

Influence of dietary calcium content on intestinal permeability in rat

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Background & objectives: Agents that increase the permeability of intestinal epithelium promote the absorption of nutrients by the gut. High calcium concentration in the gut has been shown to enhance passive transport of glucose in the rat intestine. An increase in the permeability of the intestinal epithelium may account for this observation. The present study was aimed at monitoring the permeability of intestine of rats fed high or low calcium diets.

Methods: Everted intestinal sacs were used to study transports of substances across the gut. While radioactive and non radioactive calcium isotopes were employed to study the active transport and passive transport of calcium, transport of labelled mannitol was taken as a measure of passive permeability.

Results: High calcium diet increased the passive transport of mannitol and calcium while decreasing the active transport of calcium by the everted gut sacs.

Interpretation & conclusion: Passive mechanisms are enhanced by high calcium diet, while low calcium diet favours active transport. Calcium in the diet may be affecting intestinal transport.

Key words Calcium - diet - intestine - permeability - transport

Many nutrients are absorbed in the intestinal tract extending from the duodenum to ileum. The epithelium lining the gut is specially developed for this process with large surface area provided by villi and microvilli. The process of absorption mainly occurs through two mechanisms - energy dependent active mechanism and electro-chemical gradient dependent passive mechanism. In addition to the gradient, the permeability of the intestinal epithelium plays an important role in passive transport¹. A number of factors seem to influence the permeability of the intestine and thereby alter the passive absorption of substances by the gut. These include

stress², inflammation³, toxins⁴, chemicals⁵, herbs⁶ and probiotics⁷. It has recently been shown that the presence of calcium enhances the glucose absorption in the perfused rat intestine⁸. Rise in passive transport mediated through glucose transporter 2 (GLUT 2) was held responsible for this increment. Whether alteration in dietary calcium would affect the passive permeability is not known. In order to answer this question, the present study was undertaken to assess passive permeability, passive and active transports of calcium on everted sacs prepared from rats fed either with low calcium diet (LCD) or high calcium diet (HCD).

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Material & Methods

Animals and diet schedules: This work was carried out in the department of Pharmacology during the years 1982 and 2006. Male albino rats weighing about 100g of the Sprague- Dawley strain were obtained from the vivarium of Texas Tech University Health Sciences Center, Lubbock, USA. Approvals of the Institutional Committee for Supervision of Animal Experimentation and of the Board of Radiation Safety (Texas Tech University Health Sciences Center, Lubbock) were obtained prior to the start of experiments. After a preliminary period of one week on a high calcium diet (HCD, Ca 1.5%), the rats were divided into two groups. The first group received the low calcium diet (LCD, Ca 0.2%) supplied by Teklad Test Diets (Madison, WI), while the second group was continued on HCD for a period of 21 days. The HCD was prepared by adding the appropriate amount of calcium carbonate to the LCD. Diets were given *ad libitum*. The passive permeability to mannitol and the active and passive transports of calcium were measured *in vitro* in the everted sacs of 5 cm lengths of duodenum and ileum removed from the two groups of rats. After the experimentation, the tissues and the sacrificed animals were disposed off in bags kept separately for radioactive and non radioactive wastes. All chemicals were purchased from Sigma, USA, unless otherwise indicated.

Preparation of the everted intestinal sacs: Everted intestinal sacs were prepared by the method described earlier⁹. After overnight fasting the rats were killed under ether anaesthesia and the intestine extending from the pyloric end to the ileocaecal junction was removed carefully. Fat and mesenteric attachments were removed carefully. The required segment was flushed gently with ice cold saline using a syringe equipped with a blunt needle. A stainless steel rod grooved at one end, was pushed into the lumen of the gut gently until it appeared at the distal end of the segment. A knot was applied over the segment at the groove. The segment was gently slid down over the knot until it was completely everted. The everted segment was detached from the rod and was placed in ice cold saline in a petri dish. After tying the distal end of the segment, the blunted needle of a microsyringe was gently introduced into the lumen at the proximal end of the segment and 0.5 ml of the required medium described below, was injected into the segment to distend it. A ligature was applied over the proximal end and was tightened while gently pulling down the distended segment. The everted sac

so prepared was then placed in a 25 ml flask containing 5 ml of the medium. After flushing with the required gas, the flask was closed with stopper and incubated in a shaker water bath.

Assessment of passive permeability: The changes in non specific passive permeability were examined with ¹⁴C- mannitol which has been used to assess intestinal permeability in rats¹⁰. The everted gut sacs from the first proximal 5 cm segment of duodenum and from ileum were filled with 0.5 ml of 150 mM sodium chloride solution and were placed in 5 ml of incubation medium containing 121 mM sodium chloride, 55 mM mannitol and 6.0 μ Ci/dl of D-[1- ¹⁴C] mannitol (Amersham, Arlington Heights, IL; 59 mCi/mole; catalog no CFA238). These were incubated on ice under an atmosphere of nitrogen for 15 min. The amount of radioactivity as cpm in a 200 μ l aliquot of serosal fluid was taken as the index of permeability.

Measurement of active and passive transports of calcium: To study the active transport of calcium, the duodenal sacs were incubated at 37 °C for 60 min under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide in 5 ml of incubation medium in 25 ml Erlenmeyer flasks in a shaking water bath. An aliquot (500 μ l) of the same medium was placed inside the sac. The composition of the incubation medium was: 2.4 mM dibasic sodium phosphate, 1.6 mM monobasic sodium phosphate, 150 mM sodium chloride, 20 mM fructose; 0.4 mM calcium chloride and 0.025 μ ci/ml ⁴⁵Ca as calcium chloride (New England Nuclear, Boston, MA). The ratio of cpm in 100 μ l aliquots taken from the serosal fluid to the cpm in the mucosal fluid at the end of the incubation period, designated S/M ratio, was used as the index of active transport. This ratio has been used for assessment of transport in many studies in the past¹. To begin with the ratio was 1.0 since the calcium concentration was identical in mucosal and serosal solutions. An increase in the ratio indicates active transport of calcium.

The passive transport of calcium was measured in everted gut sacs prepared from the 5 cm segment of the ileum and of the duodenum immediately distal to that used for active transport measurement. A calcium gradient was established across the gut wall by employing buffers differing in calcium concentration between the mucosal and the serosal sides. The composition of the mucosal buffer was: 23.2 mM sodium barbiturate, 16.8 mM hydrochloric acid, 150 mM sodium chloride, 20 mM fructose and

10 mM calcium chloride. The serosal buffer had a similar composition except for calcium chloride, which was 1 mM, and an additional amount of sodium chloride to equalize the osmolarities on the two sides. Incubations were carried out in 25 ml flasks which were shaken for 15 min under an atmosphere of nitrogen in an ice bath. The S/M ratio was calculated as mentioned above. The ratio was kept at 0.1 at the beginning and any improvement in this ratio denotes transport of calcium. Unpaired Student's t-test was performed to analyse the results.

Results & Discussion

High calcium diet (HCD) caused a significant ($P<0.05$) increase in mannitol transport and in passive transport of calcium ($P<0.01$) when compared to low calcium (LCD) fed group. Active transport of calcium was significantly less in HCD when compared to LCD (Table).

While effect of calcium intake on active transport of this divalent cation has been widely reported, there is a paucity of published work on passive transport¹¹. One of the reasons for this is the simultaneous operation of the active and passive modes of transport in the gut, which makes the interpretation of the results difficult. In a bid to measure passive transport in isolation,

Table. Passive permeability of mannitol and active and passive transport of calcium in the everted gut sacs from rats fed LCD or HCD

Transport	Site	Index	LCD	HCD
Passive mannitol	Duodenum	Serosal cpm	804 ± 138.9 (n=6)	1402 ± 190.5* (n=6)
	Ileum	Serosal cpm	597 ± 119.5 (n=6)	1178 ± 149.5* (n=6)
Passive calcium	Duodenum	S/M ratio	0.15 ± 0.012 (n=6)	0.21 ± 0.012** (n=6)
	Ileum	S/M ratio	0.15 ± 0.008 (n=6)	0.19 ± 0.006** (n=6)
Active calcium	Duodenum	S/M ratio	9.9 ± 1.02 (n=6)	6.3 ± 1.01* (n=5)

Rats were placed on the respective diets for 21 days. Each value is mean ± standard error of the mean. The number of the rats per group is given in parenthesis. Passive transport of mannitol: Serosal cpm: cpm in 200 µl aliquot of serosal fluid at the end of 15 min incubation at 0°C under N₂ after adding ¹⁴C-mannitol to mucosal fluid only at the start of incubation. Passive transport of calcium: S/M ratio: ratio of serosal ⁴⁰Ca/mucosal ⁴⁰Ca at end of 15 min incubation at 0°C under N₂ (ratio of 0.1 at start). Active transport of calcium S/M ratio: ratio of serosal ⁴⁵Ca/mucosal ⁴⁵Ca at the end of 60 min incubation at 37°C under 95 per cent O₂ / 5% CO₂ (ratio of 1.0 at start). LCD, low calcium diet; HCD, high calcium diet. $P^*<0.05$, $^{**}<0.01$ compared to LCD group

experimental conditions that completely inhibit the active transport were employed. Previous studies have shown that incubating the tissue under an atmosphere of nitrogen or on ice abolished the active transport of calcium by the everted gut sacs. Both these techniques have been combined to ensure elimination of active transport¹. Gut tissue has been shown to tolerate cold temperature very well. In experimental and clinical transplantation studies, the gut tissue is routinely stored in cold temperature for 6-8 h^{12,13}. In the present study the everted sacs were incubated on ice under an atmosphere of nitrogen only for 15 min, thus ensuring tissue viability.

The changes observed in active transport of calcium with HCD and LCD were in agreement with the earlier reports¹ indicating the adaptation of the duodenal segment to the changes in the dietary calcium levels. Change in passive permeability measured with mannitol indicates opening of paracellular pathways in the duodenal and ileal segments of the rats fed a high calcium diet. This may be responsible for the increase in passive transport of calcium observed in this group of rats. However, Mace *et al*¹⁴ in acute intestinal perfusion experiments found no change in mannitol transport, when they incorporated calcium in the infusate. But the transport of glucose showed an increase in their experiments which was attributed to changes in passive transcellular pathway. Differences in the experimental conditions employed may be responsible for these differing observations. Passive transcellular calcium transport in rat, enhanced by vitamin D has been reported¹¹. Since high calcium diet suppresses vitamin D synthesis, it is unlikely that this mechanism is involved in elevation of passive calcium transport noted in this study in HCD fed rats. Therefore the enhancement of paracellular transport path is most likely to be responsible for increased passive transport of calcium in the intestine of rats fed high calcium diet.

In summary, the findings reported here indicate that calcium content of the diet can possibly alter the transport by changing the permeability of the intestine in rat. The exact mechanism responsible for this change remains to be investigated.

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