Duodenal Calcium Transport in Hyperthyroidism.* (32108)

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It has been known for many years that rats made hyperthyroid with either thyroxine or thyroid extract excrete excessive amounts of fecal calcium(1,2). This increase in fecal calcium excretion might be explained by a decrease of calcium absorption or an increase of the movement of calcium into the gastrointestinal tract from the animal, or a combination of these possibilities. Since the rat duodenum is known to actively transport calcium both *in vitro* and *in vivo*(3,4), this study was undertaken to investigate the influence of hyperthyroidism on calcium absorption and unidirectional fluxes in the rat duodenum by an *in vivo* perfusion technique.

Methods. Male Sprague-Dawley rats weighing 305 to 420 g served as experimental and control animals. Nine animals were injected daily with 125 μg of l-thyroxine subcutaneously for 13 days and were studied one day following the last injection. Food was withheld for 24 hours before study but rats were permitted tap water *ad libitum*. Eleven control animals were similarly restricted from eating for 24 hours before study. During the 2 weeks before study, all animals were fed laboratory chow containing 1.2% calcium.

Anesthesia was produced with intraperitoneal *Dial*^{R} with urethane solution (Ciba) (0.7 ml/kg). Through a midline incision the distal end of the bile duct was ligated and transected. The proximal 8-13 cm of small intestine was cannulated with metal adapters attached to polyvinyl tubing. The inflow cannula was inserted through an incision in the pylorus and the tubing tied in place with suture. Similarly, the outflow cannula was positioned and tied 1 to 2 cm distal to the ligament of Treitz. The abdominal skin was clamped over the cannulas and body temperature was maintained by a heating pad upon which the animals lay during the experiment.

Fluid was perfused from a reservoir containing 17 ml of solution by recirculation at a rate of 1.8 ml/min for 2 hours by use of a proportioning pump (Technicon). The perfusing solution of pH 6.5 contained 1.7 mM $CaCl_2$, 141 mM NaCl, 10 mM THAM, 0.2% polyethylene glycol and .01 μc/ml $Ca^{45}Cl_2$ (Nuclear Chicago specific activity $>$ 10 curies/g). Before perfusion the isolated segment of gut was washed with the perfusing solution as prepared but devoid of any radioactive material and then emptied with air with the proportioning pump at a rate of 1.8 ml/min.

Polyethylene glycol was analyzed turbidometrically with a spectrophotometer (Research Specialties Model 4000 Spectromatic). Calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 303) and radioactivity was estimated in a liquid scintillation spectrometer (Packard Tri-Carb).

At termination of the 2-hour perfusion period, the gut was removed and the contents and any excess fluid expressed by gently pressing the tissue flat on absorbent paper. The perfused segment of gut was weighed on a torsion balance (Bethlehem Instrument Co.).

In this paper the term absorption refers to the net movement of calcium from the lumen of the gut into the experimental animal while lumen to plasma flux (LP) and plasma to lumen flux (PL) are unidirectional movements. The following equations, adapted from the work of Wasserman, Kallfelz and Comar (4) were used to calculate absorption, LP and PL fluxes:

$$\text{Absorption} = \frac{V[Ca^{40}_i - (Ca^{40}_f)(PR)]}{W}$$

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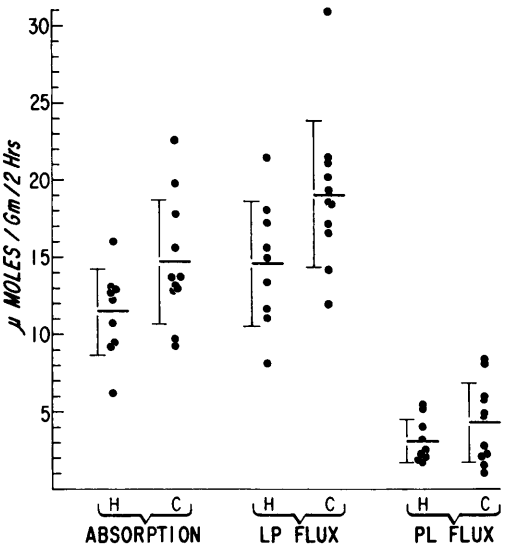

FIG. 1. Absorption rates, lumen to plasma and plasma to lumen fluxes of calcium in control and hyperthyroid rats. C = control animals. H = hyperthyroid animals. Heavy bars represent the mean. Light vertical lines represent ± one standard deviation.

$$\text{LP Flux} = \frac{V[Ca^{45}_i - Ca^{45}_f)(PR)]}{[(SA_i + SA_f)/2]\ W}$$

$$\text{PL Flux} = \text{LP Flux} - \text{Absorption}$$

Where Ca^{40} = chemical calcium content of the fluids in μmol/ml; Ca^{45} = radioactive calcium content of the fluid in cpm/ml; V = volume of perfusate in ml; W = wet weight of the gut in grams; SA = specific activity of calcium in cpm/μmol and PR = polyethylene glycol ratio, which corrects for water movement and is calculated by dividing the polyethylene glycol concentration of the initial perfusate by its concentration in the final perfusate. The subscripts "i" and "f" refer to the initial and final values.

Results. The animals which received the daily injections of l-thyroxine suffered a loss of body weight of approximately 5% as well as developing alopecia, weakness and diarrhea.

Absorption and flux data for all experiments are shown in Fig. 1. The mean 2-hour absorption rate ± standard deviation was 11.5 ± 2.8 μmol per gram wet weight of intestine for hyperthyroid animals compared with a mean of 14.7 ± 4.0 for control animals. Mean LP flux in hyperthyroid rats was 14.6 ± 4.0 while that of control animals was 19.0 ± 4.8. The PL flux means were 3.1 ± 1.4 and 4.3 ± 2.5 for hyperthyroid and control rats respectively.

Analysis of these means by a two sample t-test(5) revealed no statistically significant difference for absorption ($p>.05$). LP Flux ($.02<p<.05$), or PL Flux ($p>.20$).

Discussion. Many metabolic functions are accelerated in hyperthyroidism and an increase of calcium flux both into and out of the gut lumen might be expected on that basis. Net movement would be determined by the unidirectional flux that was most accelerated. Thus, an acceleration in the PL flux in excess of that of the LP flux might result in an increase in fecal calcium excretion. On the other hand, decreased calcium absorption might occur either in the presence of a decrease in LP flux or in the presence of an increased LP flux which was not as accelerated as the PL flux. The increased fecal calcium present in hyperthyroidism might be explained by one or more of these possibilities.

Althausen(6), studied calcium absorption in hyperthyroid rats giving calcium lactate by stomach tube and analyzing the residue in the digestive tract after a specified period. He found the same amount of calcium present in the recovered material as was present when the substance was administered. More recent studies using concentration gradients rather than net movements in *in vitro* preparations have suggested a decrease in calcium movement. Finklestein and Schachter(7) using everted gut sacs of rat duodenum from hypophysectomized animals found an increase in inhibition of calcium transport when l-thyroxine was administered subcutaneously, but no significant effect was observed with TSH replacement. Transport was expressed as a concentration gradient of serosal medium/mucosal medium of radioactive calcium. No hyperthyroid animals, however, were studied. Friedland *et al*(8) measured the serosal/mucosal concentration gradients in duodenal sacs of 45-50 g rats which had been made hyperthyroid with TSH or L-triiodothyronine and found a significant decrease in the gradient as compared to the gradient of control animals. This was much more apparent

in rats treated from 1 to 28 days, however, than in the rats treated for 98 days.

The results of the present study indicate no significant change in the net movement or the unidirectional fluxes of calcium in the duodenum of hyperthyroid rats. Although the means of the values for all 3 movements, 2 of which were measured and one of which was calculated from the other data, were lower in the hyperthyroid animals compared to the control animals, suggesting a generalized decrease in transport across the duodenum, the differences were small and not statistically significant.

The explanation for the difference in findings in this study compared to that of Friedland *et al* is not apparent. They used very low concentrations of calcium and measured only concentration gradients. The more physiological *in vivo* preparation used in our experiments measures actual transport and presumably simulates more closely what is occurring in the hyperthyroid animals. In addition, the rats used in Friedland's study were weanling and weighed only 45-50 g at the onset of the experiment. Therefore, some of the effect on calcium movement seen in these animals might have been explained by their young age. When the rats were treated for 98 days, and thus matured, there was very little difference in the hyperthyroid and control rats. In fact, the concentration gradients were slightly less than one in the experimental animals while the control animals' gradient was slightly more than one in striking contrast to the results in the younger animals. The ages and weights of those rats were more comparable to those used in this study.

The duodenum is not the only site of calcium absorption in the rat and the length of time available for calcium absorption may be an important factor in the total amount of calcium absorbed in the rat despite the demonstration that the duodenum and upper jejunum absorb the ion most avidly. It is possible, therefore, that the over-all calcium absorption in hyperthyroidism is decreased in the rat. On the other hand, factors such as increased motility and intestinal hurry may explain the increase in calcium excretion. In this study, where motility and contact times were the same in both control and hyperthyroid rats, there was only a slight decrease in the net absorption of calcium which was not statistically significant. These data suggest that the hyperthyroid rat is capable of absorbing calcium normally through the duodenum and give no support to the theory of decreased duodenal absorption of calcium as a major factor in the increase of fecal calcium excretion in hyperthyroidism.

Summary. This study investigated the influence of hyperthyroidism on intestinal calcium absorption with an *in vivo* perfusion technique in the rat duodenum. Net movement, plasma to lumen and lumen to plasma fluxes, although slightly decreased, were not statistically significantly different in hyperthyroid animals compared to normal animals. These results do not support the theory of decreased calcium absorption in the duodenum as a major factor contributory to the increased fecal excretion of calcium seen in hyperthyroid rats.

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