, indirect measurements of cecal Ca absorption calculated from arterio-venous Ca gradients across the cecum suggest that the cecum can absorb Ca [10]. In addition, an analysis [11] of the time-course of intestinal Ca absorption in women suggested that the colon accounts for 4% of the Ca absorbed.

The purpose of the present study was to employ a sensitive *in vivo* method [9] to examine intestinal Ca absorption in cecectomized rats receiving a dose of calcitriol that readily stimulates total Ca absorption.

MATERIALS AND METHODS

Male rats of the Fischer 344 strain were purchased from IFFA-CREDO (Les Oncins, France) at 4 weeks of age, housed individually and fed a modified AIN-76A diet formulated to contain 0.5% Ca (as $CaCO_3$), 0.5% P and 1 unit/g vitamin D_3. This purified diet contained 20% casein as the protein source, 15% sucrose, 50% cornstarch, 5% corn oil and 3% cellulose

*Correspondence to A-L. Carrié.
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Table 1. Influence of calcitriol and cecectomy on intestinal Ca absorption

Treatment	n	Food consumption (g/day)	Fractional Ca absorption (%)	Ca absorption (mg/day)
Sham	6	20.7 ± 0.5	16.9 ± 2.0	17.6 ± 2.6
Sham + calcitriol	9	17.8 ± 1.2	42.2 ± 1.8	37.7 ± 3.3
Cecectomy	8	22.0 ± 0.6	20.1 ± 1.4	22.2 ± 1.9
Cecectomy + calcitriol	7	17.8 ± 1.5	46.8 ± 1.8	41.3 ± 3.0
Cecectomy ANOVA P value		0.56	0.03	0.16
Calcitriol ANOVA P Value		0.004	< 0.001	< 0.001
Interaction ANOVA P Value		0.54	0.65	0.87

On the day of the study rats were injected with 2 nmol of calcitriol 9 h prior to their being given the radioactive diet. Rats were cecectomized or SHAM-operated 9 weeks prior to the study. All data are presented as means ± SEM. A two-way ANOVA was performed to identify statistical probabilities of effects due to cecectomy, calcitriol injection and the interaction between these two treatments.

as fiber. At 14 weeks of age, one-half of the rats were cecectomized by standard procedures [12]. At 23 weeks of age, some rats in each group were anesthetized with ether and injected intraperitoneally with 2 nmol of calcitriol dissolved in 50 μl of ethanol 9 h prior to measuring intestinal Ca absorption. Control rats not given calcitriol were neither anesthetized nor injected.

Fractional intestinal Ca absorption was determined from the decrease in the ratio of $^{47}Ca/^{47}Sc$ in feces relative to the diet as described in detail previously [9]. Since Sc is not absorbed by the intestine, ^{47}Sc serves as a nonabsorbable marker [13]. These γ-emitting isotopes and green food dye were mixed into the diet described above and provided to the rats for one night with the green-colored feces collected and counted for radioactivity 3 days later. Total Ca absorption was calculated from knowledge of the quantities of diet consumed. A two-factor ANOVA (SigmaStat software) was employed for the statistical evaluation of the data. ^{47}Ca with its daughter isotope ^{47}Sc was purchased from Risø Isotoplaboratoriet (Denmark).

RESULTS

As presented in Table 1, cecectomized rats consumed normal quantities of diet whereas calcitriol injections decreased food consumption ($P = 0.004$) by approx. 15%. Cecectomy increased fractional Ca absorption slightly ($P = 0.03$) but this effect disappeared when total Ca absorption ($P = 0.16$) was calculated. In both sham-operated and cecectomized rats, fractional and total intestinal Ca absorption values were both dramatically increased ($P < 0.001$) by previous treatment with calcitriol. There were no interactions between cecectomy and calcitriol treatment on any of these parameters.

DISCUSSION

The advantage of this intestinal Ca absorption assay is that rats consume their habitual diet during a single night and that only representative samples of diet and feces need to be analyzed. We have previously employed this assay to show the decline in intestinal Ca absorption with age [9], the influence of the normal estrous cycle [14] and the stimulatory effect of dietary lactulose [9].

The hypothesis that calcitriol stimulates Ca absorption *in vivo* in the large intestine, and particularly the cecum, has received considerable support during recent years as a result of numerous *in vitro* and *in situ* studies. The *in vitro* studies have typically involved voltage-clamped tissue in Ussing chambers [3–5] whereas the *in situ* studies have employed perfusions of emptied cecum [2] or infusions of $CaCl_2$ into the cecum [8]. The Ussing chamber studies have shown that calcitriol stimulates Ca transport from the low values observed in vitamin D-deficient rats but has no effect in normal, vitamin D-replete rats [4, 5].

The major unanswered question resulting from the above studies is whether the Ca present within the cecum is normally sufficiently soluble and diffusable to be available to the calcitriol-dependent active Ca transport system. Our results show that the cecum is not required for the normal stimulation of intestinal Ca absorption induced by calcitriol and thus confirm and extend earlier observations. We have previously shown with identical methodology that the stimulation of intestinal Ca absorption by dietary lactulose does not require the cecum [9]. When examined by classical balance techniques, vitamin D repletion promoted a normal stimulation of intestinal Ca absorption in cecectomized rats [6]. Another study [8] measured intestinal Ca absorption using [^{3}H]polyethylene glycol (PEG) as a nonabsorbable marker and observed no decline in Ca/PEG ratio between the cecal contents and feces and thus concluded that Ca absorption throughout the large intestine was negligible. These last results were similar when total intestinal Ca absorption was stimulated by calcitriol or depressed by a 5-day treatment with a bisphosphonate.

Evidence that the cecum does not normally absorb Ca has also been obtained in rats fed diets containing 0.2% TiO_2 as a nonabsorbable marker by analyzing

Ca/TiO_2 ratios in various segments of the intestinal tract [7]. Ca absorption in each segment was calculated from the decrease in the Ca/TiO_2 ratio observed in that segment. There was no evidence of Ca absorption in the cecum in conventional rats fed diets either with or without 10% lactose and the stimulatory effect of lactose on Ca absorption was manifested in the small intestine. In contrast, germ-free rats absorbed more Ca than conventional rats and the additional Ca absorption occurred in the cecum. Germ-free rats have extremely large cecums and these investigators [15] also observed that the quantity of ultrafilterable Ca in the cecal fluid is greatly increased in germ-free rats.

In summary, despite clear evidence that the cecum contains the necessary components to actively absorb Ca when in contact with physiological salt solutions [2–5], cecal Ca absorption *in vivo* appears to be minimal [6–9] and, as demonstrated in this report, does not contribute significantly to the elevated Ca absorption following injections of calcitriol. However, two exceptions to these findings are germ-free rats [7] and rats fed extremely high levels of digestible fiber [10]. In both cases the cecum undergoes hypertrophy and the viscosity of the cecal contents is greatly reduced. Further studies are required to determine the exact quantity and type of dietary fiber necessary to allow sufficient Ca in the cecal fluid to be ionized and diffusable and thus available to the Ca transport system present in the cecal mucosa.

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