Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans

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ABSTRACT To determine the effects of acetate and propionate on calcium absorption from the human distal colon and rectum, six healthy human subjects were given rectal infusions containing 50 mmol CaCl₂/L on four separate occasions. Addition of 56.3 mmol acetate/L, 18.7 mmol propionate/L, or acetate and propionate together increased calcium disappearance (expressed as the change in the ratio of calcium to polyethylene glycol) from -5.5 ± 1.4 to -22.6 ± 2.8, -23.2 ± 3.2, and -19.7 ± 4.6, respectively; P < 0.05. To determine the effects of different acetate and propionate concentrations, six different subjects were studied further. The effects of 18.7 or 56.3 mmol acetate/L on calcium absorption were the same as those of 18.7 mmol propionate/L (-15.7 ± 1.4), and less than those of 56.3 mmol propionate/L (-20.3 ± 2.4, P < 0.05). We conclude that both acetate and propionate enhance calcium absorption from the human distal colon, but that propionate has a greater effect at higher concentrations. Further studies are needed to determine the mechanism of calcium absorption from the colon. Am J Clin Nutr 1996;63:574–8.

KEY WORDS Calcium absorption, acetate, propionate, butyrate, distal colon

INTRODUCTION

Increased intake of dietary fiber is now being recommended for good health (1). A potential adverse effect of dietary fiber is a reduction of mineral availability because of fiber’s ability to bind minerals (2–5). However, our previous in vitro study suggests that although some fibers may bind with minerals in the small intestine, fermentation of fiber in the colon can cause a release of minerals that then may be absorbed from the colon (6). The products of fiber fermentation, short-chain fatty acids (SCFAs), may in turn enhance the absorption of minerals from the colon (7–9).

The mechanism by which SCFAs increase the absorption of calcium is obscure and we only have indirect evidence that this occurs in humans (10, 11). We showed previously, in six healthy male subjects, that rectal infusion of an isotonic solution containing calcium plus SCFAs resulted in a significantly greater increase in serum calcium than did an infusion of calcium alone (10, 11). This suggests that calcium absorption from the colon is enhanced by SCFAs. However, serum calcium is an indirect measure of calcium absorption. Moreover, the SCFAs used in the study were a mixture of acetate and propionate (3:1). Therefore, we performed two studies to investigate the direct effects of acetate and propionate, alone and combined, on the disappearance of calcium from the rectum and distal colon of human subjects. In the first study, acetate and propionate were given at normal physiologic concentrations and molar ratios (ie, acetate:propionate 3:1). In the second study the molar ratios were reversed (acetate:propionate 1:3).

SUBJECTS AND METHODS

Two studies were conducted on subjects in the morning after an overnight fast. The night before the study they were provided with a standard, low-fiber dinner to reduce colonic residue. In the first study six healthy subjects (two males and four females; 33 ± 5 y of age; 100 ± 2% of ideal body weight) were given rectal infusions on four separate occasions whereas in the second study six healthy female subjects (29 ± 4 y of age; 100 ± 2% of ideal body weight) were given rectal infusions on six separate occasions. The details of the different treatment solutions are summarized in Table 1. The average calcium intake from a usual Western diet is ~25 mmol/d (12, 13) and ~30–40% is absorbed in the small intestine (13). Therefore, ~15.0–17.5 mmol Ca reaches the colon for potential absorption. The amount of calcium infused in this study is within the above range. The sodium concentration of all treatment solutions in both studies was adjusted to 75 mmol/L with sodium chloride. In addition, each solution contained 0.625 mmol polyethylene glycol (PEG)/L, which was used as a nonabsorbable marker. Subjects served as their own controls and comparisons were done by study group. The length of the washout period between infusions was 3–7 d, depending on the availability of the subjects.

The volume of each solution infused in each of the two studies was 300 mL and the treatments were given in random order. The subjects administered their own rectal infusions. Before infusing the test solution, the subjects were asked to clean their colon by infusing an enema of 500 mL double-distilled water into the colon through a 1-m Tygon flexible
TABLE 1
Composition of the colonic treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca</th>
<th>Ac</th>
<th>Pr</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca+NaCl</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>75.0</td>
</tr>
<tr>
<td>Ca+3Ac</td>
<td>50</td>
<td>56.3</td>
<td>—</td>
<td>18.7</td>
</tr>
<tr>
<td>Ca+1Pr</td>
<td>50</td>
<td>—</td>
<td>18.7</td>
<td>56.3</td>
</tr>
<tr>
<td>Ca+3Ac+1Pr</td>
<td>50</td>
<td>56.3</td>
<td>18.7</td>
<td>—</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>75.0</td>
</tr>
<tr>
<td>Ca+Ac</td>
<td>50</td>
<td>18.7</td>
<td>—</td>
<td>56.3</td>
</tr>
<tr>
<td>Ca+1Pr</td>
<td>50</td>
<td>—</td>
<td>18.7</td>
<td>56.3</td>
</tr>
<tr>
<td>Ca+3Ac</td>
<td>50</td>
<td>56.3</td>
<td>—</td>
<td>18.7</td>
</tr>
<tr>
<td>Ca+3Pr</td>
<td>50</td>
<td>—</td>
<td>56.3</td>
<td>18.7</td>
</tr>
<tr>
<td>Ca+1Ac+3Pr</td>
<td>50</td>
<td>18.7</td>
<td>56.3</td>
<td>—</td>
</tr>
</tbody>
</table>

*The volume of each solution infused was 300 mL. All solutions contain 0.625 mmol polyethylene glycol/L.
*Calcium chloride (CaCl₂·2H₂O); Fisher Scientific, Nepean, Ontario, Canada.
*Sodium acetate, anhydrous S-8750; Sigma, St Louis.
*Sodium propionate, Food Grade; Van Waters and Roger Ltd, Ontario, Canada.
*British Drug House, Darmstadt, West Germany.

plastic tubing (inside, outside diameters of 1.2 and 4.0 mm, respectively; Norton Tubing and Molded Products, Akron, OH) connected to a 60-mL dispensing syringe. The enema was collected and discarded. Then 5 cm of one end of tubing was reinserted into the rectum and 300 mL test solution was infused over 5 min. Immediately after the infusion was complete, a 5-mL sample (0 min) was withdrawn through the infusion tube; 5-mL samples were obtained 10, 20, and 30 min later. Before each sample collection, rectal contents were mixed by withdrawing and reinfusing 10 mL fluid at least three times. After the 30-min sample collection, colonic contents were collected into a plastic bag in a fecal collection frame that fit under the toilet seat.

Samples were centrifuged at 450 × g for 10 min at 25 °C and analyzed for calcium with an atomic absorption spectrophotometer (model 1275; Varian Canada Inc, Mississauga, Ontario, Canada) after dilution with 10 mmol lanthanum chloride/L. PEG was measured by a turbidimetric method (14). The pH was measured with a pH meter (model 50; Beckman, Fullerton, CA) for the following treatments: 50 mmol Ca/L + 75 mmol NaCl/L (Ca+NaCl), 50 mmol Ca/L + 56.3 mmol acetate/L + 18.7 mmol NaCl/L (Ca+3Ac), and 50 mmol Ca/L + 56.3 mmol propionate/L + 18.7 mmol NaCl/L (Ca+3Pr).

Results were expressed as means ± SEMs. To correct for incomplete collection and fluid absorption or secretion by the colon, the calcium concentrations were expressed relative to those of PEG (calcium:PEG). Calcium absorption was expressed as disappearance, i.e., the change in calcium:PEG from baseline. Estimates of calcium absorbed (in mmol) were calculated by multiplying the percentage disappearance by the millimoles of calcium in the infused solution. Calcium disappearance from the samples of rectal fluid withdrawn at 10-min intervals for 30 min represents rectal absorption whereas that from the sample of fluid obtained when the subjects emptied their colons at the end of each study represents rectal plus distal colonic absorption. The protocol for this study was approved by the Human Subjects Review Committee at the University of Toronto.

Differences between treatments and time were examined by two-way repeated-measures analysis of variance and Tukey’s studentized range test using the Statistical Analysis System program (SAS Institute Inc, Cary, NC).

RESULTS

All the solutions were retained by the subjects for 30 min without difficulty. The presence of fluid in the rectum and distal colon for all subjects resulted in little or no sensation of distention or fullness. No side effects, such as diarrhea or rectal bleeding, were experienced by any of the subjects after the infusion.

The mean total volume of infused solutions recovered was 159 mL (53% of the volume infused) and there were no significant differences between treatments. The disappearance of calcium in the distal colon increased with time, from 0 to 30 min (study 1: r = 0.94, P < 0.0001; study 2: r = 0.77, P < 0.002) for all treatments in both studies (Figure 1; Table 2). In study 1, acetate and/or propionate significantly increased calcium disappearance (decrease in calcium:PEG) at 30 min compared with the control (Table 2; P < 0.05). However, there was no significant interaction between time and treatment. Despite the fact that acetate was three times the concentration of propionate, both SCFAs were equally effective in enhancing calcium absorption (Table 2). For each treatment, calcium absorption from the rectum and distal colon (total collection) was 1.2–2.0 times greater than that from the rectum alone at 30 min (Table 2; P < 0.05). Differences in calcium absorption between males and females was not significant so either sex could be used in future experiments.

In study 2, two of the treatments [Ca+3Ac and 50 mmol Ca + 18.7 mmol propionate/L + 56.3 mmol NaCl/L (Ca+1Pr)] from the first study were repeated to determine the effect of the different concentrations of acetate or propionate on calcium absorption in the same subject. In addition, a control treatment without calcium (NaCl group) was used to determine whether calcium is actually secreted in the colon. Results showed that

![FIGURE 1. Calcium disappearance in the rectum over time after the control treatment (calcium + sodium chloride; □) or after treatment with calcium and acetate, propionate, or both (■). Means (± SEMs) for all treatments in study 1 are provided because there were no significant differences. PEG, polyethylene glycol.](https://academic.oup.com/ajcn/article-abstract/63/4/574/4651257/6345665651257.png)
TABLE 2
The effect of sodium acetate (Ac) and/or sodium propionate (Pr) on calcium absorption in the rectum and distal colon of humans over 30 min

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca + NaCl</td>
<td>0.0 ± 0.1ba</td>
<td>-1.9 ± 0.1ha</td>
<td>-2.3 ± 0.8e</td>
<td>-4.0 ± 1.0f</td>
<td>-5.5 ± 1.4g</td>
</tr>
<tr>
<td>Ca + 3Ac</td>
<td>0.0 ± 1.0ha</td>
<td>-3.0 ± 1.0ha</td>
<td>-8.6 ± 2.7e</td>
<td>-18.3 ± 5.5g</td>
<td>-22.6 ± 2.8ha</td>
</tr>
<tr>
<td>Ca + 1Pr</td>
<td>0.0 ± 0.7ha</td>
<td>-2.0 ± 0.7ha</td>
<td>-5.8 ± 1.5e</td>
<td>-13.4 ± 3.1f</td>
<td>-23.2 ± 3.2ha</td>
</tr>
<tr>
<td>Ca + 3Ac + 1Pr</td>
<td>0.0 ± 3.5ha</td>
<td>-6.0 ± 3.5ha</td>
<td>-9.6 ± 3.5e</td>
<td>-10.2 ± 4.7f</td>
<td>-19.7 ± 4.6g</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0 ± 0.1ha</td>
<td>-0.02 ± 0.1ha</td>
<td>-0.06 ± 0.2be</td>
<td>-0.05 ± 0.2be</td>
<td>-0.1 ± 0.4ac</td>
</tr>
<tr>
<td>Ca + 1Ac</td>
<td>0.0 ± 1.1by</td>
<td>-2.4 ± 1.1by</td>
<td>-6.6 ± 1.7y</td>
<td>-11.1 ± 1.2de</td>
<td>-14.4 ± 0.4fxy</td>
</tr>
<tr>
<td>Ca + 1Pr</td>
<td>0.0 ± 1.1by</td>
<td>-4.9 ± 1.1by</td>
<td>-7.2 ± 1.4y</td>
<td>-10.8 ± 1.3f</td>
<td>-15.7 ± 1.4fxy</td>
</tr>
<tr>
<td>Ca + 3Ac</td>
<td>0.0 ± 3.2by</td>
<td>-1.2 ± 0.3by</td>
<td>-3.2 ± 0.6xy</td>
<td>-4.3 ± 0.6xy</td>
<td>-11.2 ± 3.6fxy</td>
</tr>
<tr>
<td>Ca + 3Pr</td>
<td>0.0 ± 1.5be</td>
<td>-6.5 ± 1.5be</td>
<td>-8.2 ± 1.5y</td>
<td>-10.9 ± 1.8z</td>
<td>-20.3 ± 2.3cz</td>
</tr>
<tr>
<td>Ca + 1Ac + 3Pr</td>
<td>0.0 ± 0.6bay</td>
<td>-2.1 ± 0.6bay</td>
<td>-5.2 ± 1.4xy</td>
<td>-7.0 ± 2.09z</td>
<td>-13.0 ± 1.11fxy</td>
</tr>
</tbody>
</table>

1 Calcium disappearance is expressed as the ratio of calcium to polyethylene glycol. a, b, c, d, and e indicate significant differences between times for different treatments within a study (P < 0.05); x, y, and z indicate significant differences between treatments within a study (P < 0.05). See Table 1 for definition of treatments.

this was not the case because no increase in calcium absorption was observed after treatment with sodium chloride. Calcium absorption after 50 mmol Ca/L + 18.7 mmol acetate/L + 56.3 mmol NaCl (Ca + 1Ac), −14.4 ± 0.4, was not different from that after Ca + 1Pr (−15.7 ± 1.4) or Ca + 3Ac (−11.2 ± 3.6; Table 2). However, at a higher concentration of propionate (56.3 mmol/L), calcium absorption (−20.3 ± 2.3) was greater than that after 18.7 mmol propionate/L and 56.3 mmol acetate/L (Table 2). Propionate (56.3 mmol/L) given alone resulted in greater calcium absorption than did a combination of acetate and propionate (Ca + 1Ac + 3Pr = −13.0 ± 1.1), although not significantly so. There was a significant interaction (P < 0.007) between time and treatment observed in this study.

The pH of the infused solution containing Ca + NaCl was significantly lower than that of Ca + 3Ac or Ca + 3Pr (Table 3; P < 0.05). Compared with the pH of the infused solution, pH increased significantly at time 0 and continued to increase at time 30 for Ca + NaCl. For Ca + 3Ac, the pH of the infused solution increased significantly at time 0 and leveled off at time 30 whereas for Ca + 3Pr, no significant differences between the pH of the infused solution at time 0 and at time 30 were observed (Table 3; P < 0.05). There was no significant difference in pH between treatments with Ca + 3Ac and Ca + 3Pr at any time.

DISCUSSION

Results showed that calcium is absorbed from the distal colon of humans and that this process is enhanced by the presence of SCFAs. These findings are consistent with the results obtained from our previous study in humans (10) and in a study on calcium and magnesium absorption in the distal colon of rats (7, 8). Propionate was more effective than acetate in enhancing calcium absorption. At a lower concentration of propionate, its enhancing effect on calcium absorption was similar to that of acetate at its higher concentration; thus, we looked at the effect of different concentrations of acetate and propionate on calcium absorption. It was observed that at higher concentrations, propionate was more effective than acetate in enhancing calcium absorption.

It has been speculated that the enhancing effect of SCFAs on calcium absorption may be due to an exchange of calcium and hydrogen, which occurs in the distal colon but not in the proximal colon (7). The proposed mechanism by which SCFAs enhance calcium absorption is that protonated SCFAs are absorbed by direct diffusion across the apical membrane (15, 16). Once the protonated SCFA molecule diffuses into the cell it dissociates because the pKa values (the negative log of the dissociation constant Kd) of SCFAs (−4.8) are lower than that of the pH of the cell (between 6 and 7). The resulting intracellular hydrogen ion (H+) is secreted from the cell in exchange for a cation, which in the distal colon is Ca2+ (7). Once outside the cell, H+ becomes available to protonate a SCFA to diffuse into the cell. Our results are consistent with this hypothesis.

The pH of the infused solutions increased immediately after infusion (time 0) and remained stable over the 30-min retention period. The increase in pH indicates the presence of bicarbonate in the colonic lumen. There is evidence that the appearance of bicarbonate in the colonic lumen is enhanced by the presence of SCFAs (17). The appearance of bicarbonate in the colonic lumen may indicate the presence of the hydrogen ion, which is needed to exchange with calcium for absorption. This may be the reason for increased calcium absorption in the presence of SCFAs. However, the difference in pH cannot account for differences in calcium absorption because calcium absorption after Ca + 3Pr treatment was significantly greater than that after Ca + 3Ac despite no difference in pH.

The ability of propionate to have a greater enhancing effect on calcium absorption than acetate may be due to its being more lipid-soluble than acetate and thus more rapidly absorbed.
by direct diffusion. It was observed in this study that propionate absorption was significantly greater than that of acetate, which was reported in another paper (18). In a study in guinea pig distal colon (19), SCFAs had a net absorption rate that corresponded to the chain length of the SCFAs, i.e., butyrate > propionate > acetate. The chain-length dependence of unidirectional SCFA fluxes in the distal colon may indicate the importance of lipid solubility for nonionic diffusion in the distal colon. Thus, propionate may have diffused into the cell faster than acetate and supplied intracellular hydrogen ions to exchange for calcium.

This study was done as a continuation of our previous study on the effect of a mixture of acetate and propionate on serum calcium response (10). Butyrate is immediately utilized in the colon and is difficult to measure in the serum, it was not included in our previous study or this study.

The calcium disappearance from the total colonic sample, which represents calcium absorption from the rectum and distal colon in this study, may be calculated from calcium:PEG. Calcium absorption (mmol calcium/30 min) was as follows in study 1: Ca+NaCl, 1.0 ± 0.2; Ca+3Ac, 4.5 ± 0.5; Ca+1Pr, 4.5 ± 0.6; and Ca+3Ac+1Pr, 4.0 ± 0.9. In study 2, calcium absorption was as follows: Ca+1Ac, 2.7 ± 0.3; Ca+1Pr, 3.0 ± 0.2; Ca+3Ac, 2.0 ± 0.6; Ca+3Pr, 3.9 ± 0.5; and Ca+1Ac+3Pr, 2.5 ± 0.2. These values represent ≈6–27% calcium absorption. Because the distribution of fluid between the rectum and distal colon is not known, we were not able to calculate accurately the actual amount of calcium absorbed. Therefore, calcium absorption from the distal colon and rectum was expressed as the differences between calcium:PEG of the samples infused and calcium:PEG of the samples collected. Nevertheless, this study provided interesting preliminary data suggesting that calcium absorption increased with time with and without acetate or propionate and that calcium absorption in the rectum was significantly lower than that from the rectum and distal colon for all treatments (Table 2).

Lutz and Scharrer (7) demonstrated that calcium is absorbed in the distal colon and not in the proximal colon of rats. The calcium absorption described in the present study represents that in the rectum, sigmoid colon, and descending colon. The average total length of the rectum is 12 cm whereas that of the colon has been reported to be 110 cm (20), with the distal segment comprising about one-third of the total length (21). The diameter of the rectum and distal colon is ≈4 cm (22). Therefore, the total volume of the rectum and distal colon is ≈150 and 460 mL, respectively. The total volume of the solution infused for each subject was 300 mL, indicating that a significant amount of the solution reached the distal colon.

Calcium absorption in the colon may have some important implications. Calcium maintains the rate of cell turnover (23–25). However, an extensive change in intracellular calcium concentration may lead to changes in the physiologic behavior of cells (26) because a rise in cytosolic calcium can cause cell aggregation, cell transformation, cell division, and activation of intermediary metabolism. These are related to cell proliferation (27) and extensive cell proliferation has been associated with increased cancer risk (23, 24, 28). Calcium absorption in the colon may then help maintain colonic health but extensive absorption may also be damaging to the cell. On the other hand, calcium released from its complex with dietary fiber after fermentation can bind with bile acids, rendering them insoluble and less damaging to the colon (29); bile acids are thought to enhance cell proliferation and risk of carcinogenesis (28, 30). Other minerals such as magnesium (31), iron (32), zinc (33), and copper (34) may have similar or different clinical implications as they reach the colon but little evidence is available in humans. The adverse or protective effect of calcium as well as other minerals reaching the colon will be worth investigating.

We conclude that calcium was absorbed in the distal colon of humans and that both acetate and propionate enhanced calcium absorption. However, propionate was more effective than acetate. The mechanism behind the enhancing effect of both SCFAs on calcium absorption needs further investigation.

REFERENCES