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THE ROLE OF INTESTINAL TRANSPORT PROTEINS IN CORTISONE-MEDIATED SUPPRESSION OF Ca^{2+} ABSORPTION

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SUMMARY

In order to more fully elucidate the mechanisms underlying the reduction of active Ca^{2+} transport in the duodenum produced by glucocorticosteroid administration, studies were undertaken to investigate the role of intestinal proteins which may function in the translocation of calcium in the small intestine. Levels of *in vitro* Ca^{2+} transport, a vitamin D-induced Ca^{2+}-binding protein, a brush border Ca^{2+}-dependent ATPase and a brush border alkaline phosphatase from rat duodenum were measured in animals raised on regular laboratory or vitamin D-deficient diets and given 3 days of glucocorticosteroid treatment. Glucocorticosteroid administration suppressed brush border alkaline phosphatase activity suggesting that intestinal alkaline phosphatase plays a role in mediating the corticosteroid induced depression of Ca^{2+} transport.

INTRODUCTION

Glucocorticosteroid administration reduces active Ca^{2+} transport by the duodenum[1–6], but the mechanisms underlying this effect, which is opposite in direction to that produced by vitamin D, have not been elucidated fully. Recent studies by Kimberg *et al.*[6] indicate that the suppression is mediated independent of any direct reaction with vitamin D or its metabolites.

Several proteins which are thought to have a role in membrane transport have been isolated from bacterial and mammalian organisms[7]. Intestinal proteins which have been proposed as playing a functional role in the translocation of Ca^{2+} in the small intestine include a vitamin D-induced Ca^{2+} binding protein[8–12], a brush border Ca^{2+}-dependent ATPase (Ca^{2+}-ATPase)[13,14] and a brush border alkaline phosphatase[15–17]. Activities of Ca^{2+}-ATPase and alkaline phosphatase may represent measures of the same enzyme[16,17].

Experiments described in this report were undertaken to evaluate the possible regulatory role of these proteins in the corticosteroid-produced diminution of Ca^{2+} transport by the rat duodenum. The results indicate that glucocorticosteroid administration reduces brush border alkaline phosphatase activity and suggest that this enzyme plays a role in mediating the steroid-induced depression of Ca^{2+} transport.

METHODS

Animal preparation

Albino male rats weighing 100–125 g were obtained from the Charles River Breeding Laboratories and fed standard laboratory chow (Ralston Purina Co., vitamin D content 5.31 I.U. per g diet), and tap water *ad libitum*. In certain experiments, weanling male rats were raised on a vitamin D-free diet (General Biochemicals, rachitogenic diet No. 2, U.S.P.) and demineralized water *ad libitum* for 4–5 weeks. All animals were housed in hanging cages in a windowless room with automatically controlled temperature and the lighting regulated so as to provide alternating periods of 12 h of light and dark daily. Animals were injected intraperitoneally with 5 mg of cortisone acetate in 0.2 ml aqueous suspension (Upjohn Co.) daily for 3 days prior to study. In certain experiments, 500 I.U. of vitamin D_2 in 0.05 ml propylene glycol (Sterling Drug) were given intragastrically through a blunt needle inserted through the esophagus 40–48 h prior to sacrifice. Animals were killed by cervical dislocation or decapitation and the intestine was exposed through a midline abdominal incision. The proximal 10–12 cm of intestine was used for all procedures.

Transport studies

Duodenal Ca^{2+} transport was measured by a method based on the *in vitro* everted gut sac technique of Wilson and Wiseman[18] as modified by Martin and DeLuca[19]. After the bile duct was ligated and transected at its point of insertion into the duodenum, the intestine was rinsed *in situ* through an incision at the level of the pylorus with 5 ml of iced 0.119 M NaCl. The intestine was dissected free, trimmed and everted over a chilled glass rod. Sacs of 5 cm in length were filled with 0.5 ml of a medium consisting of 0.125 M NaCl, 0.010 M fructose, 0.00025 M $CaCl_2$, 0.030 M Tris–HCl buffer (pH 7.4), and sufficient $^{45}CaCl_2$ to provide approx. 25000 cpm/ml of medium. The sacs were incubated at 37 °C with continuous bubbling of 100 % O_2 for 90 min in 25 ml Erlenmeyer flasks, which contained 10 ml of an identical medium. At the termination of incubation, the sacs were removed, blotted, and drained. Aliquots (0.1 ml) of the medium inside of the sacs were counted in 10 ml of liquid scintillation fluid containing 2,5-diphenyloxazole as a primary scintillator and α-naphthyl-phenyloxazole as a secondary scintillator in polyethylene vials in a Packard Tricarb spectrometer using automatic external standardization. The transport data are expressed as a concentration ratio of final concentration after 90 min of the tracer inside the sac (serosal medium) over that outside the sac (mucosal medium) written S/M.

Ca^{2+} binding activity

Assay of Ca^{2+} binding activity was based on the method of Kallfelz *et al.*[10]. After the small intestine was rinsed *in situ* with 0.119 M NaCl, through an incision at the level of the pylorus, the excised intestine was opened lengthwise, blotted and mucosa scraped with a chilled glass slide. A 20 % (w/v) homogenate from a pool of mucosa obtained from 6–10 rats was prepared in a buffer composed of 0.12 M NaCl, 0.0047 M KCl and 0.0137 M Tris–HCl buffer (pH 7.4), using a motor-driven Potter–Elvehjem homogenizer with a Teflon pestle. The supernatant obtained after the

homogenate was centrifuged for 20 min at 38000 × *g* in a refrigerated centrifuge was held at 60 °C for 10 min, cooled, and again centrifuged at 38000 × *g* for 20 min and the supernatant was removed for assay. 1 ml of this heat-treated supernatant was mixed with 0.5 ml of Chelex-100 cation exchange resin, which had previously been equilibrated with Tris–HCl buffer and diluted to make a suspension in which the resin represented 50 % of the total volume. To the mixture of resin and supernatant, 0.5 μCi of $^{45}CaCl_2$ were added and the mixture agitated for 15 s. After standing for 5 min, the mixture was again agitated for 15 s and, after an additional 5 min, spun at 1500 × *g* for 5 min. Radioactivity was determined on 0.2 ml aliquots of the supernatant obtained by liquid scintillation spectrometry as described above. Data are expressed as a percentage of the initial radioactivity added to the assay tube per mg of protein. Protein was determined by the method of Lowry *et al.*[20].

Enzyme studies

Brush border preparation. Purified brush borders for enzyme determinations were prepared by the method of Forstner *et al.*[21] as modified by Melancon and DeLuca[14]. The intestine was rinsed *in situ* with iced 0.005 M EDTA (pH 7.4). After centrifugation at 450 × *g* for 10 min, the sediment was washed 3 times with 10 ml of 0.005 M EDTA (pH 7.4). This sediment was suspended in 10 ml of 0.09 M NaCl–0.0008 M EDTA (pH 7.4), and mixed for 30 min. The sediment and supernatant were filtered through 300 mg of glass wool and the glass wool washed through with 20 ml of 0.005 M EDTA (pH 7.4). After centrifugation of the combined washings at 450 × *g* for 10 min, the pellet was washed 3 times with 10 ml 0.1 M Tris–HCl buffer (pH 7.4) before final suspension for determination of enzyme activity.

Alkaline phosphatase activity. The assay was based on the method of Forstner *et al.*[21]. Incubations were performed on brush border suspensions containing less than 10 μg of protein per assay. The assay system contained 0.015 M *p*-nitrophenyl phosphate as a substrate, 0.042 M glycine buffer (pH 9.2), 0.005 M KCl, 0.0042 M $MgCl_2$ and 0.0008 M $ZnCl_2$ in a final volume of 0.6 ml. The mixture was incubated at 37 °C for 15 min before terminating the reaction by the addition of 2.5 ml of 0.02 M NaOH. *p*-Nitrophenol was determined spectrometrically at 400 nm.

Adenosine triphosphatase assay. The assay procedure was based on that of Melancon and DeLuca[14]. Incubations were performed on brush border suspensions containing about 20–100 μg of protein per assay. Reactions were started by the addition of 0.40 ml of iced brush border suspension to an incubation mixture containing 0.040 M Tris–HCl buffer (pH 7.4), 0.005 M K_2ATP, 0.0025 M $MgCl_2$ or 0.005 M $MgCl_2$ + 0.010 M $CaCl_2$. (The latter combination of cations results in a 0.0024 M Mg^{2+}-ATP complex[14].) The mixtures of a total volume of 1.0 ml were incubated at 37 °C for 10 min before terminating the reaction with 1.0 ml of iced 2.5 M H_2SO_4. Phosphate was determined by the method of Gomori[22]. Mg^{2+}-ATPase activity represents phosphate released in the 0.0025 M $MgCl_2$ assay. Ca^{2+}-ATPase is calculated by subtracting the phosphate released in the 0.0025 M $MgCl_2$ assay from that released in the 0.005 M $MgCl_2$ + 0.010 M $CaCl_2$ assay according to the method of Melancon and DeLuca[14].

Statistical analysis. Statistical analyses were performed using paired and non-paired *t* tests and analysis of variance on experimental values or natural logarithms (ln) of experimental data. If the *F* values indicated a statistically significant difference

when analysis of variance was applied, a Newman–Keuls test was used to determine which specific differences were present[23].

RESULTS

The *in vivo* administration of cortisone acetate resulted in a significant depression of Ca^{2+} transport by the rat duodenum in animals fed standard laboratory chow (Table I). The effects of an identical treatment regimen on duodenal transport in rats raised on a vitamin D-deficient diet are shown in Table II. Cortisone administration resulted in an insignificant depression of the S/M ratio when compared to vitamin D-deficient controls. The administration of cortisone did not interfere with the ability of vitamin D to increase the rates of transport when given to the vitamin D-deficient group of animals.

Experiments were conducted in rats raised on normal laboratory chow and on vitamin D-deficient diets in parallel with the above studies in order to demonstrate whether or not cortisone administration affects the levels of intestinal Ca^{2+} binding activity in the duodenum. Cortisone administration had no significant effect on the

TABLE I

EFFECT OF CORTISONE ADMINISTRATION ON ACTIVE TRANSPORT OF CALCIUM IN THE DUODENUM

Animals were fed normal laboratory chow and tap water. Cortisone acetate (5 mg) was administered intraperitoneally daily for 3 days prior to study. Everted gut sacs filled with 0.5 ml of the standard medium were incubated in flasks containing 10 ml of the identical medium for 90 min as described in Methods. Values are calculated from non-paired *t* tests.

Group	*Number of experiments*	*Mean wt* $\pm$ *S.E.* (*g*)	*Mean* $^{45}Ca^{2+}$ *concn ratio* (*serosal/mucosal*) $\pm$ *S.E.*
Control	15	138 ± 5	5.8 ± 0.4
Cortisone	15	129 ± 4	4.2 ± 0.2
P		>0.10	<0.005

TABLE II

EFFECT OF CORTISONE AND VITAMIN D ADMINISTRATION ON ACTIVE TRANSPORT OF Ca^{2+} IN THE DUODENUM IN RATS RAISED ON A VITAMIN D DEFICIENT DIET

Animals were raised on a vitamin D-deficient diet and deionized water for 4–5 weeks. Cortisone acetate (5 mg) was administered intraperitoneally daily for 3 days prior to study. Vitamin D_2 (500 I.U.) was given intragastrically 40 h before sacrifice. *P* values are calculated from analysis of variance and Newman–Keuls tests.

Group	*Number of experiments*	*Mean wt* $\pm$ *S.E.* (*g*)	*Mean* $^{45}Ca^{2+}$ *concn ratio* (*serosal/mucosal*) $\pm$ *S.E.*	*P*
Vitamin D deficient	10	79 ± 2	2.0 ± 0.1	—
Vitamin D deficient + cortisone	8	74 ± 3	1.7 ± 0.2	—
Vitamin D deficient + vitamin D_2	10	69 ± 2	4.1 ± 0.2	<0.01*
Vitamin D deficient + cortisone + vitamin D_2	9	70 ± 3	4.0 ± 0.4	<0.01*

* Compared to vitamin D-deficient and vitamin D-deficient + cortisone.

level of Ca^{2+} binding activity in rats raised on normal laboratory chow (Table III). The level of Ca^{2+}-binding activity was not suppressed by cortisone in animals raised on a vitamin D-deficient diet and the vitamin D induction of Ca^{2+} binding protein was not blocked by cortisone administration (Table IV).

Table V lists the results of experiments designed to measure the effect of cortisone on alkaline phosphatase and ATPase activity of duodenal brush border preparations. Since it has been suggested that calcium dependent ATPase and not Mg^{2+}-ATPase is involved in Ca^{2+} transport (*vide infra*) the activities of both these enzymes were measured as described in Methods. Because of the variability of enzyme activity of preparations made on different days and from different batches of rats, it was necessary to treat each experiment as a paired observation. Alkaline phosphatase activity was depressed by cortisone treatment in animals fed standard laboratory chow ($P < 0.005$). Similarly Ca^{2+}-ATPase-levels were depressed in 5 of 6 experiments but statistical analysis indicated the differences to be of questionable significance ($0.05 < P < 0.1$). Mg^{2+}-ATPase was not significantly affected by the administration of cortisone.

The results of experiments designed to measure duodenal phosphatase activities in animals raised on a vitamin D-deficient diet which were treated with cortisone

TABLE III

EFFECT OF CORTISONE ADMINISTRATION ON DUODENAL Ca^{2+} BINDING ACTIVITY

Each experiment was performed on a pool of tissue obtained from the duodenal mucosa from 6–7 animals. Ca^{2+} binding activity was determined on the heat treated supernatants as described in Methods. P values are calculated from non-paired t tests.

Group	*Number of experiments*	*Ca^{2+} binding activity*	
		Mean protein ± S.E. (mg/ml)	*Mean percentage $^{45}Ca^{2+}$ in supernatant bound per mg protein ± S.E.*
Control	10	4.5 ± 0.3	4.8 ± 0.7
Cortisone	10	4.4 ± 0.3	5.1 ± 0.9
P		>0.1	>0.1

TABLE IV

EFFECT OF CORTISONE AND VITAMIN D ADMINISTRATION ON DUODENAL Ca^{2+} BINDING ACTIVITY IN RATS RAISED ON A VITAMIN D-DEFICIENT DIET

Results are from a single experiment in which determinations were made on a pool of intestinal mucosa derived from the proximal 10 cm of small intestine from 10 animals in each group.

Group	*Mean wt (g)*	*Ca^{2+} binding activity*	
		Protein (mg/ml)	*Percentage $^{45}Ca^{2+}$ in supernatant bound per mg protein*
Vitamin D-deficient	78	4.7	1.2
Vitamin D-deficient + cortisone	72	4.2	1.0
Vitamin D-deficient + vitamin D_2	78	5.6	3.8
Vitamin D-deficient + cortisone + vitamin D_2	72	4.1	4.6

TABLE V

EFFECT OF CORTISONE ADMINISTRATION ON DUODENAL BRUSH BORDER ALKALINE PHOSPHATASE AND ATPase ACTIVITIES

Brush border suspensions were incubated in a medium containing 0.015 M *p*-nitrophenyl phosphate, 0.042 M glycine buffer (pH 9.2), 0.005 M KCl, 0.0042 M $MgCl_2$ and 0.0008 M $ZnCl_2$ in a final volume of 0.6 ml for determination of alkaline phosphatase activity. The medium contained 0.04 M Tris–HCl buffer (pH 7.4), 0.005 M K_2ATP and 0.0025 M $MgCl_2$ or 0.005 M $MgCl_2$ + 0.010 M $CaCl_2$ for ATPase activity. The Ca^{2+}-ATPase activity is calculated by subtracting the phosphate released in the 0.0025 M $MgCl_2$ assay from that released in the 0.005 M $MgCl_2$ + 0.010 M $CaCl_2$ assay. Each value is the average of at least duplicate determinations performed on brush border preparations from a pool of tissue obtained from the duodenal mucosa of 6–10 animals. *P* values are calculated on ln of experimental values from a paired *t* test.

Expt. No.	*Alkaline phosphatase* (*μmoles p-nitrophenol per mg protein per min*)		*Mg^{2+}-ATPase* (*μmoles P_i per mg protein per min*)		*Ca^{2+}-ATPase* (*μmoles P_i per mg protein per min*)	
	Control	*Cortisone*	*Control*	*Cortisone*	*Control*	*Cortisone*
1	155	140	0.6	0.4	1.1	0.6
2	207	187	0.4	0.3	0.5	0.5
3	229	172	0.3	0.3	1.1	1.0
4	279	211	0.5	0.7	1.7	1.5
5	445	346	1.2	0.7	2.6	1.8
6	242	209	2.1	1.8	0.6	0.2
P	<0.005		>0.1		$0.05<P<0.1$	

TABLE VI

EFFECT OF CORTISONE AND VITAMIN D ADMINISTRATION ON DUODENAL BRUSH BORDER ALKALINE PHOSPHATASE AND ATPase ACTIVITIES IN RATS RAISED ON A VITAMIN D-DEFICIENT DIET

P values are calculated on ln of experimental values from analysis of variance and Newman–Keuls tests.

Expt. No.	*Alkaline phosphatase* (*μmoles p-nitrophenol per mg protein per min*)			*Mg^{2+}-ATPase* (*μmoles P_i per mg protein per min*)			*Ca^{2+}-ATPase* (*μmoles P_i per mg protein per min*)		
	Vitamin D-deficient	*Vitamin D-deficient + cortisone*	*Vitamin D-deficient + cortisone + vitamin D*	*Vitamin D-deficient*	*Vitamin D-deficient + cortisone*	*Vitamin D-deficient + cortisone + vitamin D*	*Vitamin D-deficient*	*Vitamin D-deficient + cortisone*	*Vitamin D-deficient + cortisone + vitamin D*
1	143	80	307	0.3	0.2	0.5	0.9	0.4	1.6
2	176	118	220	0.2	0.2	0.2	0.2	0.3	0.7
3	44	49	66	0.3	0.7	0.7	0.2	0.2	0.7
4	43	50	74	0.7	0.1	0.4	1.5	1.1	1.8
5	141	80	173	1.8	0.2	1.2	2.7	3.5	4.8
*P**	—	>0.05	<0.01	—	>0.05	>0.05	—	>0.05	<0.01

* Compared to vitamin D-deficient.

alone or in conjunction with vitamin D are listed in Table VI. Alkaline phosphatase activity was not appreciably affected by the administration of cortisone to D-deficient animals and its administration did not prevent the rise in alkaline phosphatase activity produced by vitamin D. Similarly cortisone administration did not suppress the activity of Ca^{2+}-ATPase levels in vitamin D-deficient rats and did not prevent the rise in Ca^{2+}-ATPase levels induced by vitamin D. Mg^{2+}-ATPase levels were unaffected by either treatment*.

DISCUSSION

Our experiments measuring S/M ratios demonstrate that 3 days of cortisone administration to animals which are not deficient in vitamin D or Ca^{2+} results in a significant depression of calcium transport by the rat duodenum. These results agree with those previously reported[1,6] although Kimberg *et al.*[6] noted a somewhat greater percent depression in animals given cortisone for a period of 7 days. In addition they found that the administration of large doses of vitamin D to cortisone-treated animals did not reverse the decreased transport and the effect appeared to be independent of vitamin D or 25-hydroxycholecalciferol. In contrast we encountered no interference with the expected rise in Ca^{2+} transport induced by vitamin D in rachitic animals treated with cortisone while Kimberg *et al.*[6] noted a diminished response to the administration of vitamin D under similar experimental conditions. The reason for this apparent discrepancy is not clear although it is conceivable that the more prolonged administration of cortisone might have affected cellular transport systems such that the animals were unable to respond to the dose of 50 I.U. of vitamin D administered 24 h before sacrifice. In our experiments 500 I.U. administered 40–48 h before study resulted in the expected elevation of S/M ratios.

Additionally in agreement with Kimberg *et al.*[6] we observed no further diminution of Ca^{2+} transport when cortisone was administered to vitamin D-deficient rats. The reason for this finding which is inconsistent with the theory that the effect of cortisone upon Ca^{2+} transport is independent of the vitamin D status of the animal is not apparent. Nevertheless it would appear from these experiments that in vitamin D-deficient animals cortisone does not decrease Ca^{2+} transport nor levels of Ca^{2+} binding protein or brush border phosphatases all of which are vitamin D dependent. This indicates that the suppression is limited in magnitude and is dependent on factors which are still unidentified.

Our experiments also show that the intestinal Ca^{2+} binding activity in rats raised on a diet adequate in vitamin D is not depressed by the administration of cortisone indicating that the decrease in Ca^{2+} transport produced by this regimen cannot be explained by the unavailability of this protein. Kimberg *et al.*[6] using 7 days of cortisone treatment, found that levels of Ca^{2+} binding protein and assayable vitamin D activity in the intestine were actually higher than in control animals. The role of this vitamin D-induced intestinal Ca^{2+} binding protein in the physiological state is still undetermined, although it has recently been shown in *in vitro* studies

* Brush border preparations from animals raised on the rachitogenic diet and administered vitamin D alone were attempted in some experiments, but the brush border pellets were too impure and gelatinous to use for enzyme studies. Experiments are in progress attempting to overcome this technical difficulty.

that the protein promotes the release of Ca^{2+} from mitochondria[24] and can form a complex with lysolecithin with subsequent reduction of the binding affinity of the protein for Ca^{2+} (ref. 25). At any rate, its level appears not be rate limiting in the rat which is not vitamin D-deficient and glucocorticosteroid administration does not interfere with its synthesis.

Although it has been known for many years that vitamin D administration may produce an increase in intestinal alkaline phosphatase activity[26] the suggestion that brush border phosphatases may play a functional role in the translocation of calcium in the small intestine has been made only recently[13–17]. Our studies which show a correlation between alkaline phosphatase and calcium absorption also suggest that phosphatase(s) may be involved in intestinal Ca^{2+} transport and may be at least one of the mechanisms through which glucocorticosteroids mediate their depression of Ca^{2+} transport. The suppression of Ca^{2+}-ATPase was not as striking as that of alkaline phosphatase but this may be a reflection of whether or not the Ca^{2+}-ATPase activity we are measuring reflects a different enzyme or whether activities measure the same enzyme as has been suggested previously by others[16,17] (*vide infra*). As noted, the results of our experiments in animals raised on vitamin D-deficient diets, indicating that cortisone did not further reduce alkaline phosphatase or Ca^{2+}-ATPase levels, correlate with transport ratios in animals similarly treated. Since the administration of cortisone to rats raised on vitamin D-deficient diets did not prevent a rise in alkaline phosphatase or Ca^{2+}-ATPase activities after vitamin D administration, it does appear that cortisone and vitamin D act on phosphatase(s) *via* different pathways. Unfortunately, the technical problem with the preparation of brush borders from vitamin D-deficient rats given vitamin D alone (*vide supra*) prevented us from observing whether or not there was a partial interference with the expected vitamin D-induced rise in phosphatase levels.

The activities of Ca^{2+}-ATPase in our experiments paralleled those of alkaline phosphatase but it cannot be determined from these studies whether or not the activities represent the same enzyme. Eaton and Moss[27] have shown that intestinal phosphatase activity can be demonstrated using a variety of organic phosphates as substrates and Melancon and DeLuca[14] in their studies of Ca^{2+}-ATPase in chicks demonstrated phosphatase activity using ADP and ITP as substitutes for ATP but found that the calcium dependency was more evident using ATP as a substrate. The strongest evidence indicating that the activities reflect the same enzyme has been provided by Haussler *et al.*[16] who showed parallel inhibitions of both alkaline phosphatase and ATPase activities by L-phenylalanine, a known stereo-specific inhibitor of alkaline phosphatase[28]. Holdsworth[17] has also concluded that alkaline phosphatase and Ca^{2+}-ATPase, as well as pyrophosphatase activities from chick duodenal brush border preparation are measures of the same protein.

The correlation of Ca^{2+}-ATPase activities with alkaline phosphatase activities in our experiments was more striking in rats raised on the vitamin D-deficient diet than in those experiments in which the animals were fed regular laboratory chow. Considering the large differences in absolute rates of activity comparing hydrolysis of *p*-nitrophenyl phosphate and ATP as substrates, however, it seems reasonable that differences actually measuring alkaline phosphatase, although under conditions designed to measure ATPase, might appear to be changes of ATPase. These changes might be more evident in situations manipulated biologically so as to provide for

optimum differences, as for example in our rats raised on a vitamin D-deficient diet and those given a single dose of vitamin D. It is possible, therefore, that the ATPase activity we measured from brush borders could merely have been a measure of alkaline phosphatase activity under conditions which are not ideal and using an alternate substrate.

Recent evidence also suggests that alkaline phosphatase may participate in the absorption of long chain fatty acids[29–31] but in evaluating the importance of alkaline phosphatase activity as related to either Ca^{2+} or fatty acid transport, it should be noted that there is evidence that the uptake of these molecules is mutually stimulating[32,33]. Although the correlation of transport rates and enzyme activity suggests that alkaline phosphatase plays a role in mediating the depression of intestinal Ca^{2+} translocation by glucocorticosteroids, whether or not the enzyme plays a physiological role in Ca^{2+} transport as well as a clearer understanding of the mechanisms involved, awaits further experimentation.

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