CALCIUM ABSORPTION IN THE HUMAN DISTAL COLON : EFFECT OF SHORT CHAIN FATTY ACIDS FROM FIBER FERMENTATION

by

Trinidad Palad Trinidad

Department of Nutritional Sciences

A thesis submitted in conformity with the requirements
for the Degree of Doctor of Philosophy
Graduate Department of Nutritional Sciences
University of Toronto

Calcium Absorption in the Distal Colon of Humans: Effect of Short Chain Fatty Acids From Fiber Fermentation

Trinidad Palad Trinidad
Doctor of Philosophy, 1997
Graduate Department of Nutritional Sciences
University of Toronto

ABSTRACT

Increased intake of dietary fiber is recommended by health agencies but there is a concern that it may reduce the bioavailability of minerals such as calcium (Ca) due to its ability to bind with minerals. However, it has been hypothesized that Ca is released and short chain fatty acids (SCFA) are produced upon fiber fermentation. The released Ca is absorbed in the distal colon and the absorption is enhanced by SCFA. To determine the amount of Ca potentially absorbed in the colon, Ca released in basal diets or single dairy foods with and without pectin, psyllium or cellulose were tested using an all in vitro method. Compared with the basal diet, pectin did not affect the total Ca release from food matrix but rather shifted the release of Ca from the small intestinal to the colonic conditions. Psyllium and cellulose caused an insignificant release of Ca in the colon. To determine the Ca actually absorbed in the colon with and without SCFA, four rectal infusion studies were done in humans. The serum Ca response was increased particularly in the presence of SCFA in a 3:1 acetate (Ac):propionate (Pr) ratio, indicating colonic absorption of Ca. Ca disappearance in the colon as a measure of Ca absorption showed that Pr was more effective than Ac in enhancing Ca absorption. Ca absorption increased linearly with Ca concentration with and without Ac, or Pr, suggesting a non-saturable diffusion process. Ac and Pr absorption were enhanced by the presence of Ca. A human

feeding study using two stable isotopes of Ca e.g. ^{42}Ca and ^{44}Ca showed that Ca absorption occurs in the colon. The presence of pectin caused a reduction in the total Ca absorption but shifted the amount of Ca absorbed from the small intestine to the colon. In summary, the in vitro, rectal infusion and oral feeding studies have shown that Ca is released after fiber fermentation and can be absorbed in the human distal colon. The colonic Ca absorption is enhanced by Ac or Pr, through a non-saturable diffusion process with Pr being more effective than Ac.

To ***MOM and DAD*** with ***LOVE***

ACKNOWLEDGEMENT

So many people have been a part of the success of this work that I have to be thankful to.

To my supervisors, Dr. Lilian U. Thompson and Dr. Thomas M. S. Wolever, for their never ending support, guidance, encouragement and interest in my work.

To the members of my advisory committee, Dr. Stephen Cunnane and Dr. Douglas Templeton and examination committee Dr. Michael Archer and Dr.Venket Rao for their valuable advise, comments and suggestions on my work.

To Dr. David J.A. Jenkins and Dr. David Kitts for the appraisal of my thesis.

To Dr. Thomas Francis and Dr. Tibor Heim for always having time to answer my questions.

To Felicia Cheung-Dang and John Roxbourgh, for their technical assistance and friendship.

To Dr. Alfred Yergey and Ms Nancy E. Vieira of the National Institute of Health, Bethesda, Maryland, USA for their collaboration in the stable isotope study.

To Andrea Ryl (Pharmacy) and Angelina Yau (Microbiology) of St. Michael's Hospital for their assistance in the preparation of the stable isotopes for human consumption.

To the Dairy Farmers of Canada and the Natural Science and Engineering Research Council of Canada for funding the research.

To my friends in our laboratory: Mazda Jenab, Paula Nesbitt, Lindy Orcheson, Sharon Rickard, Dr. Maria Serraino, Hans Skariah, Janet Tou, Dr. Yvonne V. Yuan, and to my friends in the department: Viola Antao, Claudia Bolognesi, Judlyn Fernandes, Safa Hamad, Nalini Shiwnarain-Irani, Dr. Ravati Koratkar, Steve Mitchell, Tina Perera, Raha Radmard, Susan Roberts, Ahmed El-Sohemy, Mykyung Sung, Jane Thorpe, Thien Tran and Anita Bentum-Williams for their camaraderie and support.

To Janette Campbell, Margaret Hardy, Brenda Rak, Elizabeth Pollack and Emilia D'Souza for their support and assistance.

To all my Filipino friends in Toronto especially Elizabeth de Leon, Violeta Turalba, Patricia Paez and to the St. Therese Group for their support and prayers.

To my FNRI friends, Divine Valdez, Nitz Madriaga, Aida Mallillin, Girlie Cruz, Dorie Sabenecio, Zoilo Villanueva, Lelibeth Ponce and Maribel Cabrera for their encouragement and help.

To my cousins, Anthony and Theresa Palad and children, Von Ryan, Rachelle, Karen and Joan for the nine happy years in Toronto; also to my cousin-in-law's parents, Roberto and Ofelia Lim, and relatives, Jose, Zenaida and Sherwin Gardiola.

To my very best friends of 35 years, Rachel Vicky Trinos-Romero and Mae D. Roque for thier thoughtfulness and friendship.

To LDF for making me a stronger woman.

To my INSPIRATION - my family - Mom and Dad, Rose, Irene, Nechie, Juliet, Jun, Seldo and to my SUPER 7 nieces, Julie Anne, Ritzie Anne, Trinidad II, Rozza Corrine, Jona Krista Marie, Janine Rose and Czarina Irene.

And, THANK YOU LORD, for making this happen.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

PUBLISHED MATERIAL

1. Trinidad TP, Wolever TMS, Thompson LU. Interactive effects of calcium and short chain fatty acids on absorption in the distal colon of man. Nutr Res 1993; 13:417-425.

2. Wolever TMS, Trinidad TP, Thompson LU. Short chain fatty acid absorption from the human distal colon: interactions between acetate, propionate and calcium. J Am Coll Nutr 1995; 14:393-398.

3. Trinidad TP, Wolever TMS, Thompson LU. Availability of calcium for absorption in the small intestine and colon from diets containing available and unavailable carbohydrates: an in vitro assessment. Int J Food Sci 1996;47:83-88.

4. Trinidad TP, Wolever TMS, Thompson LU. The effect of acetate and/or propionate on calcium absorption in the distal colon of humans. Am J Clin Nutr 1996;63:574-578.

5. Trinidad TP, Wolever TMS, Thompson LU. Calcium availability from dairy foods as affected by dietary fibers. Trace Elements and Electrolytes, 1996; 13:123-125.

6. Trinidad TP, Wolever TMS, Thompson LU. Calcium absorption in the distal colon of man: effect of schort chain fatty acids from fiber fermentation. Symposium on Calcium: Current Controversies and Future Directions Langdon Starr, Toronto, 1995; pp. 68-70.

7. Trinidad TP, Wolever TMS, Thompson LU. Effects of short chain fatty acids on calcium absorption in humans. Dietary Fiber in Health and Disease, eds. D. Krichevsky and C. Bonfield, Plenum Press, New York, 1996, in press.

Published papers number 3 and 5 are based on Chapter 4; Paper number 1 on Chapter 5; papers number 2 and 4 on Chapter 6; and papers 6 and 7 on partial data from Chapters 4 to 7.

CHAPTER 1

INTRODUCTION

I. INTRODUCTION

Dietary guidelines recommend increased intake of dietary fiber (Scientific Review Committee, 1990) but a concern is that it may reduce the bioavailability of minerals such as calcium (Ca) and result in a deficiency state (Ink, 1988; Furda, 1979; James et al, 1978; Branch et al, 1975). However, it has been suggested that minerals bound or entrapped by fiber in the small intestine may become available for absorption in the colon if the fiber is fermented by colonic bacteria (Thompson et al, 1991). This process depends upon the fermentability of the complexing agent to which Ca is bound, the release of Ca and also the short chain fatty acids (SCFA) produced during fermentation.

Ca has been shown to be transported and absorbed in the colon of animals (Nellans and Goldsmith, 1981; Nellans and Kimberg, 1978; Demigne et al, 1989; Partridge, 1978; Harrison and Harrison, 1969; Crammer and Copp, 1960; Amman et al, 1986; Petith and Schedl, 1977; Lutz and Scharrer, 1991) but little evidence is available in humans (Grinstead et al, 1984; Sandstrom et al, 1986). Short chain fatty acids (SCFA) mainly acetic (Ac), propionic (Pr) and butyric (Bu) acids, which are major products of fermentation of dietary fibers, have been suggested to enhance the absorption of Ca and magnesium in the rat colon (Demigne et al, 1989; Lutz and Scharrer, 1991; Lutz et al, 1991) but this has not been demonstrated in humans. Therefore, the overall goal of this work is to determine the amount of Ca that reaches the human colon and whether this Ca is absorbed and the absorption is facilitated in the presence of SCFA. A further goal is to provide insight regarding the potential mechanism of Ca absorption in the colon.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Calcium

2.1.1. Calcium in Foods

Calcium (Ca) is an essential nutrient for normal growth and development. Ninety-nine percent of total body Ca is in the bones and teeth (Scientific Review Committee, 1990). The remaining 1% is located in the soft tissues where it is involved in metabolic processes, including enzyme activation, nerve transmission, membrane transport, blood clotting, muscle contraction and hormone function (Linder, 1991). To maintain positive Ca balance, most adults need to consume 800 mg/day of elemental Ca; 1000-1100 mg/day, for younger men and women and an additional 500 mg/day for pregnant and lactating women (Scientific Review Committee, 1990).

Dairy products such as milk, cheese and yogurt supply three-quarters of the Ca in the Canadian food supply while vegetables, fruits, and grain products supply the rest (Scientific Review Committee, 1990). Meat, fish, and poultry supply a very small amount. Ca in foods exists in the ionic, bound or complexed forms. Ca is present in milk as ionic Ca and colloidal Ca phosphate; in cheese as Ca paracaseinate; and in yogurt primarily as ionic Ca (Farrell and Thompson, 1974). In fruits, vegetables and grain products, Ca is in the form of Ca complexes such as ascorbate, citrate, fiber, oxalate and phytates (Heaney and Weaver, 1992; Heaney et al, 1990; Leichsenring et al, 1957). A study on the form of Ca utilized by rats has shown that Ca as Ca caseinate was the best utilized; colloidal Ca phosphate was greater than Ca carbonate; and ionic Ca was as

effective as Ca carbonate (Wong and La Croix, 1980). The same study showed that ionic Ca is less utilized than colloidal Ca indicating that Ca availability in dairy products is affected by the form of the Ca complex present. However in another study, the form of Ca in whole milk, chocolate milk (containing oxalate), yogurt, imitation milk, cheese and Ca carbonate did not affect Ca availability (Recker et al, 1988; Yuan et al, 1991). This was supported by the work of Sheikh et al (1987) on the absorption of Ca from different Ca salts with different solubilities and from milk in human subjects where differences in Ca absorption did not show significant differences. They hypothesized that acid dissolution in the gastrointestinal tract may be responsible for similar absorption of Ca salts. Moreover, lactase deficient and lactose tolerant subjects fed with either yogurt (unflavored) or milk also did not show significant differences in Ca absorption (Smith et al, 1985).

Certain foods such as beet greens, rhubarb, spinach and peanuts contain subtances which can impair Ca absorption (Weaver et al, 1991). They all contain high amounts of oxalic acid which can form insoluble complexes with Ca and may remain unabsorbed in the small intestine. Cereal grains contain phytic acid which can also form insoluble complexes with Ca. High fiber meals may also impair Ca absorption. A study on premenopausal women given the same amounts of Ca showed that Ca in milk was better absorbed than a similar quantity of Ca in the presence of oxalate (Heaney et al, 1990). Ca absorption from whole wheat cereal was less than a similar Ca load from milk, but leavened bread made from the same whole wheat had a higher Ca absorption than milk in humans (Weaver et al, 1991). During the leavening process, the phytate content of the

bread is destroyed (Reinhold et al, 1976).

When 10 g cellulose (insoluble fiber) was added to a low fiber diet consumed by men for 20 days and 16 g cellulose was added to the diet of women for 30 days, negative Ca balance resulted (Ismael-Beigi et al, 1977; Slavin and Marlett, 1980). However, 36 g pectin (soluble fiber) added to a diet consumed for 6 weeks did not affect the Ca balance of 5 men (Cummings et al, 1979). In another study 15 g of pectin added to the diet (3-day study) did not impair the apparent mineral absorption from the small intestine except for iron (Sandberg et al, 1983). All of the above studies showed that Ca absorption is affected by dietary factors such as oxalate, phytates and fibers.

2.1.2. Calcium Absorption

2.1.2.1. Physiology of Calcium Absorption

A large proportion of Ca in foods exists in the bound or complexed forms and thus digestion is required to liberate Ca prior to absorption (Nordin,1976). Ca released from the complex should be soluble and in an ionized form for absorption to take place (Schachter et al, 1960; Miller and Berner, 1989; Smith and McAllan, 1966). This conversion is affected by endogenous factors such as hydrochloric acid in the gastric secretion and pH, and by dietary constituents such as fibers, phytates, carbonates and phosphates (Champagne, 1988). While digestion is necessary for the release of Ca from food complexes and its conversion to the soluble and ionized form in which it is absorbed, the limiting factor influencing the amount of Ca absorbed is the Ca solubility in the intestinal cells (Nordin, 1976).

Ca, along with other products of digestion, enters the luminal fluid from the chyme as a result of mechanical and enzymatic action. It then crosses the intestinal membranes and cell junctions and enters the lymph and blood (Bronner, 1987; Figure 2.1). The transepithelial Ca movement in the small intestine occurs by two independent processes: (a) saturable, transcellular transport which takes place in the proximal intestine, i.e. duodenum and the upper jejunum (Pansu et al, 1983). This process is subject to physiological and nutritional regulation via Vitamin D. (b) A non-saturable, paracellular route i.e. movement between the mucosal cells. This process is essentially independent of nutritional and physiological regulation and is concentration-dependent which occurs all along the small intestine (Zornitzer and Bronner, 1971; Pansu et al, 1983; Behar and Kerstein, 1976).

The transcellular movement involves three steps: (1) entry across the brush border membrane of the enterocyte; (2) intracellular movement; and (3) extrusion across the basolateral membrane. Ca entry into the cell is down an electrochemical gradient (Bronner, 1991) and is described as the sum of saturable and non-saturable steps based on uptake measurements that use right side-out brush border membrane vesicles (Rasmussen et al, 1979; Miller and Bronner, 1981; Schedl amd Wilson, 1985; Takito et al, 1990). The saturable step may represent the established and significant binding of Ca on to the inner brush border membrane vesicle (Miller and Bronner, 1981; Miller et al, 1982) and a carrier-mediated transport step (Schedl and Wilson, 1985; Wilson et al, 1989).

Ca moves through the cytoplasm inside the cell. The rate of diffusion of Ca is too

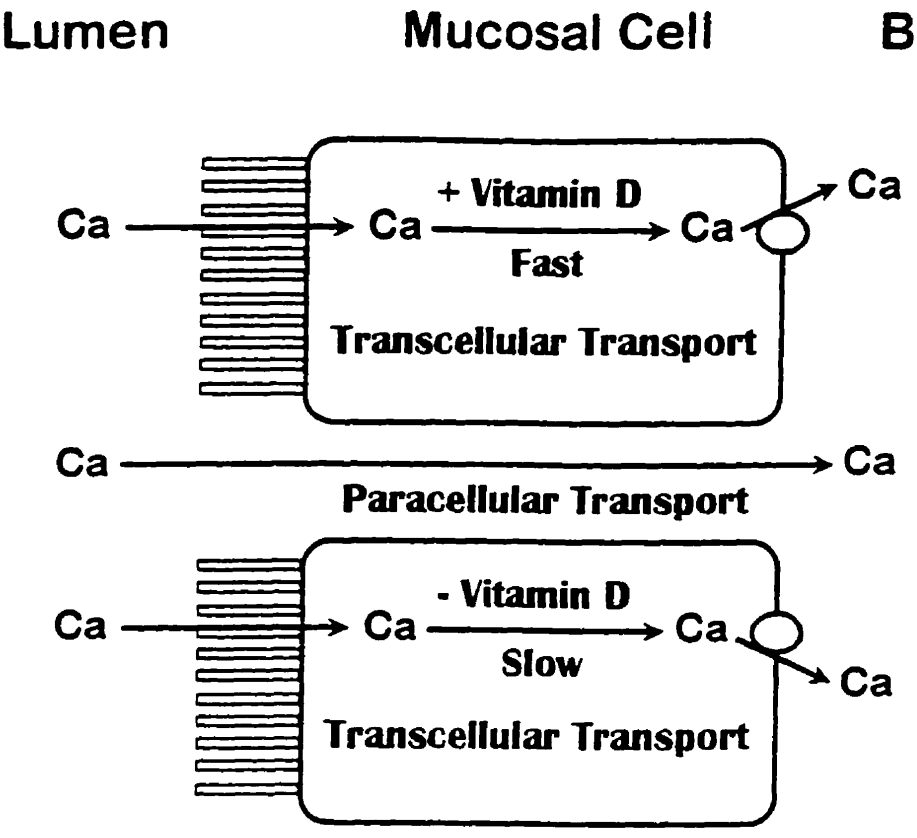


Figure 2.1. The potential pathways and mechanism for Ca transport across the small intestine epithelium Taken from Wasserman, 1992 and Nellans, 1988.

slow to allow Ca transfer to occur in an enterocyte such as the ileal cell (Bronner et al, 1986). Thus, a cytosolic Ca-binding protein, calbindin-9K (Wasserman et al, 1978), occurs in duodenal cells at high concentration (Bronner et al, 1986) to assure a rate of intracellular flux sufficient to satisfy the established transcellular transport rate.

Extrusion is against an electrochemical gradient (Bronner, 1992). However, the overall transport from the intestinal lumen to the fluid bathing the serosal pole of the intestinal cell is usually downhill, at least from a concentration viewpoint. The luminal Ca concentration is usually higher than the body fluid concentration of approximately 1 mmol Ca^{+2}/L.

Paracellular Ca movement is down a chemical gradient and consists of three sequential stretches: the tight junction, the intermediate junction, and a much wider basolateral space (Trier, 1968). The rate of Ca movement through the region of the tight junction is far slower than would be expected on the basis of simple diffusion (Bronner et al, 1986). Hence the structure of the junction, with its compressed protein-lipid space, must hinder fluid and Ca movement appreciably. Hyperosmolar solutions, regardless of their chemical nature, can cause the rate of passive Ca transport to double or triple (Pansu et al, 1976). This occurs because water moving into the hyperosmolar space causes the tissue to expand and the junctions to widen and this in turn modifies the cytoskeleton of the cell walls lining the junctions. Some amino acids are responsible for the contraction of cytoskeleton of the cells lining the junction causing an increase in Ca flow (Pappenheimer, 1987; Pappenheimer and Reiss, 1987; Madara and Pappenheimer, 1987).

It has been shown that the active transport of Ca is most highly developed in the duodenum in rats, rabbits, guinea pigs and mice (Schachter and Rosen, 1959; Schachter et al, 1960; Kimber et al, 1961; Schachter, 1963; Wasserman and Taylor, 1969). To support the above studies, Ca absorption was assessed at the different sites of the intestine by in vivo perfusion studies which allow an assessment of total Ca absorption in relation to various concentrations of Ca in the gastrointestinal lumen (Wilkenson, 1971). At any given concentration of Ca, the duodenum has a greater ability to absorb Ca per unit length than the jejunum or ileum as concluded from the everted gut sacs technique (Wilkensen, 1971; Wensel et al, 1969). However, the site at which the major part of the dietary Ca is absorbed in vivo depends not only on the efficiency of absorption but also on the length of the intestinal segment and concentration of Ca in the lumen (Nordin, 1976).

Cramer and Copp (1960) deduced that in the rat, strontium (which is equated as a measure of Ca) was absorbed primarily in the ileum (65%), followed by jejunum (17%), colon (8%), duodenum (7%) and stomach (2%). Marcus and Lengeman (1962) observed that the rat ileum was the major site of absorption of alkaline earth metals. Moreover, Cramer (1965) estimated that the amount of dietary Ca absorbed was five times more in the dog's ileum than in the jejunum, but the Ca absorption in the jejunum was four times greater than that in the duodenum.

Although several studies have examined Ca transport in the small intestine, there are few studies which have investigated Ca transport in the colon. Using everted loops of rat colon incubated in vitro in bicarbonate solution, Ca transport has been observed from mucosal to serosal compartments against an electrochemical potential producing a

concentration ratio Ca serosal/Ca mucosal (Cs/Cm) considerably greater than 1.0 (Harrison and Harrison, 1969). Vitamin D is required for measurable transport of Ca in the colon since it has been shown to increase, in vitro, the permeability of colonic mucosa to Ca. This may depend on the Vitamin D required to increase the rate of penetration of Ca across the luminal surface of the mucosal cell which will increase the concentration of intracellular Ca to the level required for maximal active transport by the energy-linked system. A similar in vitro study in the descending colon of the rat also showed an enhancing effect of Vitamin D on Ca absorption (Favus et al, 1981). Ca absorption in the descending colon is by a carrier-mediated, active transport mechanism whereas Ca secretion occurs by a non-saturable process via the paracellular pathway. Karbach and Rummel (1986) studied whether short (6h) and long time (4 days) pretreatments with 1,25-dihydroxy vitamin D act differently on the transcellular and the paracellular Ca transport across the descending colon mucosa of the rat using a modified Ussing chamber (Karbach et al, 1986). Tissue just 1.5 cm proximal to the lymph node found regularly at the distal end of the colon was used. Results showed that after a short time exposure to 1,25-dihydroxy vitamin D, only the transepithelial potential (PD)-independent transcellular mucosal to serosal Ca transport was activated while after a long time induction, both the PD-dependent paracellular and transcellular mucosal to serosal Ca transport are activated.

Ca absorption was roughly 100 mg /day when the colons of healthy human subjects were perfused with 5 mM Ca-containing solution in response to administration of endogenous Vitamin D (Grinstead et al, 1984). However, a linear correlation was not observed between the change in Ca transport and the increase in Vitamin D levels. There

was no Ca absorption in the colon before treatment with Vitamin D. Net movement indicated that Ca was secreted into the lumen although the secretion rate was not statistically significantly different from zero. Whether Vitamin D induces the Ca-binding soluble protein responsible for Ca to be absorbed in the small intestine is still a question. However, studies have shown that the concentration of Ca binding protein along the length of the entire intestine of rats decreased in the distal segment of the small intestine where active transport is negligible (Ambrecht and Wasserman, 1976; Ambrecht, 1987). Therefore it is possible that Ca transport may occur by a paracellular route or non-saturable diffusion process in the colon.

Ca transport in the rat distal colon in the presence of short chain fatty acids (SCFA) such as Ac and Bu was speculated to be by a Ca/H exchange similar to the sodium (Na)/H exchange that exists in the rat proximal colon (Lutz and Scharrer, 1991). In the above study, Ca was perfused through the distal and proximal colon of rats and the luminal disappearance of Ca was determined. Absorption rates were determined following a 30 min equilibration period.

Based on the above studies, Ca transport in the colon may be both saturable and non-saturable in the presence of 1,25-dihydroxy vitamin D. However, the effect of other substrates present in the colon such as SCFA, on Ca absorption is not clear. The transport mechanism of Ca in the presence of SCFA in the human colon needs further investigation.

2.1.2.2. Methods for Measuring Calcium Absorption

Several methods have been developed to determine Ca absorption in the small intestine: the chemical balance technique which includes chemical analysis of food eaten and feces from normal individuals or ileal effluent of ileostomate volunteers, radioisotope and stable isotope methods, and in vitro methods simulating conditions that prevail in the stomach and small intestine.

2.1.2.2.1. Chemical Balance Techniques

The absorption of Ca in the gastrointestinal tract in man is usually assessed by the metabolic balance technique (Harrison et al, 1973; Marshall and Nordin, 1981; Nordin et al, 1979; Reeve et al, 1980). The apparent Ca absorption is the difference between dietary Ca intake and fecal Ca. It can be expressed as either: (1) an absolute amount, $B_{Ca} = i_{Ca} - f_{Ca}$ where B_{Ca} represents the net Ca absorbed, i_{Ca}, the Ca intake and f_{Ca}, the fecal Ca; or (2) a percent of the dietary Ca intake, $B_{Ca} = (i_{Ca} - f_{Ca})/i_{Ca} \times 100$ (Nordin, 1976). At intakes below 3 mg/kg/day, the net Ca absorbed is negative, meaning that some of the digestive juice Ca secreted into the intestinal lumen is not being reabsorbed and is appearing in the feces with the unabsorbed dietary Ca. In this situation, the fecal Ca is greater than the dietary Ca. At a Ca intake of 3 mg/kg/day, the total Ca absorbed is equivalent to the digestive juice Ca content and the net Ca absorbed is zero. At higher intakes, Ca absorbed is greater than the Ca of the digestive juice and net Ca absorbed becomes positive. As dietary Ca intake increases, net Ca absorbed increases and, over the dietary range studied, never reaches a maximum. The relative inaccuracy of the balance

procedure may be due to biological variation including age, vitamin D status and season. It may also arise from the process of adaptation because many studies are carried out acutely utilizing dietary intake which is different from the subjects' habitual diets (Nordin, 1976).

2.1.2.2.2. Radioisotope and Stable Isotope Techniques

True absorption has been measured using a chemical balance approach and correcting for endogenous fecal secretion by the use of an intravenous Ca (^{47}Ca) label (Blau et al, 1957; Marshall and Nordin, 1981; Phang et al, 1969). Other techniques used for determining true Ca absorption are the use of a single oral tracer (^{45}Ca) (Barltrap et al, 1977; Ehrenkranz et al, 1985; Harrison et al, 1973; Miller et al, 1989) or a single tracer twice, once orally and a second time intravenously (Lutwak, 1969; Marshall and Nordin, 1981). Appreciable variability is associated with any of the chemical balance or fecal recovery methods while the use of a single label twice is limited by the assumption of constancy in the subject between the two separate studies.

Over the last 20 years, methods for determining true Ca fractional absorption were developed employing two isotopic radioactive labels simultaneously, one orally (^{45}Ca) and the second, intravenously (^{47}Ca). These dual isotope methods used a variety of experimental schemes for label administration and sample collection. The method was first proposed by Bronner (1962) and developed in the current form by de Gracia et al (1965). Bronner's (1962) approach involved determining the ratio of isotopes excreted in complete urine collection which is one of the oldest approaches developed for

measurement of fractional Ca absorption. De Gracia (1965) originated the approach which considers a delay between the administration of the oral and intravenous labels. The approach was used subsequently by a number of other investigators (Harrison et al, 1973; Szymendra et al, 1972). Ratios of isotope were measured in samples of serum or urine collected at predetermined times after administration (Harrison et al, 1973; Miller et al, 1989; Price et al, 1990) or urine obtained from complete collections over a period of time.

The last decade has seen the emergence of the use of stable Ca isotopes (^{42}Ca and ^{44}Ca) as labels in absorption measurements. This has been used in studies of Ca absorption in segments of the general population inappropriate for study using radioactive labels, i.e. pregnant women, infants and children (Abrams et al, 1993, 1992; Mauras et al, 1994). Yergey et al (1994) compared four dual isotopic label methods for determining true fractional absorption of dietary Ca in 23 subjects. They measured (a) α_{24}, which is the fractional absorption of Ca obtained from the ratio of oral (^{44}Ca) to intravenous (^{42}Ca) label in urine from 0-24 hours; (b) α_{spot}, which is the ratio of fraction of oral to intravenous label in a single urine specimen; (c) α_{lag}, which is the ratio of the dose corrected enrichment of the oral label in serum 4 hours after administration to the dose corrected enrichment of the intravenous label 2 hours after administration. This approach is physiologically equivalent to a two hour delay between administration of the oral and intravenous label; and (d) α_{Dec}, which is calculated from the deconvolution of the observed responses to the oral and intravenous labels. It was observed that the value of fractional absorption of dietary Ca are method dependent (Yergey et al , 1994). However, the mean

values for the four above methods were not significantly different. Both α_{24} and α_{Dec} gave results that are better approximations of the correct value for fractional absorption of dietary Ca because of their relative freedom from physiological assumptions. α_{lag} and α_{spot} are accepted for use in studies of changes of response within similar groups (Yergey et al, 1994).

Measurements of Ca absorption using radioisotopes and stable isotopes have been well established. However, most studies have made assumptions that Ca is absorbed in the small intestine. A study on the time course of Ca absorption in humans using radiolabled Ca (oral/intravenous) in serum and urine showed that Ca absorption was 80.9% complete at 3 hours and 95.8% complete at 7 hours; 4.2% of Ca was assumed to be absorbed in the colon (Barger-Lux et al 1989; Heaney et al. 1989). Nevertheless, it is difficult to determine the exact time Ca reaches the colon.

2.1.2.2.3. In Vitro Methods

Several investigators have developed in vitro methods of estimating the availability of iron (Miller et al, 1981; Hurrell et al, 1988). These were based on the release of dialyzable iron from foods subjected to treatment with pepsin-HCl at pH 2.0 followed by adjustment to pH 7.5 and the addition of pancreatin-bile mixture, simulating conditions that prevail in the stomach and small intestine, respectively. A correlation was found between the above in vitro method and in vivo measurements (Miller et al, 1981; Hurrell et al, 1988). Dietary factors such as ascorbic acid (Cook and Monsen, 1977; Conrad and Schade, 1968), coffee (Rossander et al, 1979), wheat bread (Bjorn-Rasmussen, 1974;

Callender and Warner, 1968), tea (Disler et al, 1975), eggs (Cook and Monsen, 1974; Monsen and Cook, 1979) and dietary fiber (Trinidad, 1990) that may enhance or inhibit iron absorption were shown to have the same effect on iron absorption in vitro as they have in vivo (Miller et al, 1981; Hurrell et al, 1988; Trinidad, 1990; Thompson et al, 1991).

Dialysable Ca as a measure of Ca availability was determined in our laboratory (Thompson et al, 1991; Trinidad, 1990) based on the method for iron of Hurrell et al (1988). The conditions used to simulate the stomach and small intestine in the determination of dialyzable Ca were similar except that the length of time of incubation was 15 hours instead of 3 hours. Percent dialyzable Ca had significant correlation with Ca absorption in ileostomate subjects (R^2 = 0.9; Trinidad, 1990). Ca availability determined by in vitro method is useful because it is faster, less expensive and offers a better control of experimental variables than by in vivo method. The in vitro method can be used to estimate Ca availability.

2.1.2.2.4. Colon

Although several studies have been done on absorption of Ca in the small intestine, studies on colonic absorption are sparse.

Ca absorption has been studied in rat colon using ^{89}Sr as a qualitative indicator (Cramer and Copp, 1960). The method involved tying the gut loop to be studied at above and below any two of the following points: pylorus, duodenum-jejunal ligament, midpoint between the ligament and caecum, ileo-caecal junctions and anal spincter and

then injecting slowly 15 microcuries of ^{89}Sr into the lumen of each loop to avoid distention of the gut. The passage of ^{89}Sr was measured along the intestinal tract and tail using a shielded Geiger counting tube, ratemeter and recording milliameter. An 8% strontium absorption was observed in the colon. While this investigation may indicate colonic absorption of Ca, it does not give the proportion of Ca that is absorbed in a food/meal.

Net absorption of Ca and Zn in the colon have been studied in pigs. Ileostomy contents were re-entered into the colon by re-entrant cannulas in the terminal ileum, 0.3 meter anterior to the ileocecal junction (Partridge, 1978). A simplified digesta-return system consisting of a polyethylene funnel connected by a polyvinyl chloride tube to the distal cannula was used. A 24-hour collection of ileal digesta was made at 6-hour intervals and analyzed for Ca, P, Mg, Na and Zn. Absorption of up to 31% of the intake of Ca and 50% of the intake of Zn was obtained from ileal digesta which were infused into the colon.

As the above method cannot be used in humans, Sandstrom et al (1986) adapted a radionuclide technique to study the quantitative uptake of Ca and Zn from the human colon during colonoscopy. The bowel of each subject was prepared by laxative and enemas. An oral saline purge was given the morning before the start of the experiment. The fiber optic colonoscope was inserted and the caecum was reached in nine patients and the hepatic flexure in four patients. The radionuclide solution was administered via the biopsy channel at maximal distant position and the colonoscope was slowly withdrawn during inspection of the mucosa. A whole body counter was used to measure absorption

of Ca and Zn from the colon. They found a 4.1% Zn and 14.1% Ca absorption after infusion of 39 umol of Zn and 6.4 mmol of Ca which was labelled with Zn-65 and Ca-47, respectively. However, this may be an underestimation of the actual absorption that takes place in the colon since absorption measurements were done in the colon that had previously been cleaned. The SCFA produced upon fermentation of undigested residue from the small intestine are present in a normally functioning colon. The Ca release and the effect of products of fiber fermentation such as SCFA may contribute to a greater degree of Ca absorption from a normal colon than from a cleaned colon.

2.1.3. Dietary Factors Affecting Calcium Absorption

2.1.3.1. Protein

Increased protein intake was associated with increased Ca excretion in the urine in 19 separate human studies consisting of 150 adult subjects (Allen et al, 1979; Chu et al, 1975; Draper et al, 1991; Hegsted et al, 1981; Hegsted and Linkswiler, 1981; Johnson et al, 1970; Kim and Linkswiler, 1979; Lutz and Linkswiler, 1982; Lutz, 1984; Margen et al, 1974; Schuette et al, 1980; Spence et al, 1978, 1983; Trilok and Draper 1989; Walker and Linkswiler, 1974;). Protein sources consisted of wheat gluten, beef, milk, egg, soy, lactalbumin or casein in the form of purified proteins or mixed foods and the study period ranged from 12 to 60 days. Although a wide variety of experimental protocols were used in all of the above studies, the relationship between dietary protein and urinary Ca excretion was linear ($r=0.70$) with protein intakes below 175 g/day, and for each 50 g increment of protein consumed, there is an extra 60 mg of urinary Ca lost (Kerstetter and

Allen, 1994). At protein intakes of 25-74 g/day, Ca balance was close to equilibrium when Ca intake was between 500 and 1400 mg/day. When protein intake was higher than 75 g/day and Ca intake less than 600 mg/day, a negative Ca balance occurred (Kerstetter and Allen, 1994).

Differences in the source of protein gave different effects on Ca absorption (Hegsted et al, 1981; Kitts et al, 1992; Schuette et al, 1982). Meats and dairy foods are high in protein and did not affect urinary Ca excretion; however both foods also contained phosphorous. Investigators have shown repeatedly that addition of dietary meat/red meat or dairy products failed to affect urinary Ca because of their high phosphorous content (Hunt et al, 1995; Spencer et al, 1978, 1985, 1988). Phosphorous intake greater than 1500 mg/day reduced calciuria in comparison to phosphorous intakes between 1000 and 1500 mg/day and 150 mg/day (Kerstetter and Allen, 1994). Isolated purified protein intake increased urinary Ca excretion (Hegsted et al, 1981; National Research Council, 1989; Schuette et al, 1980, 1982).

Paracellular Ca absorption in the distal segment of the small intestine was studied in rats fed with different sources of protein, i .e. casein and soybean-protein isolates (Kitts et al, 1992). It was found that the absorption of Ca from casein was significantly greater than that from the soybean-protein isolates. The ability of casein to enhance the absorption of Ca was partly explained by the presence of bioactive peptides i.e. caseinophosphopeptides (CPP), which inhibit the precipitation of calcium phosphate , thus, resulting in increased Ca solubility (Kitts et al, 1992). The presence of phytate in the soybean-protein isolates was an additional factor responsible for the reduced

Ca absorption. Thus, it appears that the type of protein plays an important role in paracellular Ca absorption.

2.1.3.2. Fat

Varying amounts of dietary fat (1 to 32% of the diet) did not show any effect on Ca balance in healthy adults (Steggerda and Mitchell, 1951). Fat prolongs gastrointestinal transit time allowing for a longer interval for Ca absorption; however, fat may form insoluble complexes with Ca making it less available (Levenson and Bockman, 1994). Interest in the possible connection between fat intake and Ca absorption was focused on the massive losses of Ca in patients with fat malabsorption or steatorrhea (Agnew et al, 1971). This is generally thought to be due to the reaction of Ca with fatty acids forming insoluble soaps in the intestinal lumen. The availability of Ca from these soaps decreased with increasing chain length and decreasing degree of unsaturation of the fatty acids . Ca absorption in the rat intestine was found to be less than 10% from C12:0 to C18:0 fatty acids but more than 50% from C6:0 and C8:0. Only 2% of the Ca from C18:0 soaps was absorbed compared to 20% of that from C18:2 (Kies, 1988; Gacs and Barltrop, 1977). Fat in the form of triglycerides did not affect Ca absorption implying that soap formation and Ca malabsorption may only occur when free fatty acids are present in the intestine. Ca soap formation occurs during the hydrolysis of emulsified fat droplets by pancreatic lipase in the presence of colipase and bile salts micelles. Addition of lipases caused the rapid formation of a liquid, crystalline Ca shell around the droplets of the oil which contained triglycerides and Ca and probably Ca soaps. The soap formation is

proportional to the amount of ionized fatty acids. Formation of ionized fatty acids decreased as the concentration of Ca increased and a lower amount of soap formation occurred. Monoglycerides also reduced the formation of Ca soaps. Formation of soaps is an integral part of lipid digestion and it is not known whether any Ca is absorbed with micelles (Patton and Carey, 1979). Medium chain triglycerides were found to enhance Ca absorption in infants (Tantibhedhyangkul and Hashim, 1978). Moreover, it was found that Ca absorption was improved by increasing the total level of dietary fat (Kies, 1985).

Diets containing varying levels of Ca and nearly fat-free or contained fat supplied primarily by butterfat, sesame oil, peanut oil, coconut oil or mustard oil was studied in young men (Kies, 1988; Basu and Nath, 1946). Feeding of all test fats except coconut oil resulted in a slight decrease in fecal Ca, thus an assumed increase in Ca bioavailability. However, feeding of coconut oil (a highly saturated fat) resulted in increased fecal and urinary losses of Ca.

2.1.3.3. Carbohydrates

2.1.3.3.1. Monosaccharide

Glucose has been shown to enhance Ca absorption in both humans and animals (Norman et al, 1980; Zheng et al, 1985; Younoszai and Nathan, 1985; Vaughan and Filer, 1960; Monnier et al, 1978). Monnier et al (1978) found that 40 g of orally administered glucose/m^2 body surface increased the rate of Ca absorption in 10 diabetic patients by 21%. Another study showed that 65-100 mM glucose increased jejunal Ca absorption by twofold when given to 9 normal subjects using triple lumen perfusion technique (Norman

et al, 1980). The primary site responsible for the enhanced Ca absorption following an oral dose of glucose is not known. Studies in rats have shown that glucose can increase Ca absorption in the duodenum (Behar and Kerstein, 1976), jejunum (Younoszai and Nathan, 1985) and ileum (Vaughan and Filer, 1960). The positive effect of glucose on Ca absorption in rats is consistent with the study of Zheng et al (1985) following the coadministration of an oral dose of Ca and glucose. However, some studies have shown no enhancement of glucose on Ca absorption (Urban and Pena, 1977; Wasserman and Comar, 1959). A 10 g oral dose of glucose did not increase fractional Ca absorption in postmenopausal women (Francis et al, 1986) but Wood et al (1987) have found that 50 g glucose can increase fractional Ca absorption in humans. The differences in the effect of glucose on Ca absorption from the above results may be related to dose or to differences in the responsiveness to glucose stimulation of Ca absorption in postmenopausal women. Moreover, Zheng et al (1985) have shown in rats that the effect of orally administered glucose on Ca absorption is dose dependent.

2.1.3.3.2. Disaccharide

Lactose, the principal carbohydrate in milk, has been reported to enhance the bioavailability of dietary Ca (Ambrecht, 1987; Buchowski and Miller, 1991; Cochet et al, 1983; Lavenson and Bockman, 1994; Miller, 1989; Pansu and Chapuy, 1970; Shaafsma and Visser, 1980; Ziegler and Fomon, 1983). Lactose facilitates the paracellular transport of Ca in both proximal and distal small intestinal segments (Ambrecht, 1987; Ambrecht and Wasserman, 1976). The effect of lactose on Ca absorption is greatest in the ileum

where active transport is negligible and the effect is significant only at higher intraluminal Ca concentrations (Lengemann et al, 1959; Pansu et al, 1976). A study on the effect of lactose on Ca absorption in infants under 8 months of age showed a Ca absorption of 60% from the infant formula, 36% from the lactose-free formula and 72% from the lactase-treated formula (Kabayashi et al, 1975). This suggests that glucose and galactose released by lactose hydrolysis were even more effective in improving Ca absorption than lactose (Griessem et al, 1986). This was confirmed by Wood et al (1987). The serum absorption curve of ^{47}Ca given in milk and lactose-free milk was significantly greater from milk with lactose in healthy adults (Kocian et al, 1973). When both milks were fed to lactose tolerant and lactose intolerant subjects, Ca absorption did not differ significantly. In the presence of lactose, Ca absorption decreased in the lactose intolerant subjects as indicated by reduced postprandial appearance of ^{47}Ca in the serum. However, this assumption may be incorrect since ^{47}Ca fecal excretion was not increased. Absorption may have occured in the colon at a later time. A similar study gave the same results where the lactose-tolerant and lactose-intolerant subjects absorbed Ca from lactose containing milk as efficiently as from milk with glucose as a substitute for lactose (Griessen et al, 1989). The lactose-intolerant subjects absorbed Ca from the lactose containing milk better than the lactose-tolerant subjects. The increase in Ca absorption may therefore be due to the lactose itself. The lactose not hydrolysed in the small intestine may be hydrolysed in the colon and increase Ca absorption. The above result suggests that lactose-intolerance has a negligible effect on Ca absorption but needs to be confirmed. In this case, lactose-intolerant individuals may only be Ca deficient when their Ca intake is low rather than

because of poor Ca absorption. When part of the lactose is hydrolyzed to glucose and galactose in the small intestine (Dahlqvist and Thomson, 1964; Kim et al, 1978) these monosaccharides are readily absorbed and the remaining lactose reaches the large intestine (Dahlqvist and Thomson, 1964; Kim et al, 1978) and is metabolized (Kim et al, 1978) to SCFA (Ac, Pr and Bu), thereby decreasing the pH of the fluid within the cecum and colon (Pansu et al, 1978).

Several hypotheses that explain the stimulatory effect of dietary lactose on intestinal Ca absorption have been proposed. First, the glucose and galactose produced from lactose in the small intestine enhance intestinal Ca transport as a consequence of an increased water absorption (Birlouez-Aragon, 1988; Schuette et al, 1989). Second, the unhydrolyzed lactose promotes Ca absorption in the jejunum and ileum because the increased volume of fluid required to maintain isotonicity in these segments increases the permeability of the intercellular junctions between enterocytes (Bronner, 1987). Third, the decrease in pH from hydrolysis of lactose in the large intestine increases the concentration of soluble Ca available for uptake by vitamin D-dependent Ca transport systems present in the cecum (Favus and Angrid-Backman, 1985, Nellans and Goldsmith, 1981) and colon (Karbach and Rummel, 1986; Lee et al, 1980). One other possible mechanism which has not been fully investigated involves the products of lactose metabolism in the colon, i.e. SCFA. The SCFA may be also responsible for an increase in Ca absorption in the colon.

2.1.3.3.3. Oligosaccharides

Lactulose, a disaccharide of fructose and galactose, is resistant to metabolism in the small intestine and is metabolized in the colon. A study with rats showed that lactulose and other sugars that are poorly absorbed in the small intestine such as xylitol, lactobionate, arabinose, raffinose, pyroglutamate, sorbitol, gluconate, and raftilose stimulate Ca absorption (Brommage et al, 1993). Thus, it was concluded from the above study that sugars resistant to metabolism and absorption in the small intestine and metabolized in the large intestine stimulate Ca absorption in the small intestine. This suggests that Ca does not bind with lactulose and with other resistant sugars in the small intestine. However, the Ca that escapes absorption in the small intestine reaches the colon where its absorption may be influenced by the products of fermentation of these sugars such as the SCFA.

Fructooligosaccharides are synthesized by many plants and are found in the human diet in onions, garlic, antichokes and chicory (Roberfroid et al, 1993). They are partly hydrolyzed by gastric acid (Nilsson et al, 1988) but in general are thought to escape digestion in the human upper intestine completely (Roberfroid et al, 1993; Roberfroid, 1993; Oku et al, 1984) and then fermented by the colonic microflora (Rumessen et al, 1990). It has also been shown that fructooligosaccharides are a good source of SCFA in rats and humans (Tokunaga et al, 1989). Feeding fructoligosaccharide to rats has been shown to increase Ca absorption (Ohta et al, 1995). About half of the increased Ca absorption took place in the colon and rectum. It was speculated that at least part of the stimulating effect of fructoligosaccharide on Ca absorption may be attributed to the SCFA

produced from its fermentation. Moreover, SCFA such as Ac and Bu have been shown to enhance Ca absorption in the distal colon of rats (Lutz and Scharrer, 1991).

2.1.3.3.4. Starches

Starches are the major storage polysaccharides of most higher plants. They are α-glucans and consist of two major types of molecules: amylopectin which is usually 70-80% of the total starch and amylose which usually comprises 20-30%. Amylopectin consists of large molecules made up of > 10,000 glucose residues held together by α-1,4 and α-1,6 linkages in a branched tree-like structure. Amylose is a smaller linear molecule with only α-1,4 linkages (Cummings and Englyst, 1995). They are normally digested and absorbed in the small intestine but some escapes digestion and reach the colon. The latter are refered to as resistant starch (RS).

The effect of different types of starch on Ca absorption was studied in rats (Schultz et al, 1993): cooked and cooled normal starch (low RS diet); uncooked high amylose starch, (high RS_2); cooked and cooled high amylose starch (high RS_3). The presence of RS_2 in the diet significantly increased apparent absorption of Ca while with the RS_3 diet, Ca absorption was similar to that in rats fed with low RS. Both RS_2 and RS_3 were poorly digested in the distal half of the small intestine as indicated by the high content of polymerized glucose. RS_3 has almost twice as much polymerized glucose as RS_2 found in the ileum. However, both have been shown to be fermentable by mammalian intestinal bacteria, in vitro (Englyst and MacFarlane, 1986; Wyatt and Horn, 1988) and in vivo (Andrieux and Sacquet, 1986; Demigne et al, 1989; Faulks et al, 1989; Gee et al, 1991;

Livesey et al, 1990; Mallett et al, 1988; Tomlin and Read, 1990). Fecal analysis for polymerized glucose was greater in rats fed the RS_3 diet in comparison to the RS_2 and RS diets suggesting that RS_2 is more fermentable than RS_3. According to the authors (Schulz et al, 1993), the stimulating effect of RS_2 on Ca absorption may be due to the lowered ileal pH which in turn raised the concentration of soluble Ca in the ileum and cecal contents. This study did not look at the effect of the products of fermentation of starch. A study done on the effect of amylomaize starch (70% amylose) on mineral absorption in adult rats showed that increased cecal volume, pH acidification and lower transit time may have contributed to the increased absorption of minerals. The increased absorption of minerals may also be partly attributed to SCFA in conventional rats (Andrieux and Sacquet, 1986).

It can be deduced from the above studies that different RS have different effects on Ca absorption. This depends on the extent of fermentation, bacterial products of fermentation and water holding capacity of fermentation residues (Wyatt and Horn, 1988). The nature and origin of starch should be taken into account in assessing possible physiological effects of resistant starch with behaviour similar to dietary fiber (Bjorck et al, 1986).

2.1.3.3.5. Dietary Fiber

Fibers include the principal components of the plant cell wall such as cellulose, hemicellulose, pectins, lignins and a number of chemically related substances including storage polysaccharides (inulin, guar), seed mucilages (ispaghula), and plant gums and

exudates (MacFarlane and Cummings, 1991). Fibers not digested in the small intestine reach the colon where they are metabolized. Dietary fibers have widely different physical and chemical characteristics (Anderson and Chen, 1979; Kelsay, 1978; Eastwood and Kay, 1979). They have been classified as water soluble (pectin, some hemicelluloses, mucilages, algal polysaccharides and storage polysaccharides) or water insoluble (cellulose, some hemicellulose and lignin). Most food fiber sources contain mixtures of fiber substances which make their charcteristics complex.

There are some disagreements, between and even within studies, on the degree and direction of a physiological response of humans to feeding specific test fiber sources. For example, wheat bran has been the fiber source most commonly used to study the effects of dietary fiber on Ca absorption but wheat bran contains not only fiber, but also phytate. Phytate impairs Ca absorption in a dose-related fashion (Heaney et al, 1992). Ca absorption from soybean of low phytate content was significantly better than absorption from high phytate containing soybean (41% and 31%, respectively; Heaney et al, 1991). Several studies have differentiated the effect of phytate and fiber in brans (McCance and Widdowson, 1942; Reinhold et al, 1973 a,b). Ca bioavailability improved with dephytinization of whole wheat in bread, however it remained inferior to that in white flour bread (McCance and Widdowson, 1942). Feeding purified phytate had little effect on Ca absorption while phytate-rich whole wheat bread had a pronounced negative effect (Reinhold, 1973a). This suggests that the fiber in whole wheat bread decreases Ca bioavailability. On the other hand, Anderson et al (1983) found no difference in Ca balance for six adults when they consumed diets containing 16, 24, or 32 grams of dietary

fiber/day from wheat bran for 24 days with phytic acid held constant at 2.0 grams/day.

Soluble and insoluble fiber may have different effects on Ca bioavailability. Cellulose, an insoluble fiber has been shown to decrease Ca bioavailability in humans (Slavin and Marlett, 1980; Ismail-Beigi et al, 1977) although other studies revealed no effect on Ca balance (Drews et al, 1979; Behallet al, 1987). Pure cellulose has no cation-binding capacity in vitro (James et al, 1978). Variability in results on the effect of cellulose on Ca absorption may be due to differences in subjects, diet and conditions used in the study.

Some soluble fibers contain uronic acid which has been shown to be a major factor in the binding of Ca to fiber (James et al, 1978). Ca-binding capacity was significantly correlated to the uronic acid content ($r=0.77$) with every mmol of uronic acid at pH 7.4 binding 0.32 mmol of Ca. However, over 80% dietary uronic acids are fermented in the human large intestine (James et al, 1978). Therefore, the Ca bound to uronic acid may eventually be released and become available for absorption in the colon. Pectin, a soluble fiber, contains a substantial amount of uronic acid; however, Cummings et al (1979) found no effect on Ca balance when 36 grams pectin/day were fed to young men for six weeks. This is probably because 80% of the uronic acids in pectin are methylated and would not bind Ca. In addition pectin is completely metabolized in the lower intestine so that Ca would be released for absorption (Cummings et al, 1979).

A series of human studies (285 human adults) were conducted for five to seven days on each experimental period (Kies, 1985). Each subject was given at least one fiber source to provide 20 grams fiber/day with ordinary food as the control diet. Results

showed that wheat bran, psyllium and cellulose decreased Ca balance in comparison to the low fiber control while pectin, red wheat bran and corn bran did not differ significantly from the control diet. Other studies have also shown that pectin and other fermentable fibers such as guar have no effect on Ca availability (Munoz and Harland, 1993).

All of the above studies did not relate the fermentabilities of dietary fiber sources to Ca absorption. Pectin and guar gum have been shown to be more fermentable than cellulose and psyllium (McBurney and Thompson, 1987; Demigne et al, 1980). The products of fermentation of these fibers such as the SCFA may have influenced the release of Ca in the colon for potential absorption.

2.1.3.4. Vitamins

Vitamin D is the most significant factor controlling Ca absorption. The inactive precursor, vitamin D_3, is modified by two sequential hydroxylation reactions, first in the liver and second in the kidney to produce the active form 1,25-dihydroxyvitamin D_3 (Wasserman and Fullmer, 1995). The rate of renal 1,25-dihydroxyvitamin D_3 synthesis is directly responsive to plasma parathyroid hormone (PTH) level and modulated by other feedback controlling factors (Holick, 1989; Reichel et al, 1989).

Vitamin D increases Ca absorption from the small intestine by 2 mechanisms: (1) an effect on gene expression and protein synthesis (genome response) which is exemplified by the synthesis of calbindins (Wasserman, 1992); and (2) a more rapid effect not dependent on gene activation (nongenomic). This process is dependent on the rapid

increases in intracellular Ca^{+2} concentration, cGMP levels and altered phospholipid synthesis (Baran and Sorensen, 1994; DeBoland and Nemere, 1992). The Vitamin D receptor associated with the genomic effect has been characterized (Minghetti and Norman, 1988; Pike, 1991) while the nature of the receptor associated with the rapid nongenomic effect is still under investigation (Dormanen et al, 1994; Nemere et al, 1994). The active form of vitamin D affects each step in the transcellular transport of Ca (saturable active process) which includes entry of luminal Ca into the enterocyte, the movement of Ca in the cell interior and the transport of Ca from the enterocyte into the underlying lamina propia (Bronner et al, 1986). The vitamin D-dependency of the paracellular transport of Ca (nonsaturable diffusion process) is the subject of some debate. Some investigators (Wasserman and Kallfelz, 1962; Dostal and Toverud, 1984) have shown that both saturable and nonsaturable processes are enhanced by vitamin D but other investigators disagree (Nellans, 1990; Pansu, et al, 1981).

The effect of vitamin C on Ca absorption is unclear. It was reported earlier (Leichsenring et al, 1957) that ascorbic acid slightly improved Ca absorption but also increased urinary Ca excretion when ascorbic acid or orange juice was added to a low-Ca diet. However, human studies have shown that long term large intake of ascorbic acid did not affect urinary Ca excretion (Fituri et al, 1983; Hanck, 1972 and 1974; Schmidt et al, 1981; Tsao et al, 1982). Similar results were obtained in another study in which 820±460 and 91±46 mg/day/person of Ca and ascorbic acid, respectively, were fed for 5 days on 22 human subjects (Tsao et al, 1986). On the other hand, when 805±492 and 64±37 mg/day/person of Ca and ascorbic acid, respectively, were fed, and urine collected

for 8 hours, the urinary Ca level of the initially low excretors was significantly elevated while that of the initially high excretors was not significantly affected. The effect of ascorbic acid on Ca absorption should be further investigated.

2.1.3.5. Other Minerals

Ca interacts with other minerals such as Fe, Zn and Mg. Cook et al (1991) have shown that Ca supplements such as Ca carbonate, Ca citrate and Ca phosphate given with ferrous sulfate on humans did not inhibit iron absorption alone, but did so when taken with a meal. Similar results were obtained by Hallberg et al (1991). Orange juice with Ca had minimal inhibition on iron absorption (Mehansho et al, 1989). Thus, citric acid and ascorbic acid in orange juice alleviate the inhibition of iron absorption caused by Ca.

The effect of Ca on zinc absorption was studied in relation to the phytate content of food. It was shown that [Ca][phytate]/[Zn] molar ratios greater than 0.5 mol/kg may reduce the bioavailability of Zn (Davies and Warrington, 1986). In another study (Hunt et al, 1995), 0.8 g protein/kg body weight that meets protein requirement of older women increased zinc retention without compromising Ca status.

Several investigators (Clarkson et al, 1967; Norman et al, 1981) have reported that increasing Ca in the diet of humans significantly reduced magnesium (Mg) absorption while increasing Mg in the diets has been reported to significantly decrease fecal Ca in humans (Clark, 1969; Hardwick et al, 1991; Heaton and Parson, 1961; Leichsenring et al, 1951). Mg concentration from 1.25 to 10 mmol/L had no effect on Ca absorption in the colon of rats (Karbach and Ewe, 1987). It was also observed in humans using a perfusion

method, that increasing Mg concentration in the lumen decreased Ca absorption in the jejunum (Brannan et al, 1976). However, direct intestinal perfusion with various concentrations of soluble Mg or Ca in an isolated segment may not reflect what is actually occuring in vivo. Differences in methodology between studies make it difficult to compare many of the human studies and to conclude whether there is any interaction between Ca and Mg in the intestine.

The mechanism by which Ca and Mg interact has not been well defined but several possible mechanisms has been proposed as follows: (1) competition for a common carrier system (Alcock and MacIntrye (1962); (2) a Ca-induced change in membrane permeability to Mg (Leichsenring et al, 1951); and (3) modulation of a specific Mg carrier by Ca (Walser, 1967). Hendrix et al (1963), found that Ca and Mg are taken up preferentially in different portions of the intestine. Specifically, Ca inhibited Mg transport in the ileum but not in the duodenum, whereas Mg inhibited Ca transport primarily in the duodenum. In short term uptake studies in rat duodenal mucosa, Mg significantly inhibited time-dependent uptake of Ca, but Ca did not significantly reduce Mg uptake (O'Donnel and Smith, 1973).

2.1.3.6. Antinutrients

Phytic acid, the storage form of phosphorus in seeds, has been implicated in the reduction of mineral absorption from cereals and legumes because of its ability to form a phytate-mineral-complex (Champagne, 1988; Heaney et al, 1992; Levenson and Brockman, 1994). The effect of phytate on Ca absorption depends on how phytate is

present in or added to a meal. When sodium phytate is added to a meal, Ca balance was reduced (Reinhold et al, 1973 a,b). Similarly foods rich in phytates added to a diet decreased the overall Ca balance (Reinhold et al, 1973 a,b; McCance and Widdowson, 1942) suggesting an effect of phytate on Ca absorption. However, when high and low phytate soybean intrinsically labelled with ^{45}Ca were fed to human subjects, no significant differences in Ca absorption were found between the high and low phytate and the control food (milk) in the same subjects (Heaney et al, 1991). Other sources of phytate such as wheat bran taken with Ca carbonate reduced the absorption of Ca (Weaver et al, 1992). In addition, in a crossover design, intrinsically labeled beans have substantially lower Ca absorption than milk presumably because of their high phytate content (Heaney and Weaver, 1992).

Foods containing oxalic acid reduced Ca absorption due to the formation of Ca oxalate. Ca in spinach is poorly absorbed presumably due to its oxalate content (Heaney et al, 1988; Weaver et al, 1987; Wien and Schwartz, 1983; Poneros-Schneir and Erdman, 1989) while low-oxalate vegetable such as kale exhibit excellent Ca absorption (Heaney and Weaver, 1990).

2.2. The Human Colon

The human colon consists of three parts: the ascending, transverse and descending colon (Figure 2.2; Christensen, 1991). The ascending colon is the part of the colon between the ileocecal junction and the hepatic flexure which is the bend of the colon at the caudal surface of the liver. Ventrally, this part of the colon contacts loops of the

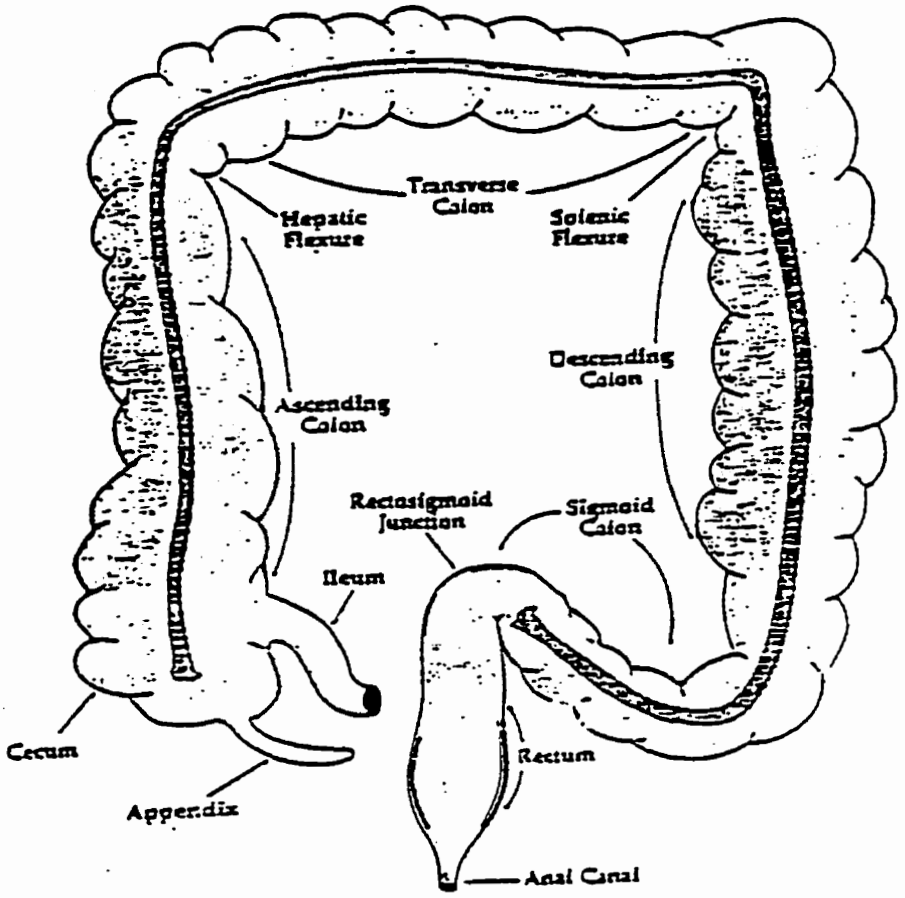


Figure 2.2. The Human Colon. The figure was taken from Christensen, 1991.

ileum and the ventral abdominal wall. The transverse colon is the part of the colon between the liver and the spleen. At the spleen, the colon usually forms an acute flexure, the splenic flexure where the colon is in contact with the caudal tip of the spleen and the tail of the pancreas. The descending colon is the part of the colon that descends from the splenic flexure toward the pelvis. This part of the colon lacks a mesentry, the investing peritoneum lying against the ventrolateral surface of the left kidney and abdominal muscles. At the upper aperture of the pelvis, the colon forms a loop, the sigmoid colon that normally lies within the pelvis. It is separated from the bladder by loops of the ileum. It joins the rectum at the rectosigmoid junction. The rectum is the part of the large intestine between the sacral vetebra and the anal canal. This segment is about 12 cm long.

The human colon has been reported to be 110 cm long (Blakebhorn et al, 1955) with the distal segment comprising approximately 1/3 of the total length of the colon (Schiller et al, 1988). The diameter of the rectum and distal colon is about 4 cm (Williams and Warwick, 1985). Therefore the total volume of the rectum and the distal colon are approximately 150 and 460 mL, respectively.

The human colon usually contains about 220 g of wet contents (range 58-904), 35 g of which is the dry matter (Cummings et al, 1990). Estimates of the microbial component of colonic contents vary depending on the analytical technique used. Values between 30 to 40% are observed with a direct microscopic counting method (Van Houte amd Gibbons, 1966; Moore and Holdeman, 1974). Gravimetric procedures demonstrated that bacteria comprised approximately 55% of the solids in feces of people on Western diets

(Stephen and Cummings, 1980) and if applied to total colonic contents this would give about 18 g bacterial dry matter equivalent to a total bacterial mass in the colon of 90 g (MacFarlane and Cummings, 1991).

2.2.1. Fermentation

Bacterial fermentation of carbohydrates, protein and other substances in the large intestine is a series of energy-yielding catabolic reactions that does not involve respiratory chains using molecular oxygen or nitrate as terminal electron acceptors. Fermentations are usually classified on the basis of their major end products, for example, lactate, alcohol, Pr, Ac and Bu (MacFarlane and Cummings, 1991). Other more specialized fermentations also occur in the large bowel such as sulfide and methane fermentations carried out by sulfate-reducing and methanogenic bacteria.

The majority of carbohydrate fermentations in the colon produce the SCFA such as Ac, Pr, Bu and the gases carbon dioxide and hydrogen as principal end products. The bacteria obtain energy from this process for growth and maintenance of cellular function. Lactate and succinate are formed as intermediates during catabolism of carbohydrate and further fermented to SCFA, i.e. succinate is converted to Pr by some species of the bacteroides fraglitis group (Macy and Probst, 1979).

Lactate is a major fermentation product of bifidobacteria, streptococci and lactobacili (Holdeman et al, 1977). It is also a common product in many other bacterial fermentations and is probably an important intermediate in the large intestinal ecosystem and particularly in the right (ascending) colon where carbohydrate availability

is greatest and fermentation rates are high.

In vitro experiments have shown that the type of SCFA formed by gut bacteria depends on the chemical composition of the polysaccharide substrate (Gottschalk, 1986). For example, starch fermentation is characterized by high levels of Bu production whereas with a more oxidized substrate such as pectin, more Ac is produced. This is partly explained by the fact that different bacteria are involved in fermenting different substrates.

The above information shows that products of fermentation are determined by the amount and type of substrate, the rate and extent to which it is broken down, the type of flora involved and host factors such as transit time (MacFarlane and Cummings, 1991).

2.2.2. The Colonic Flora

Studies on the composition of the colonic microflora come from fecal data. However, the fecal material may not reflect the type of bacteria present in the colon. Moore et al (1978) suggested that fecal bacteria are representative of those occuring at the different regions of the large bowel with respect to generic distribution of species and their relative proportions.

It has been shown that diet, age and the country in which a person lives are insignificant determinants of the composition of the colonic microflora (Gorbach et al, 1967; Finegold et al, 1974; Tannock, 1983) and that an individual's fecal flora appears to be comparatively stable over long periods (Holdeman et al, 1976; Simon and Gorbach, 1984). However, another study which followed anaerobic bacterial populations in 10 healthy volunteers over a period of 1 year found intraindividual recoveries of some

species which varied up to as much as 1,000 fold (MacFarlane and Cummings, 1991).

Diet has been shown to influence gut bacterial population in mice (Dubois and Schaedler, 1962), rats (Chung et al, 1977), and ruminants (Latham et al, 1972) but studies in humans are less convincing (Drasar and Jenkins, 1976; Drasar et al, 1976; Cummings et al, 1978; Moore and Holdeman, 1975; Hentges, 1978, Cummings, 1983).

The major polysaccharide-degrading bacteria in the colon and their fermentation products are as follows: bacteroides - Ac, Pr, succinate; ruminococcus - Ac, lactate, succinate; eubacterium - Ac, Bu, lactate; lactobacillus - lactate, Ac; bifidobacterium - Ac, lactate; and clostridium - Ac, Pr, Bu, lactate (MacFarlane and Cummings, 1991). Pectin is degraded by bacteroides, eubacteria and bifidobacteria and cellulose by bacteroides; starch in cereals and some vegetables is degraded by many bacterial species (MacFarlane and Cummings, 1991).

2.3. Sources and Production of Short Chain Fatty Acid

2.3.1. Starch

It is evident from breath hydrogen studies that starch is incompletely digested in the small intestine (Anderson et al, 1981; Calloway, 1966; Flourie et al, 1988; Levitt et al, 1987), direct intubation of the ileum (Flourie et al, 1988; Stephen et al, 1983) and study of ileostomates (Chapman et al, 1985; Englyst and Cummings, 1985, 1986, 1987; Sandberg et al, 1981, 1983; Wolever et al, 1986)

Appearance of hydrogen in breath has been used as an index of starch breakdown. The amount of hydrogen in breath is compared to that released during fermentation of a

known amount of carbohydrate such as lactulose and from this ratio, quantitative data for starch fermentation in the colon are derived. In a series of studies using breath hydrogen as a marker of carbohydrate malabsorption, it was found that 10 to 20% of the 100 g of carbohydrate in a meal of white bread, 8% in oat bread , 6% in corn bread, 13% in potato and 18% in navy beans was not absorbed (Levine and Levitt, 1981). A comparison of starch malabsorption estimated by breath hydrogen or ileostomy study showed similar results as follows: breath hydrogen - white bread, 11%, wholemeal bread, 8%, lentils, 18%, while from the ileostomy study - white bread, 10%, wholemeal bread, 8% and red lentils, 22% (Wolever et al, 1986). However, other studies found that the breath hydrogen technique gave higher values for starch malabsorption in comparison to the data from ileostomy studies and direct intubation (MacFarlane and Cummings, 1991). Despite the problems in interpretation, it is clear that significant quantities of starch arrive in the colon. Also, starch produces high amounts of Bu (MacFarlane and Cummings, 1991).

2.3.2. Dietary Fiber (Non-starch Polysaccharides)

Purified fiber supplements such as pectin, tragacanth gum, psyllium gum, guar gum, soy fiber and cellulose were fermented in vitro using human fecal microbiota (McBurney and Thompson, 1989). Pectin produced relatively more Ac and cellulose the least. Tragacanth and guar gums produced more Pr while guar gum tended to produce more Bu. Pectin was the most rapidly fermented followed by psyllium gum, tragacanth gum, guar gum, soy fiber and finally cellulose.

2.3.3. Sugars and Oligosaccharides

Lactose, lactulose and the monosaccharides which make up the dietary fibers can influence SCFA production in the colon (Mortensen et al, 1988). In an in vitro fecal intubation system, SCFA are formed from all mono- and disaccharides tested such as D-glucose, D-galactose, D-fructose, D-mannose, L-rhamnose, D-sorbitol, D-arabinose, D-xylose, D-ribose, D-galacturonate, D-glucuronate, lactose and lactulose except L-glucose. All of the above saccharides increased Ac formation. Pr was increased from rhamnose, arabinose, xylose, ribose, galacturonic and glucoronic acid while Bu was increased from assays with sorbitol, galaturonic and glucuronic acid. Other oligosaccharides probably escape digestion in humans but only few studies have been done to test this. The most common substrates tested are the fructo-oligosaccharides found in vegetables such as artichokes, onions and some root crops. They are rapidly fermented by the flora (Hidaka et al, 1986; Hosoya et al, 1988).

2.4. Absorption of Short Chain Fatty Acids in the Colon

About 95% of SCFA produced from microbial fermentation are absorbed in the large intestine and colon of mammals (Cummings, 1981; Engelhardt and Rechkemmer, 1983). In vivo and in vitro perfusion techniques showed that SCFA transport into the cell is a linear, concentration-dependent process whereby the flux rate is in direct proportion to the SCFA concentration in the perfusate (Bugaut, 1987; Rimmer et al, 1987). It has been suggested that SCFA absorption occurs by nonionic diffusion; SCFA are protonated from either the luminal hydration of carbon dioxide into bicarbonate and hydrogen ions or from

the intracellular secretion of hydrogen as a result of a sodium/hydrogen exchange in the proximal colon and by a potassium/hydrogen exchange in the distal colon (Figure 2.3; Engelhardt, 1995; Engelhardt and Rechkemmer, 1992). SCFA are weak acids with pKa values ranging from 4.75 to 4.81. As calculated from the Henderson-Hasselbach relationship, 95% or more of SCFA in the colonic lumen are present in the dissociated form at the physiological pH (pH 6-7) of the intestine (Titus and Ahearn, 1992). Because of the dependence of SCFA uptake on the availability of protons, a proportional increase in SCFA transport would be expected with a corresponding decrease in pH. However, absorption of SCFA has been found to be independent of luminal bulk pH in guinea pig (pH range 5.0-9.0), goat, rabbit and human (Argenzio et al,1975; Gasaway, 1976; Murer et al, 1984; Miller et al, 1979; Rechkemmer, 1979). To account for this, it was proposed that there exists an acidic microclimate at the epithelial surface whereby the ionized form of SCFA becomes protonated and therefore more soluble in the membrane. In such a constant pH microenvironment there would be a steady concentration of hydrogen available for association with the luminal SCFA anions. It was proposed that anionic diffusion may contribute to the overal SCFA permeability (Rechkemmer et al, 1988). Once the SCFA enters the cell, it is transported across the basolateral membrane to the blood by diffusion in the ionic or nonionic form. Alternatively it can be metabolized intracellularly, resulting in the production of carbon dioxide which diffuses back into the lumen, thus perpetuating the process that results in the diffuson of SCFA across the luminal membrane into the cell (Titus and Ahearn, 1992). The diffusive uptake of protonated SCFA is proportional to the increase in chain length of the acid, Ac < Pr

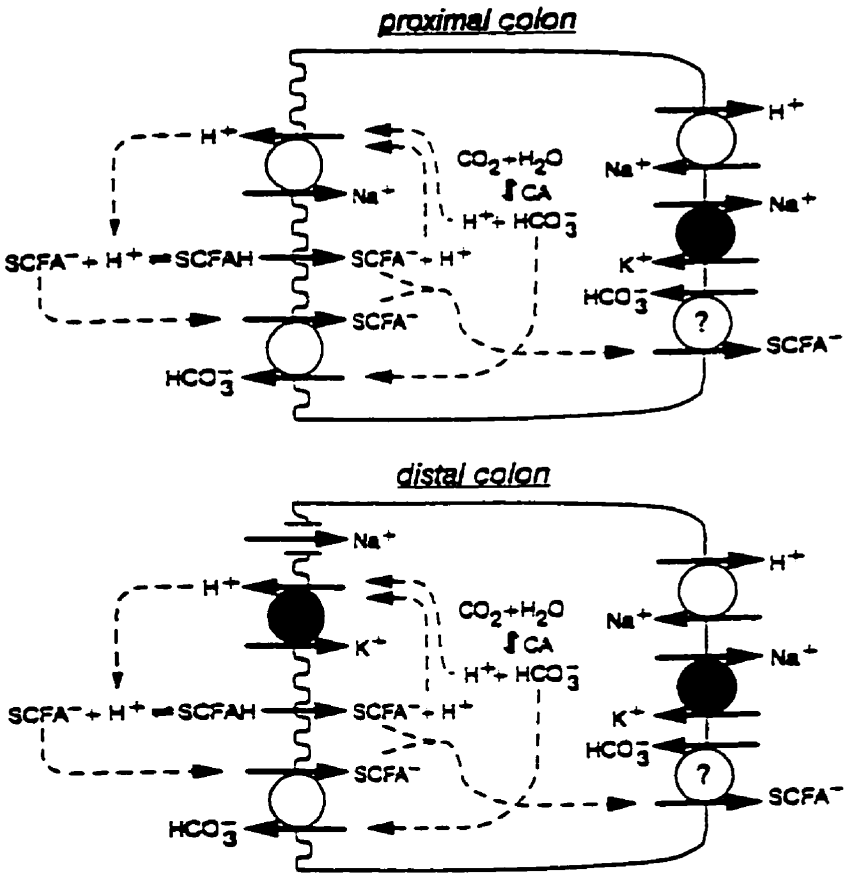


Figure 2.3. Model for cellular mechanism involved in absorption of SCFA in the proximal and distal colon of guinea pig. SCFAH, the non-ionic form; $SCFA^-$, the ionic form. This figure is taken from Engelhardt, 1995.

< Bu (Watford et al, 1979).

SCFA transport by a carrier mediated process was studied by Ruppin et al (1980) using in vivo perfusions of the human colon. Ionized SCFA enter the enterocyte by anion exchange with intracellular bicarbonate. This model was based on indirect evidence involving bicarbonate appearance in the colonic lumen (near-neutral pH) in proportion to SCFA disappearance from the luminal bulk. This result was similar to the results obtained from in vivo intestinal perfusion of rats (Umesaki et al,1979; Watson et al, 1990), pig (Argenzio et al, 1974) and goat (Argenzio et al, 1975). It was noted that the carrier-mediated characteristics indicate the presence of a saturable step in lumen-to-blood substrate transport. The rate-limiting step that results in saturation was cited as the finite supply of luminal hydrogen ion available for protonation; hence it follows that competitive inhibition results from two or more anionic species of SCFA competing for the same source of protons (Titus and Ahearn, 1992). However, the above studies were not able to state conclusively that carrier-mediated transport does occur. The techniques used cannot eliminate physiological interferences such as unstable pH, acid microclimate, unstirred water layers and cellular metabolism.

2.5. Effect of Short Chain Fatty Acid on Calcium Absorption

Fermentation of carbohydrates such as bran, pectin and guar gum in the colon of rats have been suggested to shift the absorption of minerals such as Ca and Mg from the small intestine into the large intestine (Demigne et al, 1989). However, this study did not determine for the proportion of Ca and Mg absorbed in the small intestine and the amount

of Ca and Mg released after bacterial fermentation. In vitro fermentation (McBurney and Thompson, 1987) and dialysis of the residue from the in vitro enzymatic digestion and dialysis showed that the insoluble mineral-fiber complex formed can release the mineral and produce SCFA (Trinidad, 1990; Thompson et al, 1991). However this study did not investigate whether Ca is absorbed in the colon and whether the presence of SCFA influence Ca absorption.

Considering that fructooligosaccharides are indigestible, readily fermentable in the colon and a good source of SCFA in rats (Tokunaga et al, 1989), at least a part of the stimulative effect of fructooligosaccharides on Ca and Mg absorption might be attributed to SCFA produced from fermentation of fructooligosaccharides. About half of the increased Ca and Mg absorption in the presence of fructooligosaccharides has been shown to take place in the colon and rectum of rats (Ohta et al, 1995).

The effect of SCFA such as Ac and Bu on Ca absorption was studied in rats (Lutz and Scharrer, 1991). Ac and Bu stimulate Ca absorption in the distal colon without influencing sodium and water absorption. In the proximal colon, sodium and water absorption but not Ca absorption were enhanced by Bu (Lutz and Scharrer, 1991; Lutz et al, 1991) through a Na-H exchanger located in the apical membrane of the colon (Argenzio et al, 1975; Lutz et al, 1991). Sodium absorption was not stimulated by SCFA in the distal colon suggesting that a Na-H exchange was not a main mechanism operating under the above experimental conditions. Since Ca absorption was found to be absorbed and stimulated by the presence of Ac and Bu in the distal colon, by analogy with the Na-H exchanger that exists in the proximal colon, a Ca-H exchanger located in the apical

membrane of the epithelium might be involved (Lutz and Scharrer, 1991). Ac was as effective as Bu at higher concentration (60 mmol/L) in enhancing Ca absorption while at lower concentration (30 mmol/L) only Bu stimulated Ca absorption significantly.

2.6. Summary and Experimental Goals

Ca absorption has been studied in animals and humans using radioisotopes and stable isotopes of Ca . Most of these studies did not account for a colonic component of Ca absorption. The presence of fermentable fiber such as wheat bran, pectin and guar gum has been shown to shift the absorption of Ca from the rat small intestine to the large intestine, but the amount of Ca absorbed in the colon was not determined (Demigne et al, 1989). Pectin has been found to have no effect on Ca balance when eaten by humans, but the investigators did not relate its fermentability to Ca absorption (Cummings et al, 1979; Kies, 1985; Munoz and Harland, 1993). SCFA, the products of fermentation of pectin, may have influenced the release of Ca in the colon for potential absorption. Fructooligosaccharides have been shown to stimulate Ca absorption in the rat colon suggesting that the absorption of Ca may be attributed to the SCFA produced from the fermentation (Tokunaga et al, 1989; Ohta et al, 1995). In peri-menopausal women Ca absorption appears to take place after initial entry into the colon based on a late rise in serum or urinary Ca (Barger-Lux et al, 1989). Moreover, a study in humans during colonoscopy demonstrated that Ca can be absorbed in the human colon (Sandstrom al, 1986) although in this case the conditions are not entirely physiological because of the absence of fecal bacteria and/or components which may have a significant role on Ca

absorption. Studies in rats on the the effect of SCFA such as Ac and Bu on Ca absorption suggest a Ca/H exchanger mechanism located in the apical membrane of the epithelium (Lutz and Scharrer, 1991).

Much information can be gathered from the above studies. However, most of the human studies did not account for the effect of SCFA from fiber fermentation. Extrapolation of the results of animal studies to humans is difficult and must be done with caution. Also, there is evidence that SCFA absorption differs in different species indicating that the effect of SCFA on Ca absorption may be different in humans. Thus, the overall hypothesis and objective and specific objectives of this study are given below.

2.6.1. OVERALL HYPOTHESIS

Fermentable fibers do not reduce the total absorption of Ca. The Ca that is bound by fermentable fibers is released in the colon upon fermentation. The released Ca is absorbed in the distal colon of humans and this process is enhanced by the presence of the SCFA from fiber fermentation.

2.6.2. OVERALL OBJECTIVE

To determine the amount of Ca that reaches the colon and whether this Ca is absorbed particularly in the presence of SCFA in the distal colon of humans.

2.6.3. SPECIFIC OBJECTIVES

a. Using an in vitro method, to determine the effect of various available and

unavailable carbohydrates on the release of Ca potentially absorbable in the small intestine and colon.

b. Using rectal infusion:

i. to determine the influence of a mixture of Ac and Pr on serum Ca response, as an index of Ca absorption.

ii. to determine the differential effect of Ac and/or Pr on Ca disappearance in the rectum and distal colon of humans.

iii. to determine the effect of Ca on Ac and Pr absorption in the rectum and distal colon of humans.

iv. to determine the kinetics of Ca absorption by examining the relationship between Ca concentration and the rate of Ca absorption from the human rectum and distal colon as affected by Ac and Pr.

c. Using a feeding study with stable isotopes of Ca, to determine the effect of pectin on Ca absorption from dairy products in the small intestine and colon of humans.

CHAPTER 3

STUDY DESIGN AND GENERAL METHODS

3. STUDY DESIGN AND GENERAL METHODS

3.1 Study Design

Ca absorption with and without SCFA was studied using in vitro and in vivo methods.

To determine the degree of Ca release from the diet or single dairy food with and without the presence of various available and unavailable carbohydrates, an in vitro method simulating conditions in the small intestine and colon was developed. The method involved enzymatic digestion of the test foods, followed by fermentation using human fecal microbiota and dialysis of the digesta.

The in vivo methods used in the study were the rectal infusion technique and an oral feeding study using two stable isotopes of Ca.

To investigate whether Ca released in the colon can be absorbed in the distal colon of human subjects, a study was conducted whereby healthy male subjects were given rectal infusions of isotonic solution of saline with and without addition of Ca and a mixture of Ac and Pr in 3:1 molar ratio. Serum Ca and Ac were then measured over time as an indication of absorption. Since this study provides only an indirect measure of Ca absorption and since the SCFA used was a mixture of Ac and Pr, the actual absorption of Ca and the differential effects of Ac and Pr on Ca absorption in the distal colon of man were investigated in the next study. Human subjects were given rectal infusions containing Ca alone (50 mM), Ca plus Ac (18.7 mM or 56.3 mM), Ca plus Pr (18.7 mM or 56.3 mM) or Ca plus Ac and Pr together (1Ac:3Pr or 3Ac:1Pr ratios) using polyethylene glycol (PEG) as an unabsorbable marker. Samples of rectal fluid were

collected at various times after which colonic contents were collected and analyzed for Ca, Ac, Pr and PEG disappearance as an indicator of their absorption.

To study the kinetics involved in Ca absorption alone, as well as in the presence of Ac or Pr, the relationship between Ca concentration and rate of absorption from the human colon as affected by Ac or Pr was then determined. Healthy male subjects were given rectal infusions containing various concentrations of Ca with either 56.3 mM Ac, 56.3 mM Pr or sodium chloride (NaCl) using PEG as an unabsorbable marker followed by measurement of Ca, Ac, Pr and PEG.

To determine Ca absorption in the small intestine and colon under physiologic conditions, an oral feeding study was conducted in human subjects. They were given an intravenous injection of a solution of ^{42}Ca, and immediately after, fed a test meal containing instant skimmed milk labelled with ^{44}Ca with and without pectin. Fractional Ca absorption was calculated over time.

3.2. General Methods

3.2.1 Rectal Infusion Technique for Measuring Calcium Absorption

All rectal infusions were done using a 1 m length of plastic tubing (inside:outside diameter, 2.4:4.0 mm; Tygon Flexible Plastic Tubing, Norton Tubing and Molded Products, Akron, OG) with one end of the tubing (5 cm) inserted into the rectum and the other end connected via a 3-way tap to a 60-mL dispensing syringe. Prior to the infusion of the test solution, the colon was cleared by infusing 500 mL of double distilled water followed by emptying.

The volume of the test solutions infused was either 600 mL or 300 mL. In the first study, 600 mL test solution was infused at a rate of 30 mL/min. The process was done by the subjects under close supervision by the investigators. Blood samples were taken fasting and at 30, 60, 90, and 120 min after the start of the infusion. Subjects emptied their bladders into plastic bottles before the start of the rectal infusion and immediately after the last blood sample to determine if there were differences between urinary Ca from baseline and after infusion of the treatment solutions.

In the studies where Ca disappearance from the distal colon was determined, 300 mL test solutions were infused at a rate of 60 mL/min. In some studies, the subjects left the tubing in the rectum after the infusion and, after mixing (withdrawing and reinfusing 10 mL fluid 3 times in 2 min), samples of fluid (5 mL) were collected at 0 (immediately after the infusion was complete), 10, 20 and 30 min. After the 30 min sample was collected, the subjects withdrew the tubing and emptied their colon into a plastic bag suspended under the toilet in a plastic frame. In the first few subjects, it was difficult to obtain rectal fluid samples at 20 and 30 min despite repositioning the tube, because the rectal mucosa blocked the opening of the tube. When this occurred, 10 mL of double distilled water was infused into the rectum, mixed and withdrawn. In later subjects, we found that cutting 2 or 3 'v' shaped holes in the tube at 5 cm from the end facilitated sample recovery. In some studies, the subjects collected samples at 0 time and after 30 minutes emptied their colon. Samples were analyzed for Ca, Ac, Pr and PEG.

The Ca absorption described in the above studies represents that in the rectum, sigmoid colon and descending colon. The average total length of the rectum is 12 cm

while the colon has been reported to be 110 cm (Blackebhorn et al, 1955) with the distal segment comprising approximately 1/3 of the total length (Schiller et al, 1988). The diameter of the rectum and distal colon is about 4 cm (Williams and Warwick, 1985). Therefore, the total volume of the rectum and distal colon are approximately 150 and 460 mL, respectively. Since the total volume of solution infused for each subject was either 300 or 600 mL, a significant amount of the above solutions should have reached the distal colon.

3.2.2 Calcium Analysis

For analysis of food samples and digesta, duplicate 0.1 g freeze-dried samples were digested with 1 mL concentrated sulfuric acid (AR, Sigma Chemicals Co., St Louis, Missouri, USA) and 3 mL of 30% hydrogen peroxide (AR, Sigma Chemicals Co.) in a flask for 15 min in a microKjeldahl digester (Kivisto et al, 1986). If the digest was not clear, another 3 mL of hydrogen peroxide was added to the flask and digested for another 10 min. The resulting digest was diluted with 50 mL of 10 mM of lanthanum chloride (AR, Sigma Chemicals Co.).

For serum and urine, a 0.25 mL sample was diluted to 10 (serum) or 25 (urine) mL with a solution of 10 mM of lanthanum chloride. Colonic samples were centrifuged at 450 x g for 10 min at 25°C and the supernatant was diluted with 25 mL of lanthanum chloride. Lanthanum chloride is a releasing agent that eliminates the chemical interferences in the air-acetylene flame that may depress Ca absorbance.

All digested samples were read in the atomic absorption spectrophotometer (AAS; Varian Model 1275, Varian Canada Inc, Ontario, Canada). Prior to reading the samples,

the AAS was calibrated with different concentrations of Ca (1.0, 2.5, 4.0 ug/mL Ca, Certified Ca Reference for AAS, Fisher Scientific, New Jersey, USA). The coefficient of variation (day to day variation) of standards and samples for Ca analysis was 5.0% between 1- 4 ug/mL Ca.

3.2.3 Acetate and Propionate Analysis

3.2.3.1. Colonic Samples

Ac and Pr were measured using high performance liquid chromatography (HPLC: Shimadzu SPD M6A, Tokyo, Japan). One-mL aliquots of colonic fluid were filtered through a 0.22 Millipore filter to remove any bacterial and fibrous matter. Thirty uL of filtrate were injected directly into a BioRad organic acid column HPX-87H kept at 37°C and absorbance measured with diode array detector at 214 nm. The eluent was 0.005N sulfuric acid at a flow rate of 0.6 mL/min. Samples were read against a mixture of SCFA standard solutions (Matreya Inc Lipids and Biochemicals, Pa, USA) .

3.2.3.2. Serum Samples

Serum Ac was analyzed using HPLC (Walter Division of Millipore, Milford, MA, USA) with Bu as an internal standard after sample preparation by vacuum distillation (Guerrant et al, 1982; Wolever et al, 1989).

One mL serum was filtered through an anisotropic hydrophilic YMT ultrafiltration membrane using a micropartition system with a 30,000 Dalton molecular weight cut off (MPS-1, Amicon Canada Ltd., Oakville, Ontario, Canada) to separate the protein-bound

microsolute. The unit containing the serum was placed in an ultrafiltration superspeed centrifuge (Sorvall RC-5B, refrigerated, DuPont Instruments, Delaware, USA) at 450 x g for 70 min at 4°C.

The vacuum distillation apparatus consisted of a Duo-seal vacuum pump (Welch Manufacturing Co., Chicago, Illinois, USA) with a Labconco vacuum gauge (Labconco Corporation, Missouri, USA). The vacuum pump was attached to a Y-shaped glass piece with a vacuum tubing. To each arm of the Y-piece, a pyrex culture tube was attached via neopyrene tubing. One tube served as the source and the other as the collecting tube for SCFAs. A high vacuum stopcock was attached to the top of the Y-piece.

A 200 uL aliquot of protein-free serum was placed in a pyrex culture tube and 50 uL of standard solution containing 2 mM Bu (Matreya Inc Lipids and Biochemicals, Pa., USA) was added. The sample was mixed and was frozen along its length using liquid nitrogen. The source tube was submerged into the container of liquid nitrogen with the stopcock open until the vacuum pressure reached 5 microns. The stopcock was then closed and the liquid nitrogen bath was removed from the source tube and transferred to the collecting tube. The source tube was then immersed in a water bath (22°C) to aid in the distillation process. The water bath facilitated the sublimation and evacuation of the sample to the collecting tube. The distillation process took approximately 5-7 min represented by the disappearance of the sample from the source tube. Once the distillation was complete, the liquid nitrogen bath was removed and the sample in the collecting tube was thawed. The thawed sample was transferred to a 250 uL glass vial for HPLC injection into the column.

The coefficient of variation (replicates for each subject) for Ac and Pr measurements was 8.0%.

3.2.4. Polyethylene Glycol Analysis

PEG analysis of colonic samples was based on the method used by Malawer et al (1978). Ten mL double distilled water, 1 mL of 10% barium chloride (anhydrous, Fisher Scientific, Nepean, Ontario, Canada) and 2 mL 0.3N barium hydroxide (anhydrous, Fisher Scientific) were added to 1 mL of the colonic sample (supernatant after centrifugation) and mixed. Two mL 5% zinc sulfate ($ZnSO_4.7H_20$, AR, Sigma Chemical Co., St. Louis, Missouri, USA) was added to the resulting solution and shaken vigorously. The solution was filtered through a Whatman Filter Paper #42. Three mL of 1% Acacia (Gum Arabic, Fisher Scientific, Ontario, Canada) was added to 1 mL aliquot of the filtrate and gently mixed. Four mL trichloroacetic acid (Fisher Scientific) was added and the solution was left to stand for 90 min. The sample was then read against a standard solution of PEG 4000 (0.1, 0.3 and 0.5 mg/mL; Mallinckrodt Canada Inc., Pointe-Claire, Quebec, Canada) in a spectrophotometer (LKB Ultrospec II, LKB Biochrom LTD, Cambridge, England) at a wavelength of 650 nm and a slit width of 0.4 mm.

3.2.5. Statistical Analysis

The area under the curve calculations were conducted by the trapezoidal rule. Results were expressed as means±SEM. Differences between treatments and time were determined by one-way or two-way repeated measures analysis of variance and Duncan's

or Tukey's studentized range test using the Statistical Analysis System Program (SAS Institute Inc., Gary, NC, USA). Analysis of variance using Neuman-Kuels procedure to adjust for multiple comparisons was also used (Snedecor and Cochran, 1980). Linear regression analysis was used to determine the relationships between Ca absorption and concentration using SAS program.

All human studies were approved by the Human Subjects Review Committee at the University of Toronto.

CHAPTER 4

AVAILABILITY OF CALCIUM FOR ABSORPTION IN THE SMALL INTESTINE AND COLON FROM DIETS OR DAIRY FOODS CONTAINING AVAILABLE AND UNAVAILABLE CARBOHYDRATES: AN IN VITRO ASSESSMENT

4. AVAILABILITY OF CALCIUM FOR ABSORPTION IN THE SMALL INTESTINE AND COLON FROM DIETS OR DAIRY FOODS CONTAINING AVAILABLE AND UNAVAILABLE CARBOHYDRATES: AN IN VITRO ASSESSMENT

4.1 Introduction

Unabsorbable carbohydrates may differ in the degree in which they bind Ca in the small intestine and the degree in which they are fermented in the colon and release the bound Ca. However, the amount of the released Ca absorbed in the small intestine and colon cannot easily be determined in vivo. Therefore an in vitro model was used to estimate the effects of various carbohydrates on the release of Ca for potential absorption in the small intestine and in the colon. This study tested the effect of an absorbable starch; a fiber (pectin) which binds mineral in the small intestine and is fermented in the colon; an unabsorbable sugar (lactulose) which does not bind with minerals in the small intestine and is fermented in the colon; and a fiber (psyllium and/or cellulose) which binds mineral in the small intestine but is not easily fermented in the colon from (1) test meals containing dairy products; and (2) from single dairy foods.

4.2 Materials and Methods

4.2.1. Study 1. Test meals containing dairy products.

Eight different meals were studied. The meals consisted of 375 g skimmed milk, 75 g cheddar cheese and 125 g apple juice (basal diet, BD) without and with 100 g white

bread (BD+WB). The foods were mixed, homogenized and freeze-dried. When there was no addition, 20 g BD or BD plus white bread were tested as the controls. In the test situations, 16 g BD or BD plus white bread was mixed with 4 g of pectin (POMOSIN, Pectin Type LM12-3CG, Foodpro National Inc., La Chine, Quebec, Canada) or 4 g lactulose (pure lactulose crystals, Inalco, Milano, Italy) or 4 g psyllium (85%, #9507, Kelloggs, Michigan, USA) were the test meals. The available carbohydrate, dietary fiber and Ca content of BD were 35.0 g/100g, 1.1 g/100g and 170 mg/100g, respectively, while those of BD plus white bread were 53.5 g/100g, 5.6 g/100 g and 160 mg/100g, respectively. The total Ca content of pectin, lactulose and psyllium were 311, 330 and 338 ug/g, respectively.

4.2.2. Study 2. Single dairy foods.

Three dairy foods; skimmed milk, skimmed milk cheese and yogurt with and without lactulose, pectin, psyllium and cellulose (alpha-cellulose, non-nutritive fiber, ICN Biomedicals, Canada, Mississauga, Ontario, Canada) were similarly tested for Ca availability. Food samples were freeze-dried and analyzed in duplicate using 20 g with no addition, or 16 g of food samples plus 4 g of the test carbohydrates. The Ca content of skimmed milk, skimmed milk cheese and yogurt were 12.1, 12.5 and 10.8 mg/g, respectively and cellulose, 96.6 ug/g.

4.2.3. In vitro method (stage 1).

Duplicate 20 g freeze-dried samples were homogenized with 80 g double distilled water. The pH of the homogenized solution was adjusted to 2.0 using 6N HCl and enzymatically digested using 3.2 mL of pepsin-HCl solution [8 g pepsin in 50 mL of

0.1M HCl] for 3 hours. Aliquots (20 g) of the digested solution were placed in a dialysis bag (Spectrapor 1, width 23 mm, 6000-8000 MW cut off) containing 5 mL of pancreatin-bile solution [1 g pancreatin (porcine pancreas Grade VI, Sigma Chemical Company, St Louis, Missouri, USA) plus 6.25 g bile extract (porcine, Sigma Chemical Company) in 250 mL of 0.1M sodium bicarbonate (Analytical Reagent, Mallinckrodt, NY, USA) and dialyzed in sodium bicarbonate solution (pH 7.5) for 12 hours to determine Ca potentially available for absorption in the small intestine (stage 1) (Thompson et al, 1991; Hurrell et al, 1988). After dialysis, the residue was freeze dried and samples (0.5 g) were fermented in vitro using human fecal innoculum (McBurney and Thompson, 1987). Ca availability in the small intestine determined by the above in vitro method has been shown to relate well to the in vivo Ca absorption in an ileostomate model (R^2 = 0.9 ; Trinidad, 1990; Thompson et al, 1991).

4.2.4. In vitro fermentation (stage 2).

A 0.5 g sample of freeze-dried residue from the in vitro digestion was weighed in duplicate serum bottles and to it was added 40 mL media containing 20 mL sodium bicarbonate buffer solution (0.4 M, pH 8.04), 20 mL macromineral solution (0.04 M $NaH_2PO_4.H_2O$ and 0.5 M KH_2PO_4), 0.1 mL of 0.1% resazurin solution and 2 mL of reducing solution (mixture of 1.25 g cysteine-HCl, 50 pellets of KOH, BDH Chemical Analar and 1.25 g Na_2S in 100 mL deionized water). The mixture was flushed with CO_2 until colourless. This was done 12-24 hours prior to the start of incubation to ensure hydration of samples. The bottles were sealed with butyl rubber stopper and crimped metal seals and stored at 4°C. The bottles were placed in a 37°C water bath 1-2 hour

prior to inoculation under constant flow of CO_2. A 10-mL sample of fecal inoculum prepared from 1:15 dilution of fresh feces from a human volunteer was injected into each bottle. The bottles were incubated for 24 hours at 37°C. The donor was a healthy female who had been eating an unspecified Western diet and had not taken antibiotics for a year or more. The bottles were then opened and 1 mL merthiolate solution (0.6 g/100 mL) was added. The fermentation digesta were transferred into 29 cm segments of dialysis tubing previously soaked in deionized water for 30 min. The mixture was dialyzed for 6 hours with dialysates replaced with 100 mL double distilled water every 3 hours to determine how much bound Ca was released under conditions simulating colonic fermentation (stage 2).

The dialysates in stage 1 and 2 were read in the atomic absorption spectrometer for Ca content (refer to general methods). Percent Ca release was determined by dividing the total amount of Ca in the dialysates by the total Ca in the sample multiplied by 100.

4.3 Results

4.3.1. Study 1

After in vitro digestion (stage 1), Ca released from BD with and without lactulose was significantly higher than that from BD with pectin or psyllium (Table 4.1; $P<0.05$). After in vitro fermentation (stage 2), BD with pectin had the highest Ca released while BD with psyllium had the lowest (Table 4.1; $P<0.05$). The total Ca released from BD with and without lactulose was significantly higher than those with pectin and psyllium (Table 4.1; $P<0.05$).

Table 4.1. Percent calcium released from basal diet (BD) with and without available and unavailable carbohydrates.

Test Meals	Stage 1*	Stage 2**	Total***
	percent		
BD	28.5±0.3[a]	11.9±1.2[a]	40.4±1.4[a]
+ Pectin[1]	20.7±0.4[c]	13.7±0.9[b]	34.4±0.6[b]
+ Lactulose[1]	28.4±0.3[a]	11.8±0.1[a]	40.2±0.2[a]
+ Psyllium[1]	22.1±0.8[bc]	4.4±0.2[c]	26.5±0.8[c]
BD + White Bread[2]	27.6±0.7[a]	14.5±0.2[b]	42.1±1.0[a]
+ Pectin	23.2±0.7[b]	18.2±1.2[a]	41.4±0.7[a]
+ Lactulose	27.5±0.3[a]	12.7±0.4[b]	40.2±0.8[a]
+ Psyllium	21.9±0.3[bc]	4.6±0.3[c]	26.5±1.0[c]

The data represents mean±SEM.
Different letters denote significant differences between meals in a column by one-way analysis of variance and Duncan's multiple range test at $P<0.05$.
* Under conditions simulating human stomach and small intestinal digestion.
**Under conditions simulating human colonic fermentation.
***Total Ca release = Ca release from stage 1 + Ca release from stage 2.
[1] Unavailable carbohydrates
[2] Available carbohydrate

Similarly, Ca released after in vitro digestion from BD plus white bread with and without lactulose was significantly higher than that of meals containing pectin and psyllium (Table 4.1; $P<0.05$). After fermentation, BD plus white bread containing pectin released significantly more Ca than the other test meals (Table 4.1; $P<0.05$). The total Ca released from BD plus white bread did not differ significantly from the meal containing lactulose and pectin but was greater than the Ca released from the meal containing psyllium (Table 4.1; $P<0.05$).

Table 4.2 shows the acetate (Ac), propionate (Pr) and butyrate (Bu) content of the fermented residues from BD with and without available and unavailable carbohydrates. The total SCFA (Ac+Pr+Bu) produced from BD with and without the unabsorbable carbohydrates was significantly different from each other in the following order: pectin and lactulose > psyllium and no addition (Table 4.2; $P<0.05$). Similar results were found for Ac alone. BD was significantly lower than all of the test meal for Pr alone while for Bu alone, none of the test meals differed significantly (Table 4.2; $P<0.05$).

The total SCFA produced from BD plus white bread with and without unabsorbable carbohydrates gave a different trend (Table 4.2). The meal with pectin was significantly greater than no addition and lactulose > psyllium ($P<0.05$). Results observed for Ac and Pr alone were as follows: for Ac, pectin > lactulose > psyllium and no addition and for Pr, lactulose was significantly lower than the rest of the meal ($P<0.05$). For Bu alone, pectin and no addition > psyllium and lactulose ($P<0.05$). The total SCFA produced from BD and BD plus white bread were significantly different from each other as well as for Ac or Pr or Bu alone (Table 4.2; $P<0.05$).

Table 4.2. Short chain fatty acids content of fermented residues from basal diet (BD) with and without available and unavailable carbohydrates.

Test Meals	Acetate	Propionate	Butyrate	Total*
	mmol/g residue			
BD	1.73±0.09^{f}	0.58±0.04^{d}	0.75±0.08^{b}	3.06±0.20^{c}
+ Pectin	3.02±0.02^{de}	0.82±0.05^{c}	1.04±0.04^{b}	4.92±0.02^{d}
+ Lactulose	2.84±0.17^{e}	1.03±0.08^{c}	1.06±0.08^{b}	4.94±0.20^{d}
+ Psyllium	1.98±0.19^{f}	0.84±0.01^{c}	0.72±0.06^{b}	3.56±0.20^{e}
BD + WB**	3.52±0.06^{c}	1.75±0.09^{a}	1.46±0.06^{a}	6.74±0.02^{b}
+ Pectin	4.42±0.07^{a}	1.76±0.02^{a}	1.58±0.20^{a}	7.76±0.20^{a}
+ Lactulose	3.94±0.18^{b}	1.32±0.20^{b}	1.04±0.30^{b}	6.30±0.06^{bc}
+ Psyllium	3.25±0.09^{cd}	1.61±0.06^{a}	1.02±0.06^{b}	5.88±0.20^{c}

The data represents mean±SEM.
Different letters denote significant differences between meals in a column by one-way analysis of variance and Duncans's multiple range test at $P<0.05$.
*Total = Acetate+Propionate+Butyrate.
**White Bread

The average ratio of Ac to Pr produced was 3:1 for BD and BD plus pectin and lactulose and 2:1 for BD with white bread and with psyllium (Table 4.2).

4.3.2. Study 2.

The Ca release after in vitro digestion from skimmed milk was significantly reduced ($P<0.05$) by pectin, psyllium and cellulose but not by lactulose (Table 4.3). The Ca released from skimmed milk cheese was reduced by the undigested carbohydrates in the following order: pectin > pysllium > cellulose and lactulose while for yogurt, pectin > cellulose and psyllium (Table 4.3; $P<0.05$).

After in vitro fermentation, Ca released from skimmed milk with and without the fiber was as follows: pectin > lactulose and no addition > psyllium and cellulose (Table 4.3; $P<0.05$). Similar results were observed for skimmed milk cheese and yogurt.

The total Ca release from all of the test foods was as follows: for skimmed milk, no addition, lactulose and pectin > cellulose and psyllium; for skimmed milk cheese, no addition > lactulose and pectin > cellulose > psyllium; and for yogurt, no addition and lactulose > pectin > cellulose and psyllium (Table 4.3; $P<0.05$). The total Ca release from all of the test foods with and without unabsorbable carbohydrates ranged from 16.6±0.4 to 37.8±0.7. The total Ca release from skimmed milk, skimmed milk cheese and yogurt with no addition ranged from 23.4±0.2 to 37.8±1.0 and is comparable to in vivo studies done on meals containing dairy products such as milk, cheese and yogurt (Allen, 1982; Heaney et al, 1988; Lewis et al, 1989). The Ac, Pr and Bu contents of fermented residues from all of the test foods with and without unabsorbable carbohydrates are shown in Table 4.4. The total SCFA (Ac+Pr+Bu) produced from skimmed milk

Table 4.3. Percent calcium released from dairy foods with and without unavailable carbohydrates.

Food	Stage 1*	Stage 2**	Total***
	percent		
skimmed milk			
no addition	18.2±0.2^{a}	16.2±0.6^{b}	34.4±0.8^{ab}
+lactulose	16.8±0.4^{ab}	15.8±0.4^{b}	32.7±0.7^{a}
+pectin	14.0±0.4^{c}	23.4±0.4^{a}	37.4±0.8^{a}
+psyllium	14.9±0.2^{c}	11.8±0.1^{c}	26.7±0.3^{c}
+cellulose	15.2±0.8^{bc}	11.9±0.5^{c}	27.2±1.4^{c}
skimmed milk cheese			
no addition	21.3±0.7^{a}	16.4±1.6^{b}	37.8±1.0^{a}
+lactulose	17.8±0.2^{b}	14.6±0.2^{bc}	32.4±0.0^{b}
+pectin	11.8±0.6^{d}	22.0±0.2^{a}	33.8±0.7^{b}
+psyllium	13.5±0.2^{c}	12.3±0.5^{c}	25.8±0.7^{d}
+cellulose	16.6±0.0^{b}	12.5±0 1^{c}	29.1±0.1^{c}
yogurt			
no addition	15.6±0.2^{a}	7.9±0.5^{b}	23.4±0.2^{a}
+lactulose	15.7±0.2^{a}	7.4±0.1^{b}	23.2±0.3^{a}
+pectin	10.0±1.2^{c}	9.8±0.0^{a}	19.8±1.2^{b}
+psyllium	13.1±0.0^{b}	3.5±0.4^{c}	16.6±0.4^{c}
+cellulose	12.8±0.1^{b}	3.8±0.0^{c}	16.6±0.1^{c}

The data represents mean±SEM.
Different letters denote significant differences between treatments in a column per test food by one-way anova and Duncan's range test at $P<0.05$.
* Under conditions simulating human stomach and small intestinal digestion.
**Under conditions simulating human colonic fermentation.
*** Total Ca release = Ca release from stage 1 + Ca release from stage 2.

Table 4.4 Short chain fatty acids content of fermented residues from dairy foods with and without unabsorbable carbohydrates.

Food	Acetate	Propionate	Butyrate	Total*
	mmol/g residue			
skimmed milk				
no addition	5.45±.70^{c}	1.25±.18^{c}	1.13±.01^{c}	7.83± .89^{c}
+lactulose	8.63±.17^{a}	1.68±.08^{a}	1.66±.07^{a}	11.97± .32^{a}
+pectin	7.02±.33^{b}	1.60±.06^{a}	1.30±.02^{b}	9.92± .41^{b}
+psyllium	4.91±.35^{c}	1.62±.08^{a}	1.00±.05^{c}	7.53± .48^{c}
+cellulose	2.75±.29^{d}	0.66±.12^{d}	0.67±.08^{d}	4.08± .49^{d}
skimmed milk cheese				
no addition	5.04±.21^{b}	1.05±.05^{b}	1.43±.01^{a}	7.52± .36^{ab}
+lactulose	6.87±.88^{a}	1.70±.19^{a}	1.62±.18^{a}	10.19±1.25^{a}
+pectin	5.78±.93^{ab}	1.32±.20^{ab}	1.45±.08^{a}	8.55±1.21^{ab}
+psyllium	3.86±.20^{c}	1.11±.06^{b}	1.17±.06^{b}	6.14± .32^{c}
+cellulose	2.83±.02^{d}	0.70±.01^{c}	0.85±.03^{c}	4.40± .06^{d}
yogurt				
no addition	1.72±.40^{b}	0.54±.12^{c}	0.93±.37^{a}	3.19± .89^{b}
+lactulose	3.16±.31^{a}	0.79±.10^{b}	0.99±.16^{a}	4.94± .57^{a}
+pectin	2.67±.10^{ab}	1.10±.10^{a}	1.25±.22^{a}	5.02± .42^{a}
+psyllium	2.34±.25^{ab}	0.72±.10^{b}	1.26±.04^{a}	4.32± .39^{ab}
+cellulose	1.66±.21^{b}	0.52±.06^{c}	0.93±.10^{a}	3.13± .37^{b}

The data represents mean±SEM.
Different letters denote significant differences between treatments in a column per test food by one-way analysis of variance and Duncan's multiple range test at $P<0.05$.
*Total=Acetate+Propionate+Butyrate.

with and without unabsorbable carbohydrates were as follows: lactulose > pectin > no addition and psyllium > cellulose; for skimmed milk cheese, lactulose > pectin> no addition > psyllium > cellulose; and for yogurt, lactulose and pectin > psyllium > no addition and cellulose.

In stage 1, the percent of total Ca release from single foods and meals with and without unabsorbable carbohydrates was lower in skimmed milk (37-56%) and skimmed milk cheese (35-57%) than yogurt (50-79%) and the 2 meals (BD, 60-83%; BD plus white bread, 56-83%) while in stage 2, it was higher in skimmed milk (44-63%) and skimmed milk cheese (43-65%) than yogurt (11-50%) and the 2 meals (BD, 17-40%; BD plus white bread, 17-44%). The percent of total Ca release in the presence of pectin in skimmed milk and skimmed milk cheese was greater in stage 2 (average of 64%) than in stage 1 (average of 36%) while for the 2 basal diets it was the reverse (stage 1, BD=60% and BD plus white bread =56%; stage 2, BD=40%; BD plus white bread=44%). Yogurt has a 50:50 ratio of the percent of total Ca release in stage 1 and 2. The total Ca release from single dairy foods was greater in skimmed milk and skimmed milk cheese than in yogurt similar to the results observed by Wong and La Croix (1980) in rats. However, the total Ca release from the two basal diets were greater than that from single foods for all treatments except for that with psyllium. The total Ca release from all samples with psyllium were almost the same except for yogurt.

4.4 Discussion

The study showed that Ca release differs between single foods and meals and that

different available and unavailable carbohydrates varied in their ability to bind Ca during in vitro pepsin-pancreatin-bile digestion simulating conditions in the small intestine (stage 1) and that the amount of Ca released during in vitro fermentation depended upon the fermentability of the carbohydrate (stage 2). The all in vitro method used in this study was similar to the method used from a previous study on different meals containing white bread, pumpernickel bread, barley and red lentils and was comparable to in vivo methods using ileostomate subjects (Trinidad, 1990; Thompson et al, 1991). The total Ca availability from the test diets and single foods with and without unabsorbable carbohydrates ranged from 16.6 to 42.2 % and is comparable to in vivo studies done on meals containing dairy products such as milk and cheese (Allen, 1982; Heaney et al, 1989; Kies, 1985; Lewis et al, 1989; Recker et al, 1988).

The results suggest that polysaccharides entrap Ca in stage 1 which may be due to viscosity or chemical binding or both. Pectin had a greater ability to bind Ca than psyllium and cellulose in stage 1 (Table 4.1 and 4.3). This may be attributed to its viscosity and the presence of carboxylic acid groups in the pectin molecule which may have chemically bound Ca. The effect of pectin on Ca release in stage 1 was more pronounced in skimmed milk and skimmed milk cheese in comparison to yogurt and the two meals. Psyllium was found to have a greater viscosity than pectin in the enzymatic digest in stage 1. It may have reduced the rate of diffusion of Ca out of the dialysis bag resulting in a lower Ca release. A similar result was observed with cellulose. Lactulose did not bind with Ca in stage 1 resulting in a similar Ca release as that of BD and BD plus white bread.

After in vitro fermentation, the Ca release from the meal containing both white bread and pectin was significantly greater than all of the test meals. Similar results were observed from the different dairy foods containing pectin. This suggested that any insoluble complex of Ca formed with pectin was broken down into SCFA with release of the bound Ca. The starch in white bread may have an additional effect in releasing more Ca in the presence of pectin in stage 2. It has been shown that 5-15% of the starch in normal foods escape digestion in the small intestine and enter the colon (Wolever et al, 1986). The starch that enters the colon may in turn serve as a substrate (in addition to pectin) for colonic bacterial fermentation and subsequently produce SCFA (MacFarlane and Englyst, 1986; Cummings, 1995). This is supported by the SCFA produced after fermentation from BD containing both white bread and pectin which was significantly greater than BD with pectin and the rest of the test meals (Table 4.2; $P<0.05$). Similarly, the total SCFA produced from BD plus white bread was significantly greater than that of BD (Table 4.2; $P<0.05$). The total Ca release from BD with white bread and pectin did not differ significantly from BD with and without white bread suggesting that in the presence of white bread, pectin did not affect total Ca availability (Table 4.1; $P<0.05$). However, psyllium continued to bind with Ca in stage 2 showing a significantly lower release of Ca in comparison to the other meals/test foods (Table 4.1 and 4.3; $P<0.05$). The presence of starch in the meal with psyllium did not help increase the release of Ca in stage 2 although a significant increase of total SCFA was observed in the meal with both white bread and psyllium in comparison to the meal with psyllium alone (Table 4.2; $P<0.05$). Like psyllium, cellulose showed a significantly lower Ca release from all of the

dairy foods in comparison to those with pectin. The total SCFA produced from BD plus psyllium with and without white bread and the dairy foods containing psyllium or cellulose was significantly lower than that from BD plus pectin with and without white bread and the dairy foods with pectin, respectively, suggesting that psyllium and/or cellulose was less fermentable. Chemical balance studies (Ink, 1988) have shown that cellulose has a negative effect on Ca balance.

The 3:1 ratio of Ac to Pr observed in this study in meals containing pectin and lactulose was similar to the ones that exist in human colonic contents (McBurney and Thompson, 1987). Other in vitro fermentation experiments done on ileal effluents of an ileostomate on different mixed diets also gave a ratio of Ac to Pr between 2.7:1 and 3.2:1 (Cummings et al, 1987).

In a balance study of 285 human adults in which the primary source of Ca was milk, Ca absorption was reduced by the addition of 20 g/day of psyllium but not with the addition of pectin (Kies, 1985). Other studies have also shown that pectin and other fermentable fibers such as guar have no effect on Ca availability (Cummings et al, 1979; Munoz and Harland, 1993). However, the above studies did not explain why pectin and psyllium have such an effect on Ca absorption. This study has demonstrated in vitro that a fermentable fibre such as pectin binds Ca in the small intestine but can be fermented producing SCFA and releasing in the colon Ca for potential absorption.

The differences between the total Ca release from single foods may be related to the nature of the Ca complex and products that contain colloidal phosphate (milk) and Ca paracaseinate (cheese) which are better sources of Ca than those that contain ionic Ca

(yogurt) (Wong and La Croix, 1980). However, other studies did not show any significant differences of Ca availability between different dairy products and Ca carbonate (Recker et al, 1988). The basal diets have higher Ca release than the single foods probably because of the presence of other food components in the diet such as apple juice. Apple juice contains citric acid which may have solubilized Ca and increased Ca release. In addition, the presence of both milk and cheese in the basal diets may have contributed to a higher Ca release as both are good sources of Ca.

Estimates showed that depending on the type of unabsorbable carbohydrates present, 35-83% of the total Ca release may be available for absorption in the small intestine while 17-65% may be available for absorption in the colon. The level of Ca released after in vitro fermentation is high and may be considered significant. The fermentation of carbohydrates to SCFA may have enhanced Ca release in stage 2. This will suggest that the release of Ca for potential absorption in the colon may depend on the complexing agent to which Ca is bound. The more fermentable and less viscous the substrate, the greater tendency that Ca will be released. The presence of unabsorbable carbohydrates such as lactulose and pectin in a meal therefore has little or no effect on total Ca availability. Pectin tends to shift Ca release potentially absorbable in the small intestine to the colon.

The all in vitro method used in this study may be used as an alternative method to estimate total Ca availability. However, a similar study should be designed in humans to further validate the above results.

CHAPTER 5

INTERACTIVE EFFECTS OF CALCIUM AND SHORT CHAIN FATTY ACIDS ON ABSORPTION IN THE DISTAL COLON OF MAN

5. INTERACTIVE EFFECTS OF CALCIUM AND SHORT CHAIN FATTY ACIDS ON ABSORPTION IN THE DISTAL COLON OF MAN

5.1 Introduction

The previous in vitro studies (Chapter 4) showed that Ca is released after fermentation of the insoluble Ca-fiber complex formed from the interaction between Ca and dietary fiber in the small intestine. The question is whether the Ca released can be absorbed in the colon and whether the Ca absorption is affected by the presence of the products of fiber fermentation, short chain fatty acids (SCFA). The SCFA, mainly acetic (Ac), propionic (Pr), and butyric (Bu) acids have been suggested to enhance the absorption of calcium (Ca) and Mg in the rat colon (Demigne et al, 1989; Lutz et al, 1991; Lutz and Scharrer, 1991). However, the mechanism by which SCFA increase cation absorption is obscure and there is no evidence that this occurs in humans. Therefore a rectal infusion technique (Wolever et al, 1989) was adapted to test if SCFA would increase the serum Ca response as an index of increased Ca absorption, after the rectal infusion of calcium.

5.2 Materials and Methods

Six healthy male subjects (27±3 years of age; 100±2% ideal body weight) were studied on six separate occasions in the morning after an overnight fast. A 600mL solution of each treatment was infused into the rectum at the rate of 30 mL/min. The different treatments per 600 mL used in the study were as follows: 45 mmoles sodium chloride (NaCl, AR, British Drug House, Darmstadt, West Germany); 30 mmoles calcium

($CaCl_2.2H_20$, AR, Fisher Scientific, Ontario, Canada) + 45 mmoles NaCl; 30 mmoles Ca + 22.5 mmoles SCFA (Na acetate, anhydrous, Sigma S-8750, St Louis, Missouri USA and Na propionate, food grade, Van Waters and Roger Ltd); 30 mmoles Ca + 45 mmoles SCFA; 10 mmoles Ca + 45 mmoles SCFA; and 45 mmoles SCFA. SCFA was a mixture of Ac and Pr in a 3:1 ratio; this ratio is similar to that present in the human colon (Cummings et al, 1987) and in the range produced by in vitro fermentation of available carbohydrates and fiber (McBurney et al, 1988; Weaver et al, 1989). The 45 mmoles dose of SCFA corresponds to the amount estimated to be produced in the colon in 4-6 hours from the amount and type of carbohydrates and fiber in the diet (McBurney et al, 1988). The amount of Ca (10 and 30 mmoles) was based on the observation that the dietary Ca intake usually ranges from 200-2000 mg/day (5-50 mmoles Ca) (Spencer and Kramer, 1985) and the amount of Ca absorbed in the small intestine is about 30-40% (Allen, 1982; Thompson et al, 1991), with approximately 3-32 mmoles of Ca reaching the colon for potential absorption.

The rectal infusion solutions were given in random order. The subjects administered their own rectal infusions using two 60-mL syringes as described in the general methods (Chapter 3). Blood samples (15 mL) were taken from fasting subjects at 30, 60, 90 and 120 min after the start of the rectal infusion. Subjects emptied their bladders into plastic bottles before the start of the rectal infusion, and immediately after the last blood sample.

Serum Ca and Ac and urinary Ca were measured. The pH of the blood and urine samples and the rectal infusion solutions was determined using a pH meter (Horiba pH meter F-8DP, Tokyo, Japan).

Serum Ca and Ac increments were calculated by subtracting each fasting value from the values at 30, 60, 90 and 120 min. The incremental area under the serum Ac and Ca curves were calculated geometrically (Wolever et al, 1989).

5.3 Results

All subjects retained the rectal infusion for all treatments for the full 2-hour experimental period with no difficulty.

Adding graded doses of SCFA to 30 mmol Ca resulted in a significant increase in serum Ca (Table 5.1 and Figure 5.1). Serum Ca did not change after 30 mmol Ca alone but increased significantly after 30 mmoles Ca + 45 mmoles SCFA ($P<0.0001$). Although the mean 30-120 min increment was not increased after adding 22.5 mmoles SCFA to 30 mmoles Ca (Table 5.1), at 120 min a significant increase in serum Ca was observed ($P<0.05$; Figure 5.1). After 10 mmoles Ca + 45 mmoles SCFA, the serum Ca increment did not differ significantly from 30 mmol Ca + 22.5 mmoles SCFA but significantly differed from the saline control solution ($P<0.05$).

A decrease in serum Ac was observed with graded doses of Ca (Table 5.2, Figure 5.2). The mean serum Ac increments did not differ significantly between 45 mmoles SCFA alone and with 10 mmoles Ca. However, Ac decreased significantly with 30 mmoles Ca ($P<0.0001$). Although there was a gradual increase in serum Ac with a peak at 90 min after 30 mmoles Ca + 45 mmoles SCFA, the mean serum Ac increments did not differ significantly from the control (Figure 5.2). This is due to variability in the serum Ac response of the subjects.

Table 5.1. Serum Ca fasting values, 30-120 min increments and area under the curve for all treatments.

Treatment	Fasting Values (mmol/L)	30-120 min increments (mmol/min/L)	Incremental area under the curve (mmol/min/L)
45 mmoles NaCl	2.17±.03	0.02±.03[b]	2.02±1.67[b]
30 mmoles Ca	2.20±.02	0.02±.04[b]	5.76±2.96[b]
30 mmoles Ca + 22.5 mmoles SCFA	2.21±.03	0.05±.05[b]	6.51±3.11[b]
30 mmoles Ca + 45 mmoles SCFA	2.15±.02	0.27±.02[a]	29.80±2.29[a]
10 mmoles Ca + 45 mmoles SCFA	2.21±.04	0.07±.04[b]	8.16±3.40[b]
45 mmoles SCFA	2.27±.02	0.02±.04[b]	5.19±3.71[b]

Mean±SEM with different letters in a column are significantly different at $P<0.0001$.
SCFA=3 mol Ac+1 mol Pr.

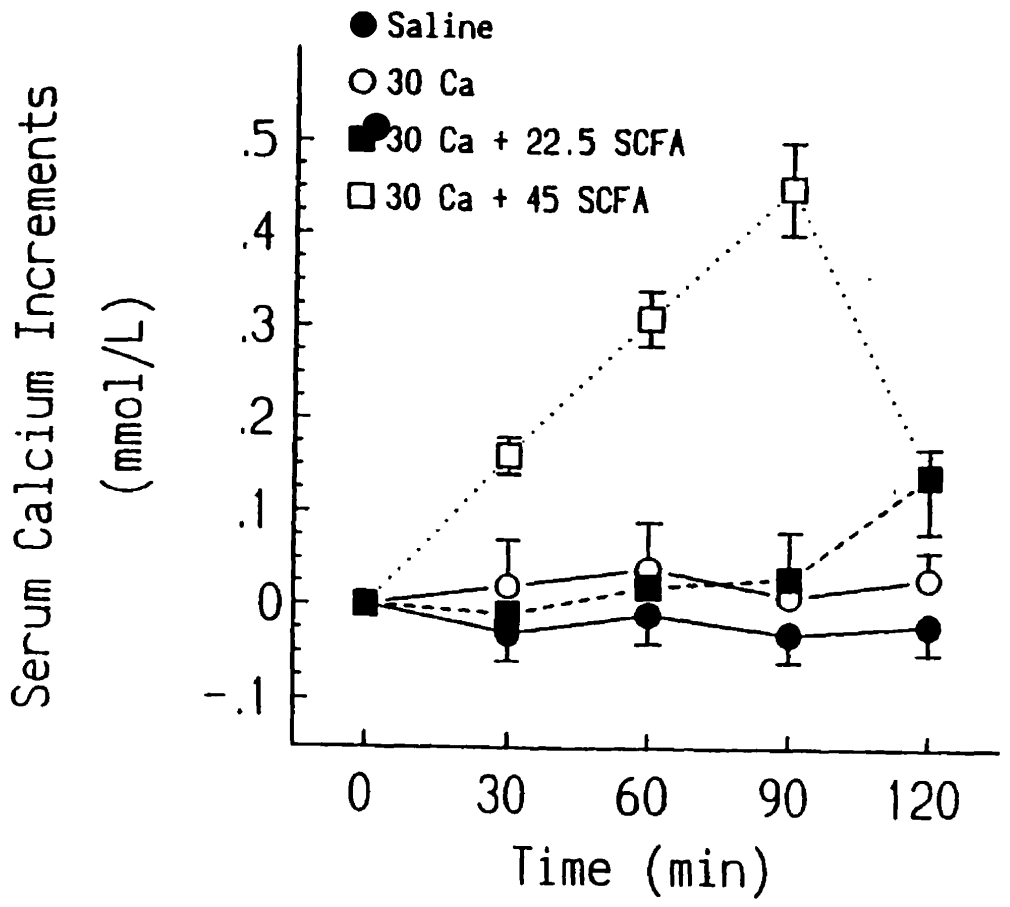


Figure 5.1. Mean±SEM serum calcium increments of six healthy men after rectal infusion of solutions containing sodium chloride or 30 mmoles $CaCl_2.2H_20$ with and without short chain fatty acids (SCFA).

Table 5.2. Serum acetate fasting values, 30-120 min increments and area under the curve for all treatments

Treatment	Fasting Values (μmol/L)	30-120 min increments (μmol/min/L)	Incremental area under the curve (μmol/min/L)
45 mmoles NaCl	105± 8	-1± 4[b]	537± 276[c]
30 mmoles Ca	89±13	-7± 3[b]	144± 136[c]
30 mmoles Ca + 22.5 mmoles SCFA	115±15	26±20[a]	3461±1860[ab]
30 mmoles Ca + 45 mmoles SCFA	98±10	6± 9[b]	1526± 682[bc]
10 mmoles Ca + 45 mmoles SCFA	107±13	26±24[a]	3862±2275[ab]
45 mmoles SCFA	119±15	40±16[a]	4996±1781[a]

Mean±SEM with different letters are significantly different at $P<0.0001$ for the mean increments and $P<0.0025$ for the incremental area under the curve.
SCFA=3 mol Ac +1 mol Pr.

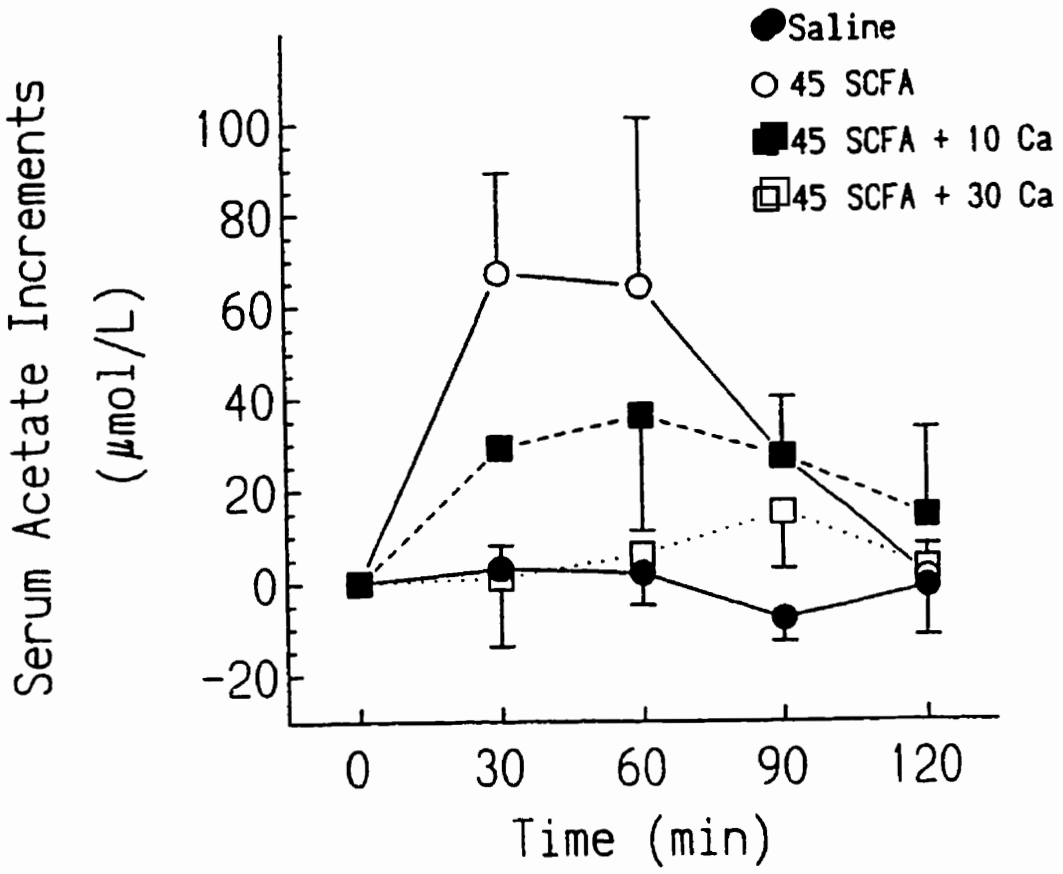


Figure 5.2. Mean±SEM serum acetate increments of six healthy men after rectal infusion of solutions containing sodium chloride or 45 mmoles short chain fatty acids (SCFA) with and without calcium.

No significant differences were found between Ca content of urine collected before and after rectal infusion for all treatments (Table 5.3). The pH of the blood did not differ significantly with time for each subject and treatment and were all in the normal range. Similar pH were obtained from urine collected before and after rectal infusion.

5.4 Discussion

The results showed that SCFA increased serum Ca and Ca decreased serum Ac. The increase in serum Ca after SCFA could be due to increased Ca absorption or reduced Ca excretion or utilization. When sodium or potassium Ac was added to total parenteral solutions, it increased blood pH and resulted in reduced urinary Ca excretion (Berkelhammer et al, 1988). Thus, it is possible that the sodium salt of SCFA may have reduced Ca excretion due to increased blood pH; leading to an increased serum Ca. However, if this were the case, SCFA alone would have been expected to increase serum Ca levels, an effect which was not observed in the study. In addition there were no significant differences in urinary Ca between the different treatments nor any differences in blood pH (Table 5.3). Therefore, the rise in serum Ca after 45 mmoles SCFA + 30 mmoles Ca cannot be explained by reduced Ca excretion or utilization and is most likely due to enhanced absorption.

It has been shown in rats that SCFA enhance Ca and Mg absorption from the distal colon (Lutz et al, 1991; Lutz and Scharrer, 1991). It was suggested that this effect was due to a Ca/H and Mg/H exchange similar to the Na/H exchange or K/H exchange proposed to exist in the proximal colon (Lutz and Scharrer, 1991) and distal colon (Lutz

Table 5.3. Urinary Ca and pH after infusion for all treatments.

Treatment	Urinary Ca Post treatment (mmol)	Volume of Urine (mL)	pH		
			Infusion Solution	Urine	Blood
45 mmoles NaCl	0.35±.11[a]	146.0±30.1[a]	4.77±.06[a]	6.10±.19[ab]	7.18±.02[a]
30 mmoles Ca	0.32±.07[a]	143.8±39.4[a]	4.91±.03[a]	5.67±.19[a]	7.20±.03[a]
30 mmoles Ca + 22.5 mmoles SCFA	0.42±.06[a]	121.8±34.6[a]	6.72±.04[b]	5.74±.38[a]	7.20±.02[a]
30 mmoles Ca + 45 mmoles SCFA	0.36±.06[a]	148.0±41.8[a]	6.72±.09[b]	5.55±.15[a]	7.12±.02[a]
10 mmoles Ca + 45 mmoles SCFA	0.44±.12[a]	221.5±72.5[a]	7.03±.03[c]	6.80±.38[b]	7.12±.02[a]
45 mmoles SCFA	0.39±.07[a]	179.7±60.6[a]	7.01±.08[c]	6.13±.32[ab]	7.22±.02[a]

Different letters in a column denotes significant differences between treatments at $P<0.05$. Values are mean±SEM.

et al, 1991), respectively. This would suggest that Ca would have no effect on Ac absorption. However, it was observed that graded amounts of SCFA increased the serum Ca response while graded amounts of Ca decreased the serum Ac response. This suggests that SCFA stimulated Ca absorption is not mediated solely by Ca/H exchange. Another potential mechanism which may be consistent with the current result is the formation of a $[CaAc]^+$ complex. Nancollas (1956) showed that Ac in the presence of bivalent metal chlorides such as Ca can form a complex, $[CaAc]^+$. The thermodynamic association constants between Ac and various metal ions was greatest for Ca in comparison to the other alkaline-earth metals such as Mg, Sr and Ba. The complex formation occurs in two stages: (a) formation of an ion pair, $Ca^{2+}(nH_20)Ac^-$ and (b) formation of a more covalent complex, $CaAc^+(H_20)_{n-1}$ or simply written as $[CaAc]^+$ (Nancollas, 1956). This complex is stable and less highly charged than the Ca^{2+} ion (Nancollas, 1956). Because cell membranes have low permeability to highly charged ions, it is likely that the $[CaAc]^+$ complex, which is less charged than Ca^{2+} passes more readily in the cell membranes than Ca^{2+} (Marshall, 1976).

After administering 30 mmoles Ca + 45 mmoles SCFA, a rise in serum Ac was observed at 90 min, after 10 mmoles Ca + 45 mmoles SCFA, the Ac peak was at 60 min and after 45 mmoles SCFA the Ac peak was at 30 min (Figure 2). The peak in serum Ca was also observed at 90 min after 30 mmoles Ca + 45 mmoles SCFA (Figure 2), and this is consistent with the possible formation and absorption of a $[CaAc]^+$ complex. However, it does not fully explain the lower serum Ac levels in the presence of Ca. Although this requires further investigation, two explanations may be hypothesized. It

could be due to an increased rate of Ac uptake by the tissues in the presence of high Ca levels and/or it could be related to the differential effect of Ac and propionate (Pr) on Ca absorption. Pr was also present in the infusion solution but was not detected in the serum probably because it is taken up readily by the liver (Cummings et al, 1987). It can also complex with Ca but less stable than Ac (Cannan and Kibrich, 1938). A possibility exists that, at high Ca and SCFA (i.e. Ac and Pr) concentrations, Pr may become more involved than Ac in the increase in Ca absorption perhaps by way of the Ca/H exchange. Pr is more lipid soluble than Ac and thus has been shown to pass faster through the cell membrane (Dawson et al, 1964; Saunders, 1991).

Analytical error cannot account for the reductions in serum Ac response after rectal Ca because the addition of Ca to standard Ac solutions alone or added to serum did not reduce Ac recovery. The osmolality of all the test solutions was not the same because their Na concentrations were controlled. However, the differences in osmolality cannot account for the effects we observed of SCFA on serum Ca responses. The serum Ca level of the subjects infused with 45 mmoles NaCl and 30 mmoles Ca + 45 mmoles NaCl did not differ significantly (Figure 5.1). Moreover, the serum Ca level of 30 mmoles Ca + 45 mmoles NaCl and 30 mmoles Ca + 45 mmoles SCFA, two solutions with the same osmolality, differed significantly ($P<0.0001$), showing the enhancing effect of SCFA on serum Ca level (Figure 5.1).

The area under the serum Ca response curve after 30 mmoles Ca + 45 mmoles SCFA represents 2.4±0.24% of the infused dose of Ca. This probably underestimated the true Ca absorption from the colon as affected by SCFA since the shape of the plasma Ca

response curve following Ca administration depends upon the rate of absorption from the gastrointestinal tract into the plasma and the rate of removal from the plasma (Marshall, 1976). This removal will be into soft tissues and bone within a few hours with only 1% of an oral Ca dose appearing in the urine within four hours. The results are consistent with this since there was no significant increase in urinary Ca after 30 mmoles Ca + 45 mmoles SCFA compared to the saline control (Table 5.3).

In conclusion, SCFA enhance Ca absorption and Ca reduces the serum Ac response in the distal colon of human subjects. The inhibiting effect of Ca on serum Ac response needs further investigation.

CHAPTER 6

THE EFFECT OF ACETATE AND PROPIONATE ON CALCIUM ABSORPTION FROM THE RECTUM AND DISTAL COLON OF HUMANS

6. THE EFFECT OF ACETATE AND PROPIONATE ON CALCIUM ABSORPTION IN THE RECTUM AND DISTAL COLON OF HUMANS

6.1 Introduction

In Chapter 5 it was found that rectal infusion of an isotonic solution containing Ca plus SCFA resulted in a significantly greater increase in serum Ca than did an infusion of Ca alone. This suggests that Ca absorption from the colon is enhanced by SCFA. Unexpectedly, Ca also reduced the rise of blood Ac after rectal infusion of Ac and Pr, suggesting that Ca reduced Ac absorption from the distal colon. This result suggested that Ca may affect Ac absorption.

However, serum Ca and Ac is an indirect measure of Ca or SCFA absorption, respectively. Moreover, the SCFA solution used in this study was a mixture of Ac and Pr in a 3:1 ratio. Therefore two studies were performed to investigate the direct effects of Ac and Pr, alone and combined, on the disappearance of Ca from the rectum and distal colon of human subjects. In the first study, Ac and Pr were given at normal physiologic concentrations and molar ratios (i.e. Ac:Pr = 3:1). In the second study, the molar ratios were reversed (Ac:Pr = 1:3). In the first study, the effect of Ca on SCFA absorption was also determined.

6.2 Materials and Methods

Two studies were conducted on subjects in the morning after an overnight fast. The night before the study, subjects were provided with a standard, low fiber dinner to reduce

colonic residue. In the first study, six healthy subjects (2 males and 4 females; 33±5 years of age; 100±2% ideal body weight) were given rectal infusions on eight separate occasions while in the second study, six healthy female subjects (29±4 years of age; 100±2% ideal body weight) were given rectal infusions on six separate occasions. The details of the different treatment solutions are summarized in Table 6.1.

The average Ca intake from a usual western diet is around 5-50 mmoles/day (Allen, 1982; Spencer and Kramer, 1985) and about 30-40% is absorbed in the small intestine (Allen, 1982). Therefore, approximately 3-32 mmoles of Ca reaches the colon for potential absorption. The Na concentration of all treatment solutions in both studies was adjusted to 75 mmoles/L with sodium chloride. In addition, each solution contained 0.625 mmoles/L of polyethylene glycol (PEG) which was used as an unabsorbable marker. Each subject served as his own control and comparisons were done by study group. The length of the washout period between infusions was 3-7 days depending upon 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 using a plastic Tygon tubing connected to a 60-mL syringe as described in the general methods (Chapter 3). Samples were collected at 0, 10, 20 and 30 min. After the 30 min sample, the tubing was removed and the total colonic sample was collected.

Samples were centrifuged and the supernatant analyzed for Ca, Ac, Pr and PEG. The pH was measured using a pH meter (Beckman 50) for the following treatments: Ca+NaCl, Ca+3Ac and Ca+3Pr.

Table 6.1. Composition of the rectal infusion solutions.

Treatment	Ca[1]	Ac[2]	Pr[3]	NaCl[4]
	mmol/L			
Study 1				
Ca+NaCl	50.0	-	-	75.0
Ca+3Ac	50.0	56.3	-	18.7
Ca+1Pr	50.0	-	18.7	56.3
Ca+3Ac+1Pr	50.0	56.3	18.7	-
3Ac	-	56.3	-	18.7
3Ac+1Pr+NaCl	-	56.3	18.7	75.0
3Ac+1Pr	-	56.3	18.7	-
1Pr	-	-	18.7	56.3
Study 2				
NaCl	-	-	-	75.0
Ca+1Ac	50.0	18.7	-	56.3
Ca+1Pr	50.0	-	18.7	56.3
Ca+3Ac	50.0	56.3	-	18.7
Ca+3Pr	50.0	-	56.3	18.7
Ca+1Ac+3Pr	50.0	18.7	56.3	-

The volume of each solution infused was 300 mL.

[1]Calcium chloride ($CaCl_2.2H_20$), Fisher Scienctific, Nepean, Ontario, Canada.

[2] Sodium acetate, NaAc, anhydrous, Sigma S-8750, St. Louis, Missouri, USA.

[3] Sodium propionate, NaPr, Food Grade, Van Waters and Roger Ltd, Toronto, Ontario, Canada.

[4] Sodium chloride, NaCl, Analytical Reagent, British Drug House, Darmstadt, West Germany.

To correct for incomplete collection and fluid absorption or secretion by the colon, the Ca, Ac and Pr concentrations were expressed relative to that of PEG (i.e. Ca/PEG ratio). Ca, Ac or Pr absorption was expressed as disappearance, i.e. change in the Ca/PEG concentration ratio from baseline. Estimates of Ca absorbed in mmoles were calculated by multiplying the percent disappearance by mmoles of Ca in the infused solution. Ca, Ac and Pr disappearance from the samples of rectal fluid withdrawn at 10 min intervals for 30 min represents rectal absorption while 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.

6.3 Results

All solutions were retained for 30 min without difficulty. The presence of fluid in the rectum and distal colon produced little if any sensation or distention or fullness. 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 Ca in the distal colon increased with time (0-30 min; study 1, $r=0.94$, $P<0.0001$; study 2, $r=0.77$, $P<0.002$) for all treatments in both studies (Figure 6.1; Table 6.2).

In study 1, Ac and/or Pr significantly increased Ca disappearance (decrease in Ca/PEG ratio) at 30 min compared to the control (Table 6.2; $P<0.05$). However, there was no significant interaction between time and treatment. Despite the fact that Ac was 3 times the concentration of Pr, both SCFA were equally effective in enhancing Ca absorption (Table 6.2). For each treatment, Ca absorption from the rectum plus distal

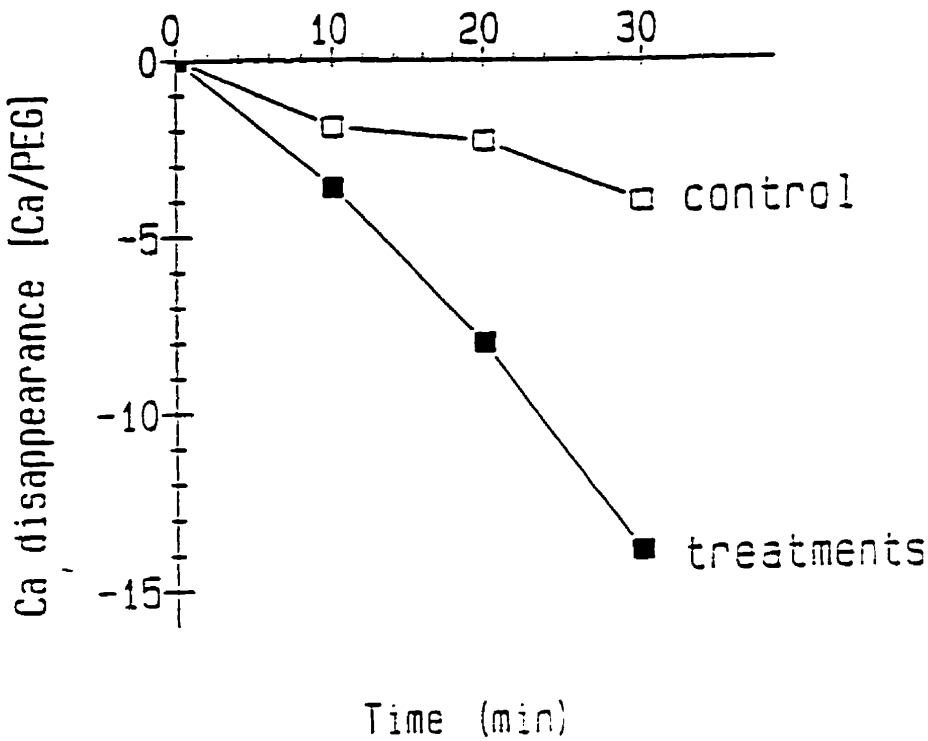


Figure 6.1. Calcium disappearance from the rectum over time for the control (Ca+NaCl) or calcium with acetate, propionate or both. Mean for all treatments was pooled since treatments did not differ significantly from each other (Study 1).

Table 6.2. The effect of acetate and/or propionate on calcium disappearance in the rectum and distal colon of human subjects.

	Ca disappearance (Ca/PEG)				
Time (min)	0	10	20	30	Total
Treatment					
Study 1					
Ca+NaCl	0^{ax}	-1.9 ± 0.1^{bx}	-2.3 ± 0.8^{cx}	-4.0 ± 1.0^{dx}	-5.5 ± 1.4^{ex}
Ca+3Ac	0^{ax}	-3.0 ± 1.0^{bxy}	-8.6 ± 2.7^{cy}	-18.3 ± 5.5^{dy}	-22.6 ± 2.8^{dy}
Ca+1Pr	0^{ax}	-2.0 ± 0.7^{bxy}	-5.8 ± 1.5^{cy}	-13.4 ± 3.1^{dy}	-23.2 ± 3.2^{ey}
Ca+3Ac+1Pr	0^{ax}	-6.0 ± 3.5^{by}	-9.6 ± 3.5^{by}	-10.2 ± 4.7^{by}	-19.7 ± 4.6^{cy}
Study 2					
Ca+1Ac	0^{ax}	-2.4 ± 1.1^{bx}	-6.6 ± 1.7^{cy}	-11.1 ± 1.2^{dz}	-14.4 ± 0.4^{ex}
Ca+1Pr	0^{ax}	-4.9 ± 1.1^{by}	-7.2 ± 1.4^{by}	-10.8 ± 1.3^{cz}	-15.7 ± 1.4^{dx}
Ca+3Ac	0^{ax}	-1.2 ± 0.3^{bx}	-3.2 ± 0.6^{cx}	-4.3 ± 0.6^{cx}	-11.2 ± 3.6^{dx}
Ca+3Pr	0^{ax}	-6.5 ± 1.5^{by}	-8.2 ± 1.5^{by}	-10.9 ± 1.8^{byz}	-20.3 ± 2.3^{cy}
Ca+1Ac+3Pr	0^{ax}	-2.1 ± 0.6^{bx}	-5.2 ± 1.4^{cxy}	-7.0 ± 2.0^{cxy}	-13.0 ± 1.1^{dx}

abcde represents significant differences between time points for treatments within a study across a row at $P<0.05$. xyz represents significant differences between treatments within a study down a column at $P<0.05$.
Ca disappearance is the change in Ca/PEG concentration ratio from the baseline.

colon (total collection) was 1.2-2.0 times greater than that from the rectum at 30 min (Table 6.2; $P<0.05$). The difference in Ca absorption between males and females was not significant so either sex could be used in future experiments.

The disappearance of Ac and Pr from the rectum is shown in Figures 6.2 and 6.3, respectively. There was no significant difference between treatments ($P<0.05$) but there was significant difference between times ($P<0.05$). When all treatments were combined (Figure 6.4), the absorption of Ac and Pr from the rectum was approximately linear over the 30 min study period.

The disappearance of Ac from the rectum by 30 min was significantly less than that from the rectum plus distal colon for the Ac+Pr and Ac+Ca treatments (Figure 6.5). As well, Pr was also absorbed less in the rectum than in the rectum plus distal colon ($P<0.01$), but there was no significant difference for any of the individual treatments (Figure 6.6).

Ac disappearance (decrease in Ac/PEG ratio) from the rectum plus distal colon was significantly enhanced by adding Pr from -4.1±4.2 to -40.2±11.9 ($P<0.05$; Figure 6.5). The addition of Ca to Ac also increased Ac disappearance significantly to -34.0±8.9 ($P<0.05$). However, adding Ca to a mixture of Ac+Pr reduced Ac disappearance to -13.2±4.6 ($P<0.05$ compared to Ac plus Ca and $P<0.05$ compared to Ac plus Pr). Ac disappearance from the Ac/Pr/Ca mixture was not significantly different from that after Ac alone.

Pr disappearance (decrease in Pr/PEG ratio) from the rectum plus distal colon when Pr alone was infused was -15.7±3.3, and this was not affected significantly when Ac,

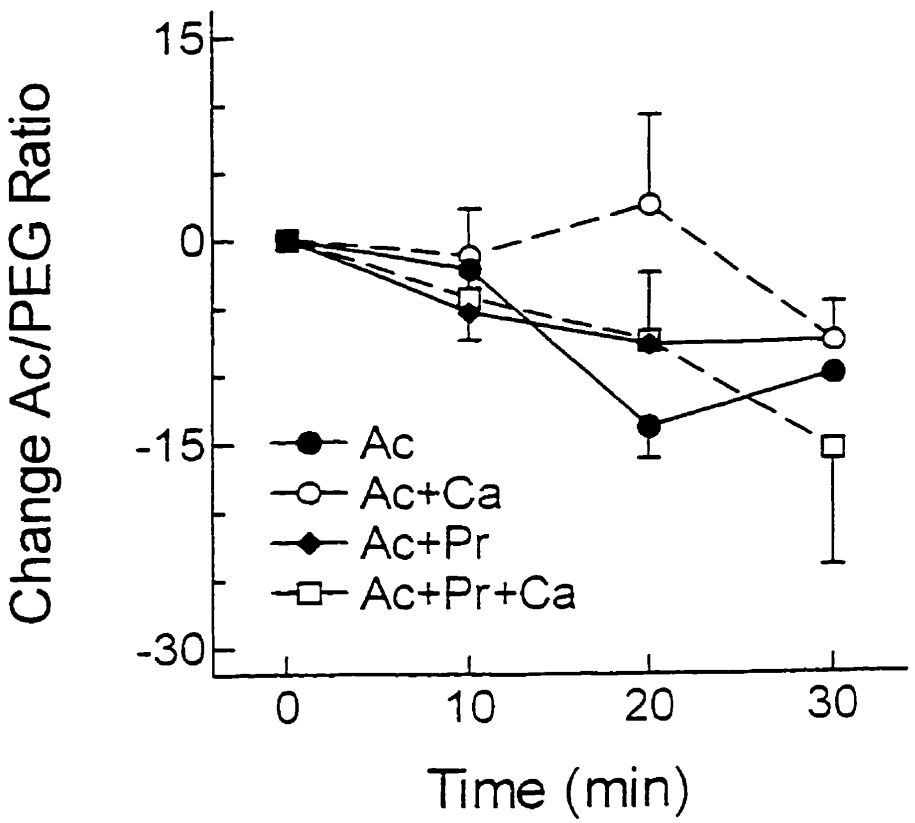


Figure 6.2. Change in Acetate/PEG concentration ratio in fluid from the rectum after infusion of the four treatment solutions (study 1).

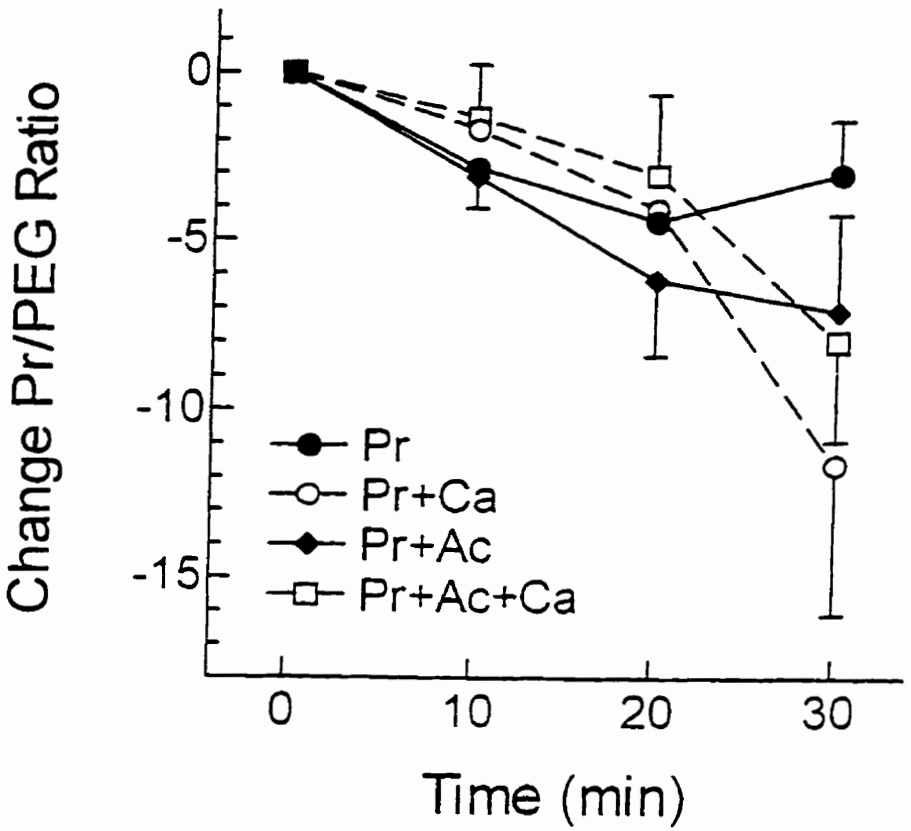


Figure 6.3. Change in propionate/PEG concentration ratio in fluid samples from the rectum after infusion of four treatment solutions (study 1).

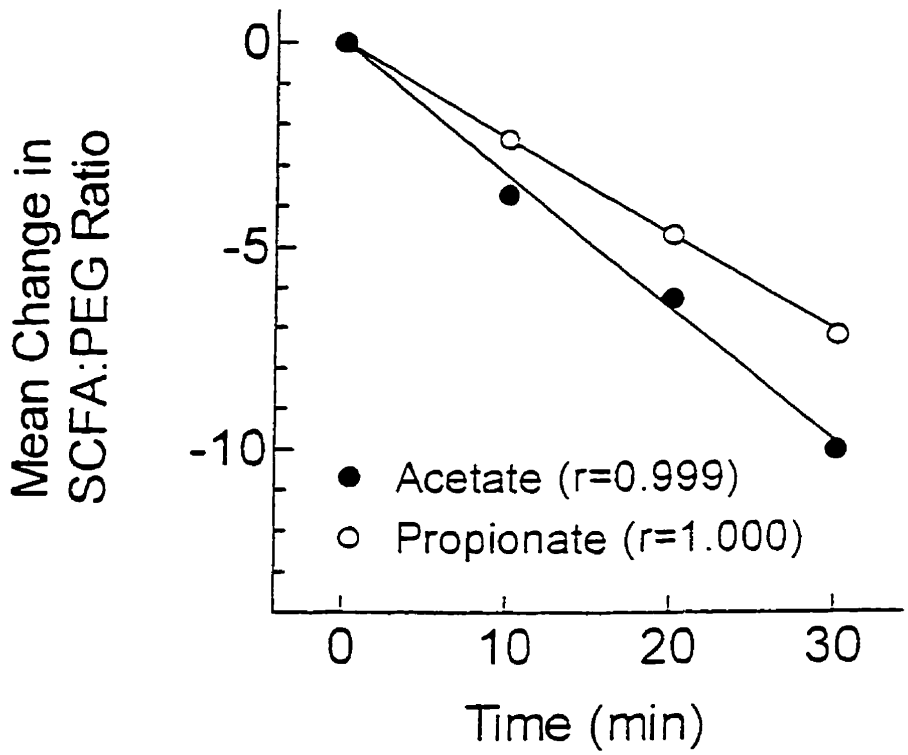


Figure 6.4. Mean changes in acetate/PEG and propionate/PEG concentration ratios in samples of fluid taken from the rectum of human subjects. Treatments were pooled for either acetate or propionate treatments.

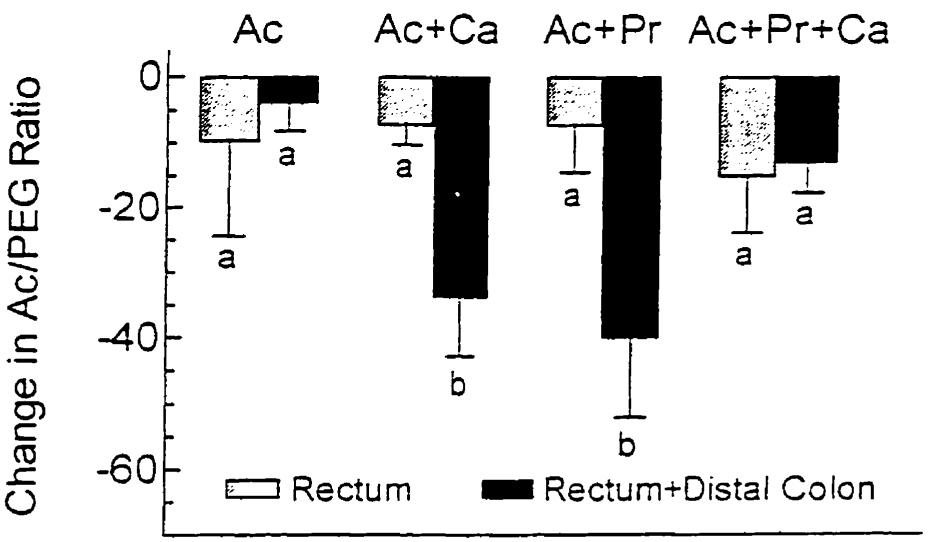


Figure 6.5. Change in acetate/PEG concentration ratios in fluid samples from the rectum and distal colon 30 min after infusion of the four treatment solutions (study 1).

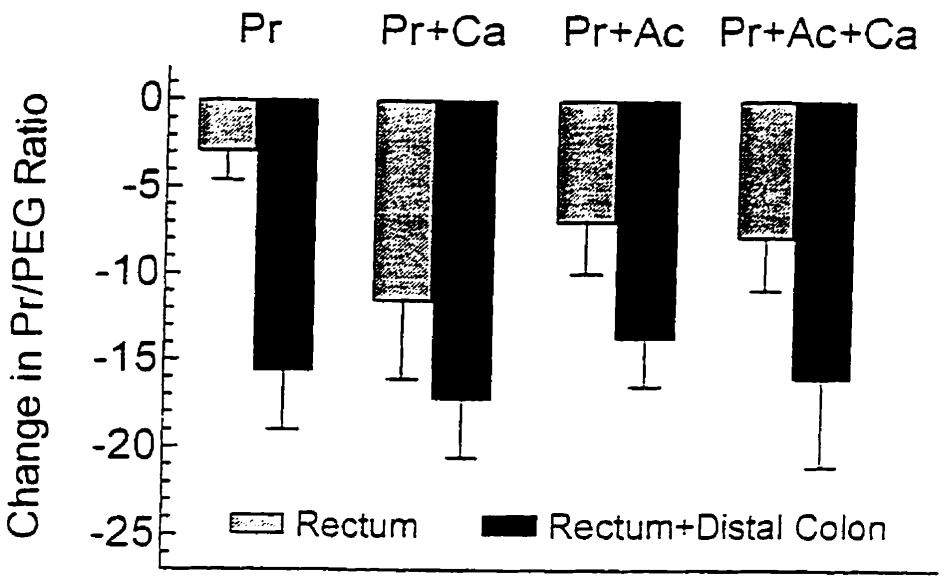


Figure 6.6. Change in propionate/PEG concentration ratios in fluid samples from the rectum and distal colon 30 min after infusion of the four treatment solutions (study 1). Pr is propionate, Ac is acetate, and Ca is calcium.

Ca or Ac plus Ca were added (Figure 6.6).

In study 2, two of the treatments (Ca+3Ac and Ca+1Pr) from the first study were repeated to determine the effect of the different concentrations of Ac or Pr on Ca absorption on the same subject. In addition, a control without Ca (NaCl group) was used to determine if Ca is actually secreted in the colon. Results showed that this was not the case as no increase in Ca absorption was observed for NaCl. Ca absorption after Ca+1Ac (-14.4±0.4) was not different from Ca+1Pr (-15.7±1.4) or Ca+3Ac (-11.2±3.6; Table 6.2). However, with a higher concentration of Pr (3Pr; 56.3 mmoles/L), Ca absorption (-20.3±2.3) was significantly greater than with 18.7 mmoles/L Pr (1Pr) and 56.3 mmoles/L Ac (3Ac; $P<0.05$; Table 6.2). The 56.3 mmoles/L Pr alone caused greater Ca absorption than the treatment with both Ac and Pr (Ca+1Ac+3Pr = -13.0±1.1) although not significant. 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 with Ca+3Ac or Ca+3Pr (Table 6.3; $P<0.05$). Compared to 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 while for Ca+3Pr, no significant difference between the pH of the infused solution, at time 0 and at time 30 was observed (Table 6.3; $P>0.05$). There was no significant difference in pH between Ca+3Ac and Ca+3Pr at any time.

Table 6.3. pH of the infused solution and recovered infusate

Treatment	pH		
		Time (min)	
	Infused Solution	0	30
Ca+NaCl	$4.9\pm.02^{ax}$	$6.0\pm.20^{ay}$	$7.2\pm.06^{az}$
Ca+3Ac	$7.0\pm.05^{bx}$	$8.2\pm.08^{by}$	$7.8\pm.10^{ay}$
Ca+3Pr	$7.1\pm.03^{bx}$	$7.6\pm.10^{bx}$	$7.5\pm.10^{ax}$

abc indicates significant differences between treatments in a column at $P<0.05$.
xyz indicates significant differences between time in a row at $P<0.05$.

6.4 Discussion

Results showed that Ca is absorbed from the rectum and distal colon of man and that this process is enhanced by the presence of SCFA. This is consistent with the results obtained from the previous study (Chapter 5) and in the study on Ca and Mg absorption in the distal colon of rats (Lutz et al, 1991; Lutz and Scharrer 1991). Pr was more effective than Ac in enhancing Ca absorption. At the lower concentration of Pr, the enhancing effect on Ca absorption was similar to the effect of Ac at its higher concentration. Because of the above result, we looked at the effect of different concentrations of Ac and Pr on Ca absorption. It was observed that at higher concentrations, Pr was more effective than Ac in enhancing Ca absorption.

It has been speculated that the enhancing effect of SCFA on Ca absorption may be due to a Ca/H exchanger which exists in the distal colon but not in the proximal colon of rats (Lutz and Scharrer, 1991). The proposed mechanism by which SCFA enhanced Ca absorption is that protonated SCFA are absorbed by direct diffusion across the apical membrane (Fleming et al, 1991; Rechkemmer and Engelhardt, 1988). Once the protonated SCFA molecule diffuses into the cell it dissociates, since the pKa values of SCFA (approximately 4.8) are lower than that of the pH of the cell (between 6 and 7). The resulting intracellular hydrogen ion (H^+) available in the lumen is secreted from the cell in exchange for a cation which, in the distal colon is Ca^{++} (Lutz and Scharrer, 1991). Once outside the cell, the 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 bicarbonate appearance in the colonic lumen is enhanced by the presence of SCFA (Titus and Ahearn, 1992). Bicarbonate appearance in the colonic lumen may indicate the presence of hydrogen ion needed to exchange with Ca for absorption. This may be the reason for increased Ca absorption in the presence of SCFA. However, difference in pH cannot account for differences in Ca absorption because Ca absorption from the treatment with Pr was significantly greater than that from the treatment with Ac despite no difference in pH.

The greater enhancing effect of Pr than Ac on Ca absorption may be due to its being more lipid soluble than Ac and thus more rapidly absorbed by direct diffusion. It was observed in this study that Pr absorption is significantly greater than that of Ac. In a study in guinea pig distal colon (Engelhardt and Rechkemmer, 1992), SCFA showed a net absorption rate that corresponds to the chain length of SCFA where Bu > Pr> Ac. The chain length dependence of unidirectional SCFA fluxes in the distal colon may indicate the importance of lipid solubility for non-ionic diffusion in the distal colon. Thus, Pr may have diffused into the cell faster than Ac and supplied intracellular hydrogen ions to exchange for Ca.

The exact mechanism by which SCFA are absorbed is not clear, but it may be species dependent (Engelhardt and Rechkemmer, 1992; Hatch, 1987; Sellin and DeSoignie, 1990), and different mechanisms exist in different parts of the intestine (Engelhardt and Rechkemmer, 1992; Rechkemmer and Engelhardt, 1988). There is much evidence that

an anion exchange mechanism operates whereby the SCFA anion is exchanged for bicarbonate (Binder and Mehta, 1989; Fleming et al, 1991; Ruppin et al, 1980; Titus and Ahearn, 1992). If this were the only mechanism for SCFA absorption, it would imply competition for the exchanger between different SCFA such that the absorption of the individual SCFA would be inhibited in the presence of others. In favor of this possibility are studies showing that SCFA containing 2 to 5 carbon atoms decreased Pr (Harig et al, 1991) and Bu (Harig et al, 1990) uptake rates by vesicles from human ileal brush border membranes and colonic apical membranes, respectively. However, competition between SCFA for a single anion exchanger is not consistent with the present results since Pr significantly enhanced Ac absorption. This suggests that there may be another mechanism, in addition to the anion exchanger, by which Ac or Pr or both are absorbed.

The absorption of Ac from the rectum and distal colon was increased in the presence of Pr alone or Ca alone, but the presence of both Pr and Ca had no effect on Ac absorption. In the previous study (Chapter 5), it was found that adding Ca to a rectally infused solution of Ac+Pr reduced the rise in serum Ac seen after Ac+Pr without Ca. The present results are consistent with this because, when Ca was added to a rectally infused solution of Ac+Pr, there was a significant reduction in Ac absorption. This is difficult to explain. One possibility is that the difference in chloride concentrations may have influenced the results since chloride may be a substrate for the anion exchanger involved in SCFA absorption (Binder and Mehta, 1989). However, the difference in chloride cannot explain the above results since adding NaCl to Ac and Pr did not affect Ac or Pr absorption. In addition, the chloride concentration in the Ac+Pr+Ca treatment (100

mmol/L) was greater than in Ac+Pr (75 mmol/L) and less than that in Ca+Ac (119 mmol) and thus did not relate to Ac absorption which was lowest for Ac+Pr+Ca. The reduction in Ac absorption by Ca could be due to Ca increasing the affinity of the anion exchanger for Pr such that Ac absorption is reduced due to competition between Pr and Ac for transport sites. There may be competition between Ac and Pr anions for the proton required for non-ionic absorption. Perhaps the most likely explanation is that Ac absorption is enhanced by a $[CaAc]^{+}$ complex and that Pr interferes with this complex. In support of this, is the fact that 18.7 mmol/L Pr had the same effect in stimulating Ca absorption from the rectum and distal colon as 3 times the concentration of Ac, suggesting that Pr interacts with Ca more avidly than Ac.

This study was done as a continuation of the previous study on the effect of a mixture of Ac and Pr on serum Ca response (Chapter 5). Since Bu is immediately utilized in the colon and is difficult to measure in the serum, it was not included in the previous study and thus also in this study. The design of this study does not only address the primary question about the effect of Ac and Pr on Ca absorption or the effect of Ca on Ac and Pr absorption, but it also gives preliminary information about the time course of Ca and SCFA absorption from the rectum, and whether there was any evidence of regional differences in absorption between rectum and distal colon. For these reasons, samples of fluid were taken from the rectum at 10 min intervals before the subjects emptied their colons and the contents were collected. This provided interesting data suggesting that regional differences in the absorption of Ca, Ac and Pr exist between rectum and distal colon. However, the drawback was that each sampling would alter the Ca/PEG

and SCFA/PEG ratio expected in the final sample. In addition, the distribution of fluid between the rectum and distal colon is unknown. Thus, the actual amount of Ca and SCFA absorbed could not be calculated accurately. The Ca disappearance from the total colonic sample which represents Ca absorption from the rectum plus distal colon in this study may be estimated from the Ca/PEG ratios. Ca absorbed (mmoles Ca/30 min) are as follows: 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, 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 approximately 6-30% Ca absorption. An estimate of absorption obtained by expressing the change in SCFA/PEG ratios at 30 min as a percent of those at baseline showed that Ac absorption from the rectum plus distal colon was about 10% for Ac alone, and increased to 40-45% by adding Ca or Pr. Pr absorption was about 40-45% for all treatments.

In conclusion, Ca is absorbed in the distal colon of man and both Ac and Pr enhanced Ca absorption. However, Pr has been shown to be more effective than Ac in enhancing Ca absorption. The absorption of Ac in the rectum and distal colon of humans may be influenced by the presence of Ca and Pr. The mechanism behind the absorption of Ca and the enhancing effect of Ac and Pr on Ca absorption as well as the influencing effect of Ca on Ac and Pr absorption in the human distal colon needs further investigation.

CHAPTER 7

CALCIUM ABSORPTION IN THE DISTAL COLON OF HUMAN: EFFECTS OF CALCIUM CONCENTRATION, ACETATE AND PROPIONATE

7. CALCIUM ABSORPTION IN THE DISTAL COLON OF HUMANS: EFFECTS OF CALCIUM CONCENTRATION, ACETATE AND PROPIONATE

7.1 Introduction

The previous study (Chapter 6) has shown that Ca provided at 50 mmol/L concentration with and without acetate (Ac) or propionate (Pr) is absorbed from the distal colon of humans. At lower concentration of Ca, Ac and Pr were as effective in enhancing Ca absorption while at higher concentration, Pr was more effective. These results suggest that Ac and Pr may have a different transport mechanism in enhancing Ca absorption. To investigate the kinetics involved in Ca absorption, the objective of this study was to determine the relationship between Ca concentration and Ca absorption from the human distal colon in the presence and absence of Ac or Pr.

7.2 Materials and Methods

Six healthy subjects (1 male and 5 females; 29±4 years of age; 100±2% ideal weight) were studied. Rectal infusions containing various concentrations of Ca as $CaCl_2.2H_20$ (4, 11, 24, 46 mmol/L) with 56.3 mmol/L Ac or Pr or sodium chloride (NaCl) were given to each subject on 13 occasions. In addition, a solution of 56.3 mM NaCl alone was used as a control. Polyethylene glycol (PEG, 0.625 mM) was added to each solution as an unabsorbable marker. Ca, Ac, Pr, NaCl and PEG were dissolved in double distilled water. A 300 mL test solution was infused to each subject with a maximum osmolality of 300 mosm similar to the previous study (Chapter 6) and was described in the general methods

(Chapter 3). Table 7.1 shows the composition of the test solutions in mmol/L. The 13 different treatments were given in random order. Samples were centrifuged and the supernatant analyzed for Ca, Ac Pr and PEG as earlier described (Chapter 3).

Ca, Ac and Pr disappearance from the distal colon was used as an index of absorption. PEG was used as an unabsorbable marker to correct for incomplete collection and fluid absorption or secretion by the colon. The percent Ca, Ac or Pr disappearance was expressed as the difference between the ratio Ca/PEG, Ac/PEG or Pr/PEG in the baseline sample (0 min) and the total colonic sample collected at 30 min. The percent Ca, Ac or Pr disappearance was then converted to mmoles by multiplying the percent disappearance by the amount of Ca, Ac or Pr present in the distal colon at baseline (0 time) in mmoles.

7.3 Results

All solutions were retained by the subjects for 30 min without difficulty. The mean total volume of infused solution recovered was 159 mL (53% of the total volume infused) and there were no significant differences among treatments or among subjects. Ca absorption was affected significantly by Ca concentration and the presence of Ac and Pr (Figure 7.1). For the control (Ca+NaCl) treatment, Ca absorption increased linearly as the concentration of Ca increased ($r=0.995$, $P<0.005$). The slope of the line was 67 umoles/30min/mM Ca (Figure 7.1; Table 7.2). When Ac was added, Ca absorption was significantly greater than the control at all concentrations of Ca. Ca absorption with Ac increased linearly with Ca concentration ($r=0.999$; $P<0.0003$; Figure 7.1) and the slope

Table 7.1. Composition of the different treatments.

Treatment	Ca[1]	Ac[2]	Pr[3]	NaCl[4]
	mmol/L			
NaCl	-	-	-	56.3
Ca+NaCl	4	-	-	56.3
	11	-	-	56.3
	24	-	-	56.3
	46	-	-	56.3
Ca+Ac	4	56.3	-	-
	11	56.3	-	-
	24	56.3	-	-
	46	56.3	-	-
Ca+Pr	4	-	56.3	-
	11	-	56.3	-
	24	-	56.3	-
	46	-	56.3	-

[1] Calcium chloride ($CaCl_2.2H_20$), Fisher Scientific, Nepean, Ontario, Canada.
[2] Sodium acetate, NaAc, anhydrous, Sigma S-8750, St Louis, Missouri, USA.
[3] Sodium propionate, NaPr, Food Grade, Van Eaters and Roger Ltd, Toronto, Ontario, Canada.
[4] Sodium Chloride, NaCl, AR, British Drug House, Darmstadt, West Germany.
All treatments contain 0.625 mM polyethylene glycol (PEG 4000), Mallinckrodt OR, Pointe-Claire, Quebec, Canada.

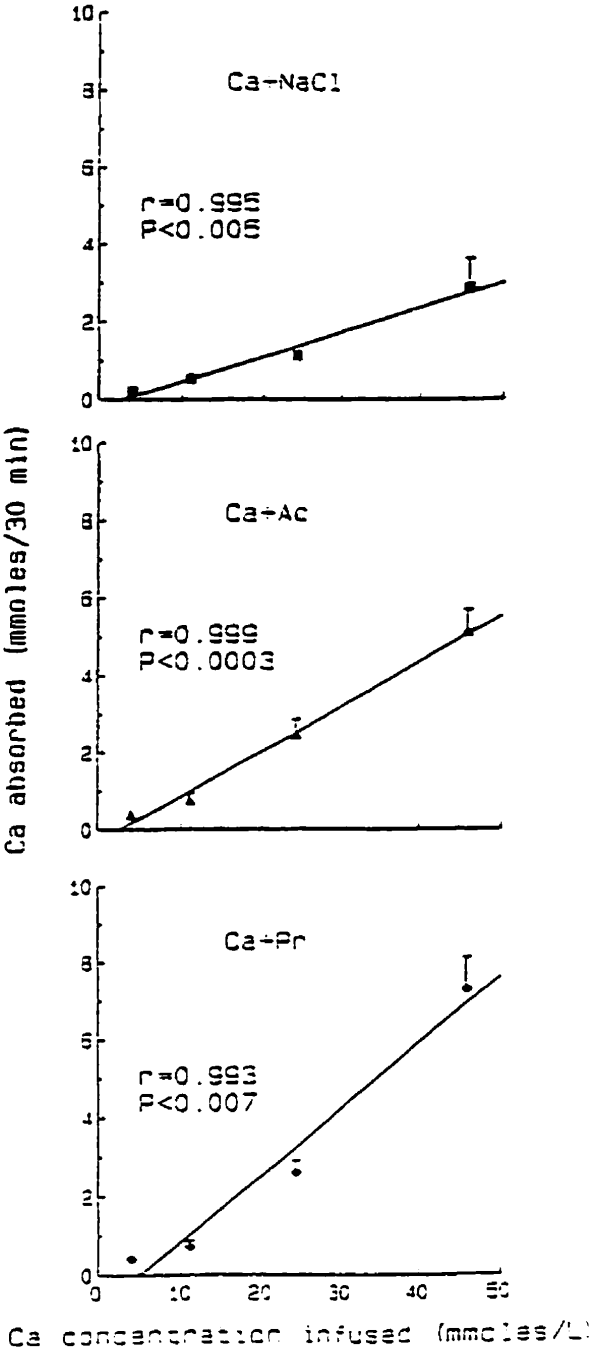


Figure 7.1. Relationship between calcium absorption and calcium concentration in the human distal colon in the presence of sodium chloride, 56 mmol/L acetate and 56 mmol/L propionate. Values are means±SEM.

Table 7.2. Ca absorption at different concentrations of Ca.

Ca*	Treatment		
	Ca+NaCl	Ca+Ac	Ca+Pr
	mmol/30 min		
4	0.20 ± 0.04^{aw}	0.38 ± 0.07^{bw}	0.41 ± 0.08^{bw}
11	0.52 ± 0.08^{ax}	0.75 ± 0.20^{bx}	0.72 ± 0.16^{bx}
24	1.13 ± 0.12^{ay}	2.45 ± 0.40^{by}	2.59 ± 0.31^{by}
46	2.86 ± 0.76^{az}	5.12 ± 0.56^{bz}	7.28 ± 0.83^{cz}

*Actual Ca concentration infused in mmol/L.
abc represents significant differences between treatments in a row at $P<0.05$.
wxyz represents significant differences between Ca concentrations in a column at $P<0.05$.

of the regression line was 123 umoles/30 min/mM Ca. Ca absorption with Pr was greater than that with Ac but the difference only reached significance at 46 mM Ca (Table 7.2; $P<0.05$). With Pr, Ca absorption also increased linearly with Ca concentration ($r=0.993$; $P<0.007$) and the slope of the regression line was 179 umoles/30min/mM Ca. The difference with Ac only approached significance ($P<0.06$).

Ac absorbed at different concentrations of Ca was almost constant from 7 to 24 mM Ca and increased significantly at 46 mM Ca ($P<0.05$) while Pr absorption increased significantly from 4 to 24 mM Ca ($P<0.05$) and reached a plateau at 46 mM Ca (Figure 7.2; Table 7.3). Pr absorption was significantly greater than that of Ac from 11 to 46 mM Ca (Table 7.3).

The pH of each treatment was not controlled. For Ca+NaCl and at 4-24 mM Ca, the pH of the infused solution significantly increased at time 0 and levelled off at 30 min (Table 7.4; $P<0.05$). Similar results were obtained for Ca+Pr at 4-24 mM Ca and for Ca+Ac at 4, 11 and 46 mM Ca. At 24 mM Ca, the pH of the infused solution for Ca+Ac and at 46 mM Ca for Ca+NaCl significantly increased at all times while at 46 mM Ca for Ca+Pr, no significant change in pH was observed at any time.

7.4 Discussion

This study showed that Ca absorption in the human distal colon is directly related to concentration of Ca, either alone or in the presence of Ac or Pr suggesting that a non-saturable diffusion process is involved. Ca may be absorbed via a paracellular pathway in the distal colon because the concentration of the Ca binding protein which is

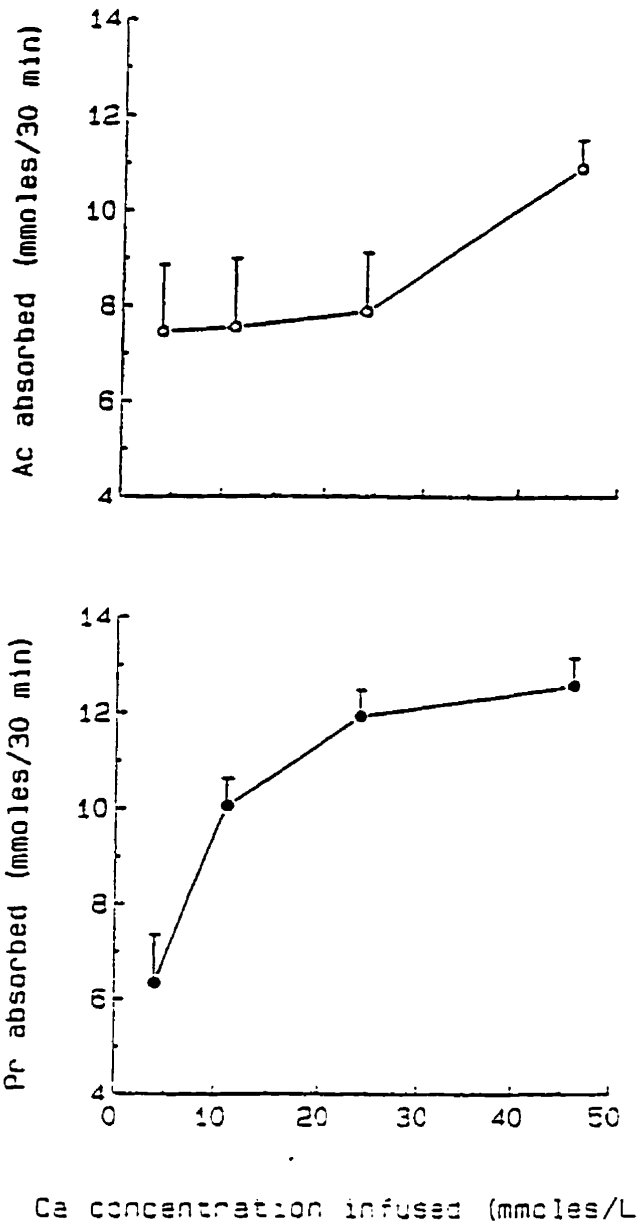


Figure 7.2. Acetate and propionate absorption from the human distal colon after infusion of calcium plus acetate and calcium plus propionate at different concentrations of calcium. Values are means±SEM.

Table 7.3. Ac and Pr absorption at different concentrations of Ca.

Ca*	Treatment	
	Ca+Ac	Ca+Pr
	mmol/30 min	
4	7.46 ± 1.40^{ax}	6.34 ± 1.01^{ax}
11	7.56 ± 1.43^{ax}	10.05 ± 0.57^{by}
24	7.88 ± 1.23^{ax}	11.92 ± 0.55^{bz}
46	10.90 ± 0.59^{ay}	12.57 ± 0.58^{bz}

*Actual Ca concentration infused in mmol/L.
ab represents significant differences between treatments in a row at $P<0.05$.
xyz represents significant differences between Ca concentrations in a column at $P<0.05$.

Table 7.4. pH of the infused solution and recovered infusate

Treatment	Time	Ca concentration (mmol/L)			
	(min)	4	11	24	46
Ca+NaCl	soln infused	$5.3\pm.01^{ax}$	$5.0\pm.10^{ay}$	$4.9\pm.04^{ay}$	$4.9\pm.02^{ay}$
	0	$8.1\pm.10^{bx}$	$7.9\pm.10^{bx}$	$7.7\pm.20^{bx}$	$6.0\pm.20^{by}$
	30	$8.2\pm.10^{bx}$	$8.0\pm.10^{bx}$	$8.0\pm.30^{bx}$	$7.2\pm.06^{cy}$
Ca+Ac	soln infused	$7.0\pm.02^{ax}$	$7.0\pm.03^{ax}$	$6.9\pm.01^{ax}$	$7.0\pm.05^{ax}$
	0	$8.5\pm.10^{bx}$	$7.9\pm.02^{bz}$	$7.9\pm.01^{bz}$	$8.2\pm.08^{by}$
	30	$8.3\pm.10^{bx}$	$8.0\pm.01^{bxy}$	$8.2\pm.07^{cx}$	$7.8\pm.10^{by}$
Ca+Pr	soln infused	$7.4\pm.03^{ax}$	$7.3\pm.02^{ax}$	$7.2\pm.08^{axy}$	$7.1\pm.03^{ay}$
	0	$8.1\pm.08^{bx}$	$8.1\pm.02^{bx}$	$8.2\pm.20^{bx}$	$7.6\pm.10^{ay}$
	30	$8.1\pm.09^{bx}$	$8.2\pm.07^{bx}$	$8.2\pm.08^{bx}$	$7.5\pm.30^{ay}$

abc indicates significant differences between time in a column per treatment at $P<0.05$.
xyz indicates significant differences between concentrations in a row at $P<0.05$.

responsible for the active transport (transcellular pathway) of Ca in the small intestine decreases towards the lower gut (Ambretch, 1987). It was speculated from the previous study (Chapter 6) that Ca is absorbed by a Ca/H exchange process in the distal colon. The colonic mucosa contains carbonic anhydrase which catalyzes the formation of hydrogen and bicarbonate ions from carbon dioxide and water (Figure 7.3a). These substrates are derived by diffusion of carbon dioxide from the blood (Rechkemmer, 1994). In this way intracellular hydrogen can be generated for a Ca/H exchange. However, the activity of carbonic anhydrase declines toward the distal part of the colonic mucosa (Carter and Parsons, 1970 and 1972; Charney et al, 1986; Lonnerholm, 1977). The present results showed that Ca is absorbed in the distal colon and that the rate of absorption is significantly greater in the presence of Ac or Pr. The presence of Ac or Pr may have enhanced the production of hydrogen needed by Ca to be absorbed (Figure 7.3b).

Intestinal SCFA transport is a linear, concentration dependent process whereby the flux rate is in direct proportion to the SCFA concentration (Bugaut, 1987; Fleming et al, 1991; Rechkemmer and Engelhardt, 1988; Rimmer and Wiebe, 1987). Non-ionic diffusion occurs when SCFA is protonated from either the luminal hydration of carbon dioxide (CO_2) to form bicarbonate (HCO_3^-) and hydrogen ion (H^+), or from secretion of H^+ produced intracellularly from the hydration of CO_2 to form HCO_3^- into the lumen (Cummings, 1981; Engelhardt and Rechkemmer, 1983; Fleming et al, 1991; Rechkemmer and Engelhardt, 1988). The intracellular H^+ subsequently facilitates the continued diffusion of SCFA (Stevens, 1986). Since the pKa values of Ac and Pr are approximately

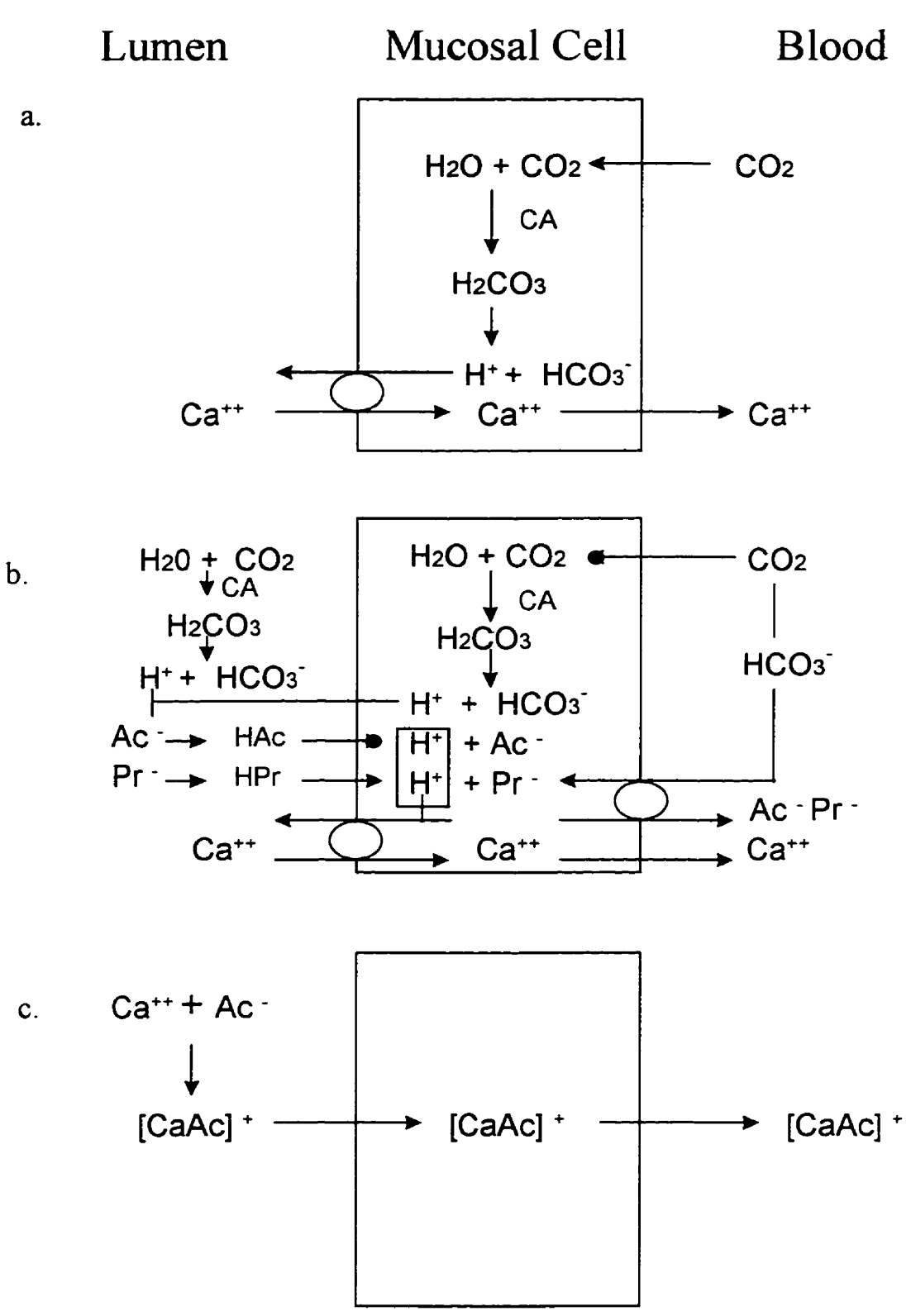


Figure 7.3. Proposed models for calcium absorption in the human distal colon.

4.75 and 4.87, respectively, and the physiological pH of the cell is around 6.5-7 (Titus and Ahearn, 1992), the SCFA that diffuses into the cell dissociates and liberates H^+ which is secreted out of the cell. This H^+ may then be available for exchange with any cation such as Ca or may again protonate another SCFA. Another mechanism whereby SCFA is absorbed is by a carrier-mediated saturable anionic exchange mechanism in which SCFA anion is exchanged for bicarbonate (HCO_3^-) (Binder and Mehta, 1989; Fleming et al, 1991; Harig et al, 1987; Mascalo et al, 1990; Ruppin et al, 1980; Titus and Ahearn, 1992). The presence of HCO_3^- in the colonic lumen in the present study was indicated by the immediate increase of pH of the different treatment solutions after infusion into the rectum of subjects (Table 7.4). There is evidence that HCO_3^- appearance in the colonic lumen is enhanced by the presence of SCFA which contribute to the regulation of the luminal pH (Rechkemmer, 1994).

The present study showed that at lower concentrations of Ca (4-24 mmol/L), Ac absorption was almost constant while Pr absorption increased with Ca concentration (Figure 7.2). At higher concentrations of Ca (24-46 mmol/L), Ac absorption increased while Pr absorption reached a point of saturation (Figure 7.2). This result suggests that Ca stimulated Ac and Pr absorption by two different transport mechanisms, a saturable process which involves a carrier-mediated anion exhange and a non-saturable non-ionic diffusion process similar to the ones previously proposed. However, whether Ca stimulated Ac or Pr absorption by a saturable or a non-saturable process, the linear relationship between Ca absorbed and Ca concentration in the presence of Ac or Pr was not affected (Figure 7.1). This is probably due to the fact that bicarbonate production in

the mucosal cell builds up protons (H^+) which are associated with the Ca/H exchange process (Figure 7.3 a and b).

Several investigators (Argenzio et al, 1975; Gasaway, 1976; Miller and Wolin, 1979; Murer et al, 1984; Rechkemmer et al, 1988) have shown that SCFA absorption is independent of the overall pH of the colonic lumen due to a constant pH microclimate at the surface of the colonic epithelium. This condition is responsible for a steady concentration of H^+ ions available for association with the luminal SCFA anions which may then be absorbed into the cell. This study showed that the pH of the infused solution did not account for the increase in Ca absorption. The treatment containing Ca+NaCl had the lowest pH and had also the lowest Ca absorbed. However, the pH of the treatments containing Ca+Ac and Ca+Pr did not differ, and Ca+Pr resulted in a significantly greater Ca absorption than Ca+Ac at the highest concentration of Ca ($P<0.05$). Although a steady state condition or chemical interaction that would precipitate Ca in the distal colon was not specifically investigated in this study, the constant pH microclimate may account for the possibility of Ca to remain in solution. Therefore, Ca disappearance can be a measure of Ca absorption in the colon.

This study showed that at higher concentrations of Ca, Ca absorption from the treatment with Pr is significantly greater than that with Ac ($P<0.05$). This is consistent with the results from the previous study (Chapter 6), and is probably due to the fact that Pr is more lipid soluble than Ac (Engelhardt, 1995). Thus, Pr is expected to diffuse faster across the colonocyte membrane. The slope of the regression line was significantly greater in the treatments with Ac or Pr in comparison to the treatment with Ca alone

($P<0.05$). The difference between the slopes of the regression lines for the Ac and Pr treatments approached significance ($P<0.06$) indicating that there is a tendency for Ca to be absorbed faster in the presence of Pr than Ac (Figure 7.1).

The formation of a stable $[CaAc]^{+}$ or $[CaPr]^{+}$ complex (Nancollas, 1976) may also be an important mechanism of Ca absorption in the distal colon (Figure 7.3c). Cell membranes have low permeability to highly charged ions [Ca^{++}] and, therefore, it is likely that the less charged complex ion passes more readily through the cell membrane (Marshall, 1976). However, the affinity of the complex over the protonated Ac (HAc) or Pr (HPr) in passing through the colonocyte membrane is unknown. This study does not have enough evidence to support this mechanism.

In conclusion, Ca absorption from the human rectum and distal colon was linearly related to Ca concentration in the presence and absence of Ac and Pr indicating that it involves a non-saturable diffusion process at least over the range of concentrations studied. Ac and Pr are also absorbed by transport mechanisms influenced by Ca. This study is the first to show the kinetics of the interactive effects of Ca and Ac or Pr.

CHAPTER 8

THE EFFECT OF UNABSORBABLE CARBOHYDRATE ON CALCIUM ABSORPTION FROM DAIRY PRODUCT IN HUMANS

8. THE EFFECT OF UNABSORBABLE CARBOHYDRATE ON CALCIUM ABSORPTION FROM DAIRY PRODUCT IN HUMANS

8.1 Introduction

In Chapter 4, the in vitro study showed that fermentable fiber such as pectin reduced the release of Ca under the small intestinal conditions but released a significant amount of Ca after fermentation while there was little or no effect on total Ca availability. In chapters 5 to 7, a series of rectal infusion studies demonstrated that Ca is absorbed in the rectum and distal colon and that SCFA stimulate Ca absorption. To support all of the above studies, this study determined, in humans, the influence of pectin on Ca absorption from milk under physiological conditions using two different stable isotopes of Ca: an intravenously administered isotope (^{42}Ca) to allow determination of the disappearance of Ca from the plasma compartment and an orally administered Ca isotope (^{44}Ca) to allow determination of Ca absorption. It is assumed that the early rise in Ca absorption denotes small intestinal absorption while a later rise in Ca absorption denotes colonic absorption.

8.2 Materials and Methods

8.2.1. Subjects

Five healthy female subjects (30±3.0 y; 55.9±2.6 kg) who could tolerate the test meal (less stable isotope) and were not lactose intolerant were studied. Lactose intolerance was determined using the breath hydrogen test. Alveolar breath samples were collected at 0, 30, 60, 90, 120, 180 and 240 min after the test food (66.1 g skimmed milk powder in 250

mL of double distilled water containing 800 mg Ca) by having the subjects exhale down a 1 m long rubber tube attached to a 20-mL syringe. Breath hydrogen was analyzed using a Quintron Microlyzer (Model DP, Quintron Instruments Co., Inc., Milwaukee, WI) and values rising over 20 ppm after the test food was taken indicated carbohydrate malabsorption, i.e. lactose intolerance.

8.2.2. Study Design

The subjects were given a standard low fiber meal (spaghetti and sauce) the night before the study. After an overnight fast (12 h; no solid foods and no liquids allowed), subjects were then given an intravenous injection of ^{42}Ca (0.1 mg/kg body weight). Immediately after, a test meal containing 50 g instant skimmed milk powder dissolved in 250 mL of double distilled water labelled with ^{44}Ca (0.3 mg/kg body weight) with or without 20 g pectin and 60 g of white bread was provided. ^{44}Ca was added to milk and allowed to equilibrate for at least 12 hours at 4°C prior to administration. Double distilled water, i.e. water without minerals (250 mL) was provided with the meal. The total Ca content of the meal was 654±0.4 mg for milk alone and 661.8±0.6 mg for milk plus pectin. Seven mL blood samples were taken at 0, 0.75, 1.5, 2.25, 3, 5, 8, 10, 12, 24, 34 and 48 hours after the meal. Lunch consisting of turkey, salmon, or strawberry jam sandwich, apple juice, and cookies was given after the 5th hour. After the 12th hour, a low fiber dinner consisting of spaghetti and sauce was given. Subjects were fasted overnight for the 24th and 48th hour blood collection. The subjects ate their usual meals after the 24th hour but were asked to record their food intakes up to the 48th hour blood

collection. The total amount of blood collected from each subject was 168 mL which is about 1/3 that of the normal volume when donating blood. Urine was collected and pooled by the subjects as follows: 0-3, 3-5, 5-8, 8-12 hours. After the 12-hour collection, urine samples were taken separately and labelled with the time of collection.

8.2.3. Isotope Preparation and Analytical Techniques.

Stable isotopes of Ca, ^{42}Ca and ^{44}Ca, were obtained from the Trace Sciences International Corporation (Richmond Hill, Ontario, Canada), as the carbonates. ^{42}Ca was obtained at 93% and ^{44}Ca at 97% enrichment.

Sterile samples were prepared at ~ 3 mg/mL ^{42}Ca or 6 mg/mL ^{44}Ca at the St. Michaels Hospital, Pharmacy Department, Toronto, Ontario, Canada. The carbonate salts were dissolved in 12N hydrochloric acid (HCl; Baker Analyzed, J.T.Baker Chemical Company, Phillipsbury, NJ, USA) and 10 mL of 0.45% NaCl (isotonic saline for injection) for ^{44}Ca or 1 mL of 0.90% NaCl for ^{42}Ca was added and mixed well. Benzyl alcohol (1%; J.T.Baker Chemical Company) was added as a preservative. The pH of the solution was determined and adjusted to pH 5.5 using 1N sodium hydroxide (NaOH; Fisher Scientific Inc., Springfield, NJ, USA). The solution was made to final volume gravimetrically (density=1g/mL) with 0.45% (^{44}Ca) or 0.9% (^{42}Ca) saline solution. The resulting solution was filtered using a sterile BD 60 cc syringe with a Millex GV sterile non-pyrogenic filter (Millipore Products Division, Bedford, MA, USA). Five mL aliquots of the solution were placed in a pre-sterilized 5-mL clear siliconized vials obtained from the National Institute of Health (NIH), Bethesda, Maryland, USA. A portion of the aliquot solution was tested

for sterility at St Michael's Hospital, Toronto, Ontario, Canada and for pyrogenicity at the Associate of Cape Cod Inc., MA, USA before use.

Ca for isotope ratio analysis was extracted from serum and urine, using an oxalate precipitation technique described by Yergey et al (1980;1990). Five to 6 drops of Ultrex ammonium hydroxide (J.T. Baker Co.) was added to 1-mL of serum or 5-mL of urine in an acid washed centrifuge tube and mixed. The pH of the solution was adjusted to pH 9-10 using pH paper. A saturated solution of ammonium oxalate (pH 9.4; J.T.Baker Co.) was added to the serum (1.5 mL) and urine (5 mL) solution. The resulting solution was allowed to stand overnight. The next day the solution was centrifuged at 2000 rpm for 10 min. The precipitate was dried in a furnace first at 100°C for 30 min, then at 150°C for 15 minutes and finally 500°C for 2 hr. After drying, the oxalate salt was dissolved in 100 uL nitric acid (Ultrex, J.T.Baker Chemical Co.). The samples were transferred to polypropylene microfuge tubes and sent to NIH for thermal ionization mass spectrometric analysis of ^{42}Ca and ^{44}Ca. A Finnigan MAT, Thermoquad THQ Mass Spectrometer (Finnigan MAT, San Jose, California, USA) was used in the dual filament mode for all measurements. A 5 uL sample droplet (approximately 10 ug Ca) was loaded onto the "evaporation' filament and dried in two stages. The first stage used 1.5 amps for approximately 45 sec to remove the water from the solution. This yields a white crystalline precipitate in the center of the filament. The second stage required 1.9 amps for approximately 1 sec during which the filament glows a dull red and the previously white precipitate changes to a pale yellow-green precipitate which firmly adheres to the filament. The sample filaments are loaded onto a 13 sample magazine which is placed

inside the THQ Mass Spectrometer for analysis. Ions are produced by heating the uncoated 'ionization' filament to 3.5 amps and the samples coated 'evaporation' filament to approximately 2 amps. Under these circumstances, about 4 x 10^{-7} amps ^{44}Ca were observed using the electron multiplier detector. Measurements were made under computer control, first by adjusting the physical position of the sample magazine and then by optimizing the lens potentials in the ion source. Ion signals for all Ca isotopes except ^{40}Ca were then measured at the center of the peak tops for fixed times. The order of measurement and fixed sampling times were: 4,4,4,8,4 sec. for ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca and ^{48}Ca, respectively. Isotope ratios were calculated for each scan relative to ^{48}Ca and corrected for fractionation by using the deviation of $^{43}Ca/^{48}Ca$ ratio from natural abundance. This correction allows one to compensate for the differential rates of isotope evaporation from the filament, and the observed $^{43}Ca/^{48}Ca$ ratio was typically within 1% of the natural abundance ratio. The controlling software averaged sets of 10 scan ratios and a typical filament was used to obtain 2 sets of 10 scan blocks. Interscan, interblock and interfilament reproducibility were typically within 1.5% of each other. Typical measurement time for 2 of the 10 scan blocks is about 10 min.

Food, serum and urine were analyzed for total Ca by atomic absorption spectrometry as described in the general methods (chapter 3).

8.2.4. Data Calculation and Statistical Analysis.

8.2.4.1. Calculation of Δ% excess of natural abundance isotope ratios.

$$\Delta\% \text{ excess} = \frac{100 \times (\text{observed ratio} - \text{natural abundance ratio})}{\text{natural abundance ratio}}$$

$$\Delta\% \text{ excess for } {}^{42}Ca = \frac{100 \times (\text{observed } {}^{42}Ca/{}^{48}Ca - \text{na } {}^{42}Ca/{}^{48}Ca)}{\text{na } {}^{42}Ca/{}^{48}Ca}$$

$$\Delta\% \text{ excess for } {}^{44}Ca = \frac{100 \times (\text{observed } {}^{44}Ca/{}^{48}Ca - \text{na } {}^{44}Ca/{}^{48}Ca)}{\text{na } {}^{44}Ca/{}^{48}Ca}$$

na = natural abundance

na $^{42}Ca/^{48}Ca$, ratio of natural abundance = 3.4276

na $^{44}Ca/^{48}Ca$, ratio of natural abundance = 11.0341

8.2.4.2. Calculation of Fractional Ca Absorption.

Fractional Ca absorption was calculated using α_{24} as follows (Yergey et al, 1994; Gibaldi and Perries, 1982):

$$\alpha_{24} = \frac{\text{AUC (oral administration recovered in urine)}}{\text{AUC (intravenous administration recovered in urine)}}$$

where AUC = area under the curve [fraction of dose] from 0-24 hours.

The oral and intravenous label were calculated from the Δ% excess of ^{42}Ca and ^{44}Ca described above.

8.2.4.3. Calculation of percent completion of Ca absorption showing comparisons between milk and milk plus pectin at different times.

Fractional Ca absorption data (urinary cumulative Δ % excess ratio of $^{44}Ca/^{42}Ca$) were pooled as follows: 0-3, 3-5, 5-8, 8-12, 12-24, 24-36 and 36-48 hours. The data were normalized as a percentage of the highest fractional Ca absorption obtained (12-24th hour for milk and 24-36th hour for milk plus pectin). The incremental differences of absorption for each time from the two treatments were determined to differentiate the Ca

absorbed in the small intestine and colon.

8.2.4.4. Statistical Analysis

Results were expressed as mean±SEM. Differences in Ca absorption between treatments and time were determined by a two-way repeated measures General Linear Model and Tukey's Studentized Range Test using Statistical Analysis System (SAS Institute Inc., Cary, NC).

8.3. Results

The mean age, weight and height of the subjects are provided in Table 8.1. The Ca intakes of all subjects were 654±0.5 and 661±0.6 mg Ca for milk and milk plus pectin, respectively, with an oral ^{44}Ca and intravenous ^{42}Ca dose of 16.8±0.8 and 5.6±0.3 mg Ca, respectively (Table 8.1). The fractional Ca absorption, α_{24}, estimated from the urine for all subjects from milk (0.15±.02) was significantly greater than that from milk plus pectin (0.11±.01; $P<0.05$; Table 8.2). The Δ % excess of ^{42}Ca and ^{44}Ca did not differ significantly between treatments except for 0-3 hours for the urinary ^{44}Ca data and 0.75 hour for the serum ^{44}Ca data ($P<0.05$; Table 8.3). The curve of ^{42}Ca for both treatments exhibited an exponential decay with R^2 of 0.990 and 0.978 for milk and milk plus pectin, respectively, for the urinary data and 0.993 and 0.996 for milk and milk plus pectin, respectively for the serum data (Figure 8.1). The Δ % excess of ^{44}Ca for the urinary data for milk alone showed a peak at 3-5 hours and plateaued at 5-24 hours and slowly went down until 36-48 hours (Figure 8.2). Milk plus pectin showed a peak at 5-12 hours and

Table 8.1. Calcium intake of subjects (n=5).

Subject	Age	Weight	Height	Calcium intake (mg)		Oral Dose	IV Dose
	y	kg	m	Milk	Milk+Pectin	mg ^{44}Ca	mg ^{42}Ca
MA	38	48.6	1.65	655.4	662.4	14.58	4.86
RK	21	55.7	1.62	654.6	662.4	16.71	5.57
LO	31	58.0	1.70	654.6	659.2	17.40	5.80
SH	26	53.0	1.62	653.0	662.4	15.90	5.30
CM	34	64.0	1.65	654.6	662.4	19.20	6.40
Mean	30	55.9	1.65	654.4	661.8	16.76	5.59
±SEM	±3.0	±2.6	±.01	±.40	±.60	±.77	±.26

Table 8.2. Fractional absorption of dietary calcium determined by α_{24} (n=5).

Subject	Milk	Milk + Pectin
MA	0.13	0.11
RK	0.17	0.10
LO	0.17	0.11
SH	0.10	0.09
CM	0.18	0.13
Mean±SEM	0.15±.02	0.11±.01*

*Significant differences between treatments at P<0.05

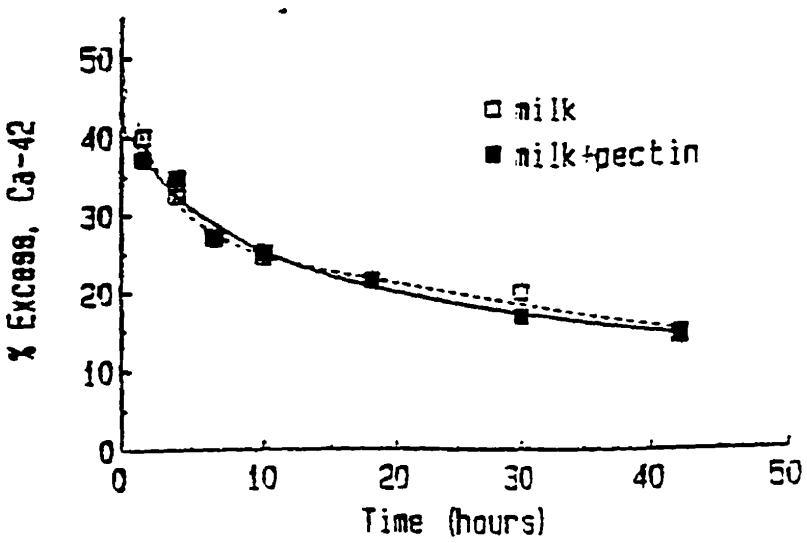
Table 8.3. Means±SEM Δ % excess for urinary and serum ^{42}Ca and ^{44}Ca from milk and milk plus pectin for all subjects (n=5).

	Milk		Milk plus Pectin	
Time (hr)	^{42}Ca	^{44}Ca	^{42}Ca	^{44}Ca
Urinary				
0-3	40.0±0.3^{a}	2.0±0.3^{d}	37.1±2.8^{a}	0.3±0.2^{d}*
3-5	32.5±2.2^{b}	3.7±1.1^{a}	34.8±2.4^{a}	2.5±0.2^{b}
5-8	26.8±2.3^{c}	3.4±0.9^{b}	26.9±2.1^{b}	3.3±0.3^{a}
8-12	24.5±1.9^{cd}	3.4±0.7^{b}	25.0±0.5^{b}	3.3±0.5^{a}
12-24	21.2±0.7^{de}	3.4±0.4^{b}	21.4±0.8^{c}	2.9±0.3^{ab}
24-36	19.9±1.0^{e}	2.9±0.4^{c}	16.8±0.7^{d}	2.2±0.3^{bc}
36-48	14.3±0.4^{f}	2.7±0.6^{c}	14.8±0.4^{e}	1.9±0.7^{c}
Serum				
0.75	48.1±2.8^{a}	0.3±0.2^{e}	46.6±2.2^{a}	1.2±0.7^{e}*
1.50	38.4±1.2^{b}	2.0±0.5^{d}	40.3±3.6^{a}	1.6±0.2^{d}
2.25	34.8±0.9^{b}	2.8±0.6^{bc}	35.2±2.7^{b}	2.7±0.3^{c}
3.00	32.8±1.0^{b}	4.1±0.5^{a}	32.3±1.9^{bc}	3.1±0.3^{b}
5.00	28.4±0.5^{c}	5.0±1.0^{a}	29.1±2.4^{c}	3.6±0.4^{a}
8.00	23.8±0.6^{d}	3.7±0.6^{ab}	24.3±2.4^{d}	3.5±0.6^{a}
10.00	23.6±0.6^{d}	2.9±0.8^{bc}	24.0±1.8^{d}	3.2±0.2^{b}
12.00	22.6±0.9^{d}	3.6±0.6^{ab}	20.7±1.9^{de}	2.7±0.4^{c}
24.00	18.6±0.8^{e}	2.8±0.5^{bc}	19.6±1.5^{e}	1.7±0.7^{d}
34.00	15.5±1.1^{f}	2.4±0.8^{cd}	16.1±2.0^{e}	1.5±0.7^{d}
48.00	13.9±0.5^{g}	2.1±0.4^{d}	13.6±1.8^{f}	1.8±0.2^{d}

abcdefg signifies differences between time for each treatment in a column at $P<0.05$.

* significantly different between treatments in a row.

A.



B.

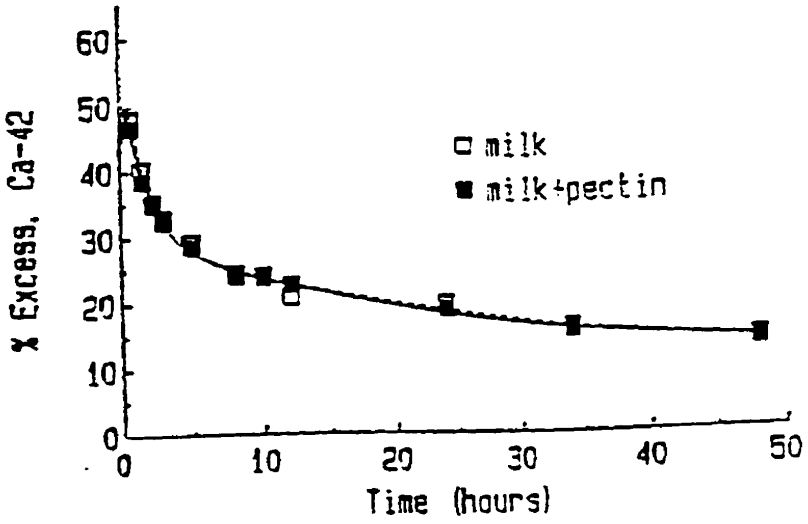
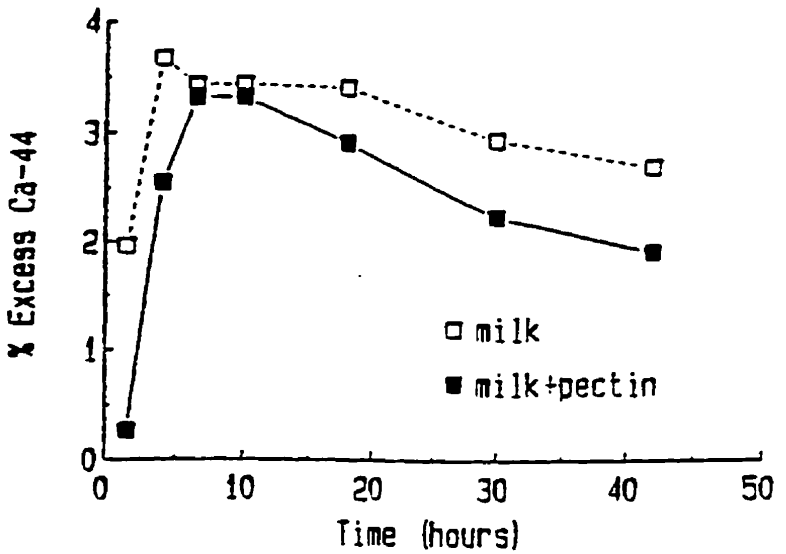


Figure 8.1. Urinary (A)and serum (B) Δ % excess of ^{42}Ca from milk and milk plus pectin at different times (n=5).

A.



B.

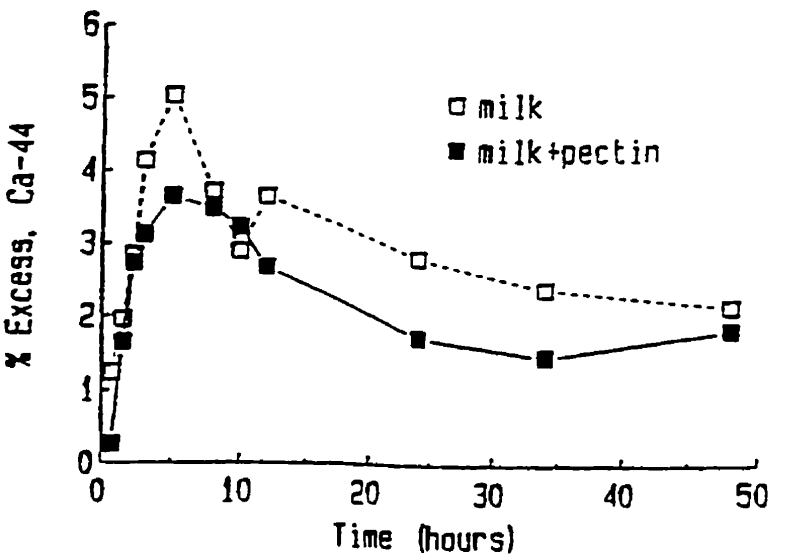


Figure 8.2. Urinary (A) and serum (B) Δ % excess of ^{44}Ca from milk and milk plus pectin at different times (n=5).

slowly went down at 36-48 hours. The Δ % excess for ^{44}Ca (serum data) showed two peaks for milk alone at 5 and 12 hours which did not differ significantly while the treatment with pectin showed a peak at 5-8 hours. The peak at 5 hours for milk and milk plus pectin did not differ significantly.

The fractional absorption data showed significant differences between the two treatments for all times ($P<0.05$; Table 8.4; Figure 8.3). To compare the pattern of absorption of the two treatments showing the small intestinal and colonic absorption of Ca, the percent incremental differences of completion of Ca absorption was calculated (Table 8.5). It was observed that Ca absorption from milk alone was greater than that from milk plus pectin at 0-3 hours while at the later hours that is 3-12 hours and 24-36 hours, Ca absorption from milk plus pectin was greater than that from milk alone (Figure 8.4). At 12-24 hours, Ca absorption from milk alone was greater than milk plus pectin.

Estimates of the small intestinal plus colonic absorption can be calculated from the above results. For milk alone, if the total fractional Ca absorption was 0.15 (Table 8.2) and the total Ca intake was 654.4 mg (Table 8.1), then the total Ca absorbed would be 98.2 mg. For milk plus pectin, if the total fractional Ca absorption was 0.11 and the total Ca intake was 661.8 mg, then the total Ca absorbed would be 72.8mg.

Considering previous studies (Haboubi et al, 1988; Jenkins et al, 1978; Magee and Dalley, 1986), and the pattern of incremental Ca absorption from the urinary data (Figure 8.4; Table 8.5) it can be assumed that 0-5 hours will denote small intestinal Ca absorption, 5-8 hours as either colonic absorption or colonic absorption plus lower small intestinal absorption, and 8-48 hours colonic Ca absorption. Thus the percent Ca absorbed

Table 8.4. Means±SEM fractional Ca absorption from milk and milk plus pectin at different times for all subjects (n=5).

Time	Milk	Milk plus Pectin
0-3	$0.0750\pm.0100^{c}$	$0.0280\pm.0007^{c}$ *
3-5	$0.0813\pm.0050^{c}$	$0.0480\pm.0008^{c}$ *
5-8	$0.1114\pm.0039^{bc}$	$0.0773\pm.0010^{bc}$*
8-12	$0.1310\pm.0037^{ab}$	$0.0943\pm.0100^{ab}$*
12-24	$0.1508\pm.0057^{a}$	$0.1017\pm.0038^{a}$*
24-36	$0.1489\pm.0040^{a}$	$0.1120\pm.0043^{a}$*
36-48	$0.1485\pm.0150^{a}$	$0.1110\pm.0175^{a}$*

abc signifies differences between time for each treatment in a column at $P<0.05$.

*significant differences between treatment in a row at $P<0.05$.

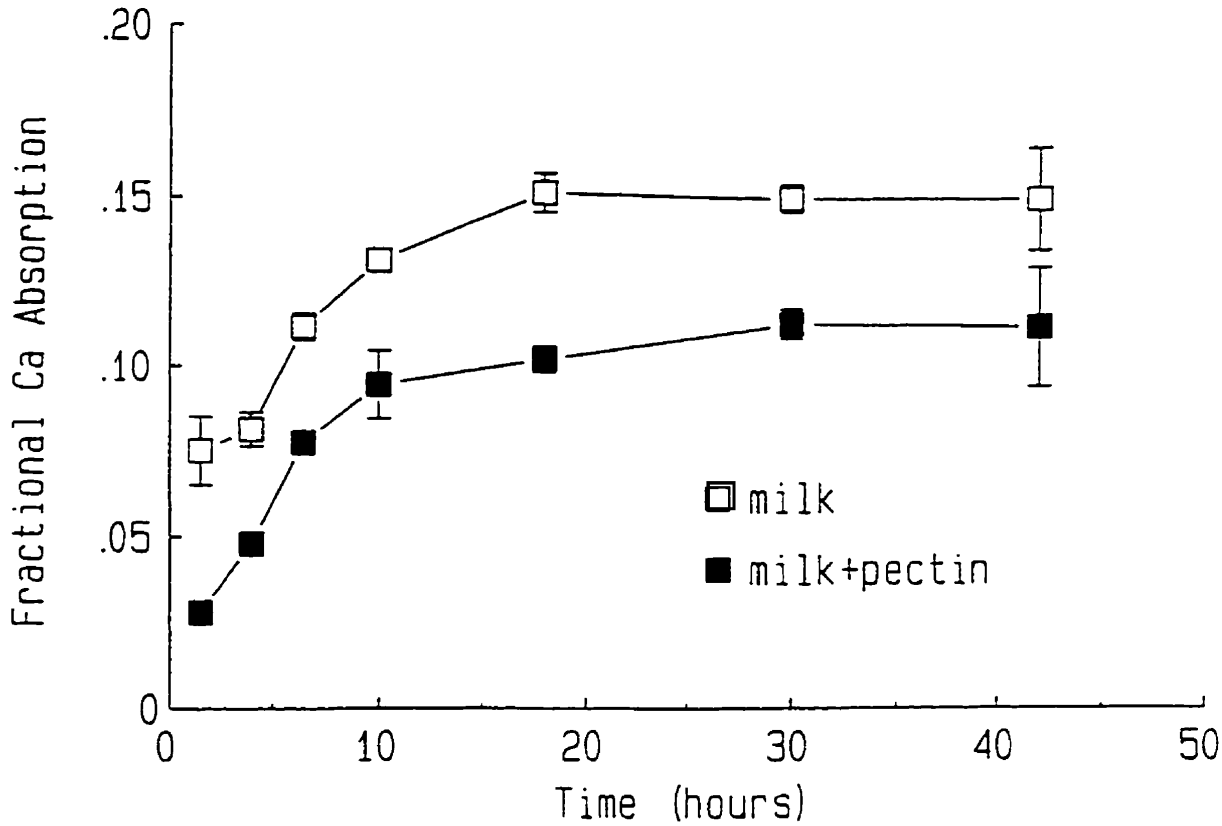


Figure 8.3. Fractional Ca absorption from milk and milk pectin at different times (n=5).

Table 8.5. Percent completion of fractional Ca absorption from milk and milk plus pectin for all subjects at different times (n=5).

	% Completion		Incremental Differences	
Time	Milk	Milk plus Pectin	Milk	Milk plus Pectin
0-3	49.7±1.8	25.0±0.2	49.7±1.8	25.0±0.2*
3-5	53.9±0.9	42.8±0.2	4.2±0.9	17.8±0.1*
5-8	73.9±0.7	69.0±0.2	20.0±0.2	26.2±0.1*
8-12	86.9±0.6	84.1±2.3	13.0±0.1	15.1±1.1
12-24	100.0±0.0	90.8±0.9	13.1±0.7	6.7±0.4*
24-36	98.7±0.7	100.0±0.0	-1.3±0.7	9.2±0.9*
36-48	98.5±2.6	99.1±1.1	-1.5±0.7	-0.9±0.4

*Significant differences in incremental differences calculated from % completion between treatments in a row at $P<0.05$.

Incremental difference is the difference between % completion of two different times i.e. $t_2 - t_1$ as in 53.9-49.7=4.2 (for milk).

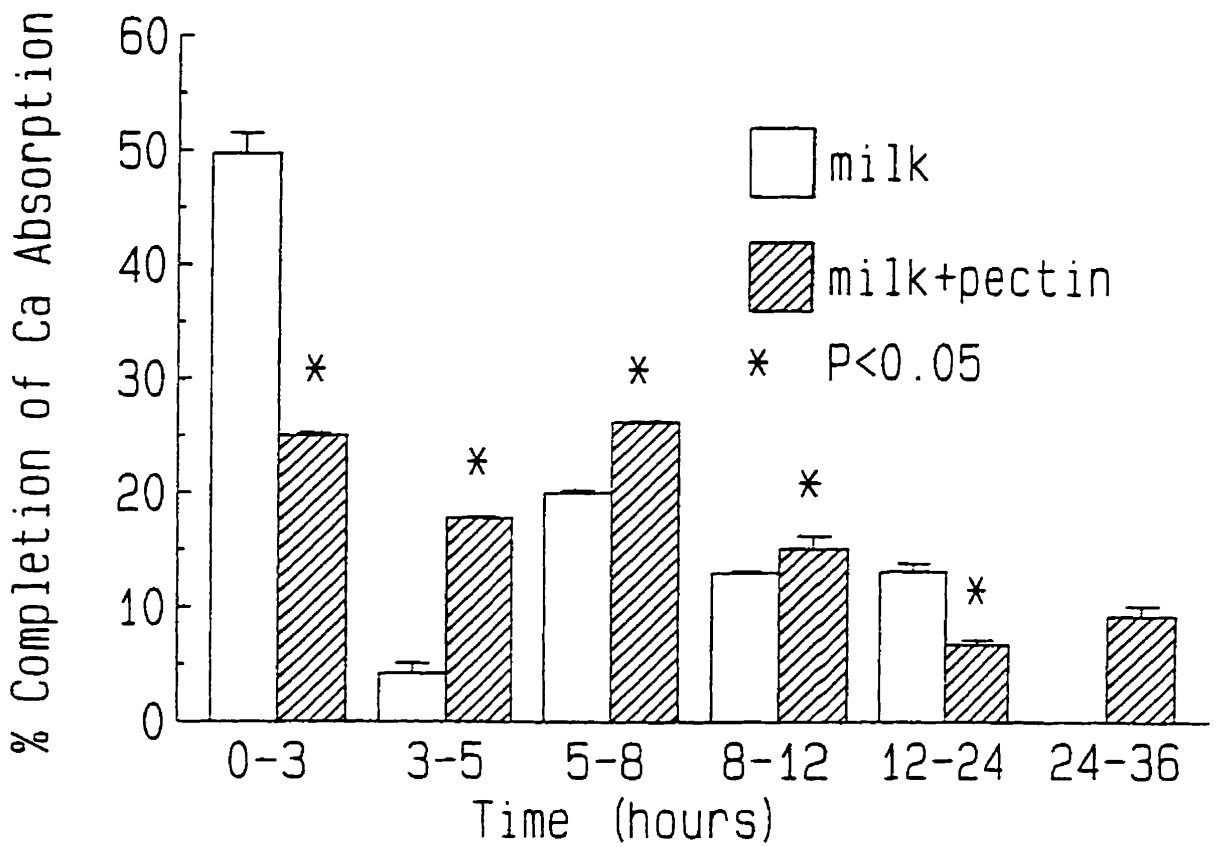


Figure 8.4. Percent incremental completion of fractional Ca absorption showing comparisons between milk and milk plus pectin at different times (n=5).

from milk could be 53.9 (i.e. 49.7+4.2; Table 8.5) and 46.1 for small intestine and colon, respectively. For milk plus pectin, the percent Ca absorbed would be 42.8 (i.e. 25.0+17.8; Table 8.5) and 57.2 for small intestine and colon, respectively. The absolute amount of Ca absorbed from milk would be 52.9 and 45.3 mg in the small intestine and colon, respectively, while from milk plus pectin, Ca absorbed would be 31.2 and 47.0 mg, respectively. If the 5-8 hours include absorption in the ileum, however, then the colonic component as estimated above may be overestimated.

8.4. Discussion

This study has clearly shown that fermentable fiber such as pectin reduced the total Ca absorption; pectin caused a greater amount of Ca released in the later time and is assumed to be absorbed in the colon.

Small intestinal transit time, from mouth to cecum, for a liquid meal was estimated to be around 2 hours for young healthy adults and 3 hours for healthy elderly adults (Haboubi et al, 1988). Jenkins et al (1978) has approximately the same estimation for a liquid meal (2 hours) but also observed a delay in transit time by 75, 30 and 15 min, respectively with dietary fibers such as guar, tragacanth and pectin. An average size meal was estimated to reach the terminal ileum in about 4 hours with most of the delays occurring in the colon and cecum (Magee and Dalley, 1986). This study used a liquid meal with and without pectin plus white bread, therefore, the estimated transit time may be similar to the studies described above, i.e. about 3-5 hours.

Ca from milk alone was absorbed rapidly during 0-3 hours and less rapidly at 3-5

hours. Addition of pectin to milk caused a reduction in Ca absorbed at 0-3 hours but a greater Ca absorption than from milk alone at 3-5 hours. This suggests that pectin slowed down the absorption of Ca in the upper small intestine probably due to its viscosity and/or binding with Ca to form an insoluble complex. An increase in Ca absorption was again observed at 5-8 hours for both treatments with values for milk plus pectin significantly greater than that for milk ($P<0.05$; Figure 8.4). As part of the meal may have entered the colon during the 5-8 hour period, the increased absorption for both treatments at this time period could be a consequence of Ca absorption that took place in the ileum and/or in the colon following fermentation of the pectin and lactose that reached the colon.

Lactose in milk could have enhanced Ca absorption at the 5-8 hour period (Ambretch, 1987; Buchowski and Miller, 1991; Miller, 1989). It has been shown to facilitate the paracellular transport of Ca in both proximal and distal small intestinal segments but more so in the ileum where active transport is negligible (Ambretch, 1987).

The lactose not hydrolyzed and absorbed in the small intestine and pectin may have been fermented in the colon and produced SCFA which influenced the absorption of Ca (Chapter 5-7). Previous studies have shown the production of significant amounts of Ac and Pr from fermentation of pectin (Chapter 4) and the enhancing effect of Ac and Pr on Ca absorption (Chapter 5-7). Although the assessment of the exact time Ca reaches the colon is difficult, the increase in Ca absorption observed at the 5-8 hours period and the slow extended release of Ca at the later time (12-24 hours for milk and 24-36 hours for milk plus pectin; Figure 8.4) may reflect fermentation and release of Ca for colonic absorption.

The use of stable and radioisotopes as tracers to measure fractional Ca absorption from foods/meals has been established (Yergey et al, 1994; Bronner et al, 1962). However, most studies have made assumptions that Ca is absorbed in the small intestine. A study on the time course of Ca absorption from milk in humans using radiolabelled Ca (oral/intravenous) showed an 80.9% completion of Ca absorption in 3 hours and 95.8% in 7 hours (Barger-Lux et al, 1989). The remaining 4.2% was absorbed in a slower late component which was completed by 23-26 hours. A different pattern of absorption was observed in this study. The difference in results may be attributed to sample size and nature of subjects in the study. This study was done in five normal premenopausal subjects while the above study was done in a population at risk for osteoporosis in 155 subjects, some of which were premenopausal or perimenopausal receiving estrogen or postmenopausal not receiving estrogen. According to the above investigators (Barger-Lux et al, 1989), absorption of Ca was higher in the estrogen-replete subjects. However, the fractional Ca absorption of milk obtained from the above study (0.156) for the low absorbers of Ca was similar to the ones obtained from this study (0.15). This value was also in the range of similar studies done on Ca absorption from milk (0.13 - 0.26; Recker et al, 1988; Smith et al, 1985).

According to results of the present study, pectin reduced the total (small intestine and colon) Ca absorption from milk by 27%. Our previous in vitro study did not show any significant reduction in Ca absorption from milk plus pectin or from the basal diet of milk, cheese, apple juice and white bread plus pectin (Table 4.1; Chapter 4). However, the in vitro study estimated the Ca released from the small intestine and colon

that could only be potentially absorbed. The unabsorbed Ca may have bound with other constituents in the colon such as bile acids and fatty acids to form soaps (Wargowich et al, 1984). Other investigators have shown that pectin did not alter total Ca absorption but provided no explanation for this lack of effect (Cumings, 1979, Kies, 1985; Munoz and Harland, 1993). The present study may have had a different effect on Ca absorption if pectin was fed for longer than a day.

In conclusion, this study has shown for the first time in humans that Ca in the presence of fermentable fiber such as pectin reduces the total Ca absorption but causes a significant amount of Ca assumed to be available for absorption in the colon.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSION

9. GENERAL DISCUSSION AND CONCLUSION

Dietary fiber is important in our diet but different sources of fiber can interfere with the absorptive processes in the small intestine and colon. Some fibers inhibit the absorption of minerals in the small intestine by forming insoluble complexes with the minerals or by entrapping them due to their viscosity. However, it is hypothesized that the fiber that reaches the colon is fermented and releases the minerals for potential absorption. Also, the product of fiber fermentation (SCFA) can in turn influence the absorption of these minerals.

Fermentable fibers such as pectin and guar gum and oligosaccharides such as lactulose did not affect the absorption of Ca (Cummings et al, 1979, Allen, 1982, Ohta et al, 1995). However, the role of SCFA on Ca absorption has not been fully investigated. Although studies in rats showed an enhancing effect of Ac and Bu on Ca absorption (Lutz and Scharrer, 1991) this has not been demonstrated in humans. In the presence of fiber, the amount of Ca that reaches the colon and gets absorbed is also unknown.

Thus, the focus of the present study was to determine using a variety of techniques, the amount of Ca that reaches the colon and whether Ca is absorbed there, particularly in the presence of SCFA. The first study used an all in vitro method which simulated the conditions that prevail in the small intestine and colon to estimate the amount of Ca release. The test was done on basal diets containing dairy products as well as single dairy foods in the presence and absence of unabsorbable carbohydrates such as lactulose, pectin, psyllium and cellulose. Results showed that except for lactulose, all of the above

carbohydrates reduced the release of Ca in conditions found in the small intestine by 16-27% (Table 4.1 and 4.3). The more fermentable pectin caused a significant increase in the proportion of Ca released in the colonic conditions (13-31%; Table 4.1 and 4.3) while the less fermentable psyllium and cellulose continued to bind with Ca. Thus, the in vitro method has shown that a large amount of Ca can be released in the small intestine but significant proportions of Ca can also be released after fermentation especially in the presence of fermentable fiber.

Very little is known regarding how much of the released Ca would actually be absorbed in the colon. Although Zn and Ca absorption in the colon has previously been reported to be 4% and 14%, respectively, the study was done in subjects whose colons were cleaned by an enema, i.e. undigested residues as well as SCFA were not present in the colon (Sandstrom et al, 1986). To further investigate the absorption of Ca in the human distal colon in the presence and absence of SCFA, a series of studies were done using the rectal infusion method. The first study determined the serum Ca response as an index of Ca absorption after infusion of two different concentrations of Ca and SCFA (a mixture of Ac and Pr in 3:1 molar ratio). The serum Ca response was significantly increased in the presence of SCFA suggesting increased colonic Ca absorption. However, the presence of Ca decreased the serum Ac response. Since serum Ca is an indirect measure of Ca absorption and also the SCFA used in this study was a mixture of Ac and Pr, the next study directly measured Ca absorption by estimating the Ca disappearance in the presence and absence of Ac or Pr from the rectum and distal colon with polyethylene glycol as an unabsorbable marker. Results supported the previous study i.e. Ac and Pr

enhanced Ca absorption. At higher concentration, Pr was more effective than Ac in enhancing Ca absorption. Ca alone (50 mmol/L) enhanced Ac absorption but, in the presence of Pr, Ac absorption was reduced (Figure 6.5). This is similar to the result obtained from the previous study; Ca reduced the serum Ac response in the presence of Pr (Chapter 5; Table 5.2). Similarly, Pr alone (18.7 mmol/L) enhanced Ac absorption. This showed that without Ca, competition between Ac and Pr absorption may not be possible.

The above findings addressed the need to investigate further the kinetics of Ca absorption in the presence and absence of Ac or Pr. Different concentrations of Ca with and without Ac or Pr were rectally infused in human subjects. Results showed that Ca absorption was linearly related to Ca concentration with and without Ac or Pr indicating a non-saturable diffusion transport process involving a Ca/H exchange mechanism similar to the ones suggested by Lutz and Scharrer (1991). Also, this study showed that Ca stimulated the absorption of Ac and Pr when they were present at higher concentrations (56.3 mmol/L). In the presence of Ca, Ac and Pr absorption probably reflected the sum of two absorptive mechanisms: (a) a carrier mediated saturable anion exchange process (Binder and Mehta, 1989; Fleming et al, 1991; Harig et al, 1987; Mascalo et al, 1990; Ruppin et al, 1980; Titus and Ahearn, 1992) with Ac at lower concentrations of Ca and with Pr at higher concentrations of Ca and (b) a non-saturable diffusion process (Bugaut, 1987; Fleming et al, 1991; Rechkemmer and Engelhardt, 1988; Rimmer and Wiebe, 1987) with Ac at higher concentrations of Ca and with Pr at lower concentration of Ca. Despite these mechanisms of Ac and Pr absorption at different concentrations of Ca, the linear

relationship between Ca absorption and Ca concentration was not affected. This was probably due to the fact that bicarbonate production which is involved in the saturable transport of Ac or Pr builds up protons (H^+) which are associated with the Ca/H exchange process (Figure 7.3). This study is the first to show the kinetics of the interactive effects of Ca and Ac or Pr.

The last experimental approach was a feeding study designed to determine in humans the influence of pectin on Ca absorption from milk using two stable isotopes of Ca. Again, it was observed that Ca was absorbed in both the small intestine and colon. Estimates showed that 53.9% of the total Ca absorbed from milk was absorbed in the small intestine and 46.1% in the colon. In the presence of pectin, 42.8% of Ca was absorbed in the small intestine and 57.2% in the colon. However, in contrast with the in vitro study (Chapter 4) a reduction in Ca absorption was observed in the presence of pectin. The in vitro study estimated the amount of Ca released in both the small intestine and the colon that could potentially be absorbed. Some of the unabsorbed Ca in the colon may have bound with other constituents present in the colon, such as bile acids and fatty acids and excreted as such (Wargowich et al, 1984). Nevertheless, the oral feeding study has shown that pectin slowed down the absorption of Ca in the small intestine but a significant amount of Ca was assumed to be absorbed in the colon.

It was shown from the rectal infusion study that at higher concentrations of Ac and Pr, Pr was more effective in enhancing Ca absorption. Pectin produces more Ac than Pr (Table 4.2 and 4.4). Other sources of fermentable fiber and/or complex carbohydrates that produced more Pr than Ac may better enhance the total Ca absorption. It is also

interesting to note that different ratios of SCFA produced after fermentation of different fiber sources may influence Ca absorption. The in vitro study showed that 34% and 44% of the total Ca released from the basal diet alone and basal diet plus pectin, respectively, can be potentially absorbed in the colon. The rectal infusion study showed that 6% and 30% of the total amount of Ca infused in the colon was absorbed from the treatment without and with Ac or Pr, respectively. The oral feeding study with stable isotopes of Ca showed that 46.1% and 57.2% of the total Ca absorbed from milk and milk plus pectin, respectively, may be assumed to occur in the colon. The difference in the percent of the total Ca absorption from the rectal infusion and oral feeding study from the treatment without pectin or Ac or Pr can be explained by the difference in the source of Ca in the study. The rectal infusion study used Ca chloride and the oral feeding study used milk as the source of Ca. Although studies have shown that different sources of Ca did not differ significantly in their total Ca absorption (Recker et al, 1988; Heaney et al, 1988), these studies did not differentiate the absorption of Ca in the small intestine and colon. Moreover, the assumed absorption of Ca in the colon from the oral feeding study may be a slight overestimation if at the 5-8 hour period after feeding, Ca absorption took place in the ileum in addition to the colon following fermentation of the pectin and lactose that reached the colon.

Overall this study was the first to demonstrate the absorption of Ca in the human colon in the presence of fermentation products i.e. SCFA. It also quantified for the first time the amount of Ca in dairy products absorbed in the small intestine and assumed to be absorbed in the colon in the presence of fermentable fibers such as pectin.

This study has several implications. Since Ca may be released and absorbed in the colon, in vitro methods for mineral availability measurements that simulate small intestinal conditions alone may underestimate the total release of minerals under investigation. In vitro methods should include colonic fermentation such as the one used here to better estimate the total absorption and site of absorption of minerals. The rectal infusion method used in this study can be a good model for studying colonic absorption of other minerals, i.e. magnesium, zinc and iron. The oral feeding technique, with stable isotopes of Ca, can measure the absorption of Ca in the small intestine and colon. This study is limited by the fact that it did not provide direct evidence that pectin formed an insoluble complex in the small intestine or that it underwent fermentation which caused the release of Ca for absorption in the colon. Assumptions were made based on the previous in vitro and rectal infusion studies. This study was not able to directly show the transport of Ca into the colonic cells. Assumptions were based on the increased Ca absorption in the presence of Ac or Pr. The absorption of Ac or Pr has been proposed to release protons in exchange for Ca for absorption.

The release of a significant amount of Ca in the colon after bacterial fermentation may have some important physiological implications. Ca is needed to maintain the rate of cell turnover of intracellular structures such as organelles and chromatin (Lipkin and Newmark, 1985; Lewis LH, 1995; Berger et al, 1991). An extensive change in intracellular Ca concentration can lead to changes in physiological behaviour of the cell (Campbell, 1990). A rise in cytosolic Ca can cause cell aggregation, cell transformation, cell division and activation of intermediary metabolism which are related to cell

proliferation (Nordin, 1988). Extensive cell proliferation has also been associated with increased cancer risk (Lewis, 1995; Lipkin and Newmark, 1985; Bird and Bruce, 1986). Therefore Ca absorption in the colon may help maintain colonic health but extensive absorption may also be damaging to the colon. Ca released from its complex with dietary fiber after fermentation can bind with bile acids rendering them insoluble and less damaging to the colon (Wargowich et al, 1984); bile acids are thought to enhance cell proliferation and risk of carcinogenesis (Bird et al, 1986; Bird and Bruce, 1986). Although this study has shown the interactive effects of Ca, Ac and Pr on absorption, very little information is available regarding their interactive effects on colonic health. Studies on the interactive effects of Ca and SCFA on the growth, differentiation and health of normal or cancerous human colon cells is necessary to predict their adverse or protective effects.

In conclusion:

a. Using the in vitro method, it is concluded that the presence of fermentable carbohydrates has little or no effect on total Ca availability; it tends to shift the Ca released from the small intestine to the colon for potential absorption.

b. Using the rectal infusion model, it is concluded that:

i. short chain fatty acids enhance Ca absorption and Ca reduces the serum Ac response in the human distal colon

ii. Ca is absorbed in the rectum and distal colon of humans. Both Ac and Pr enhance Ca absorption, with Pr being more effective.

iii. Ac absorption in the rectum and distal colon of man is enhanced by the

presence of either Ca or Pr but not both. Pr absorption is not affected by the presence of Ac or Ca or both.

iv. Ca absorption in the human distal colon is linearly related to Ca concentration either alone or in the presence of Ac or Pr indicating that it involves a non-saturable diffusion process. Ac and Pr are also absorbed by transport mechanisms influenced by Ca.

c. Using a feeding study with stable isotopes of Ca, the presence of fermentable fiber such as pectin reduced the total Ca absorption but shifted a significant release of Ca assumed to be absorbed in the colon.

Overall, Ca is absorbed in the human distal colon and short chain fatty acids produced from fiber fermentation enhance Ca absorption.

CHAPTER 10

REFERENCES

10. REFERENCES

Abrams SA, Esteban NV, Vieira NE, Sidbury JB, Specker BL, Yergey AL. Developmental changes in Ca kinetics in children assessed using stable isotopes. J Bone Min Res, 1992, 7(3):287-293.

Abrams SA, Lipnick RN, Vieira NE, Stuff JE, Yergey Al. Ca absorption and metabolism in children with juvenile rheumatoid arthritis assessed using stable isotope. J Rheunatol, 1993, 20:1196-1200.

Agnew JE, Holdsworth CD. The effect of fat on calcium absorption from a mixed meal in normal subjects, patients with malabsorption disease and patients with a partial gastrectomy. Gut 1971, 12:973-977.

Alcock N, MacIntyer I. Inter-relation between calcium and magnesium absorption. Clin Sci 1962, 22:185-193

Amman P, Rizzoli K, Fleish H. Calcium absorption in rat large intestine, in vivo: availability of dietary calcium. Am J Physiol 1986, 251:G14-G18.

Armbretch HJ. Age and the effect of lactose on calcium and phosphorous uptake by rat small intestine. Nutr Res 1987, 7:1169-1177.

Armbretch HJ, Wasserman RH. Enhancement of calcium uptake by lactose in the rat intestine. J Nutr 1976, 106:1265-1271.

Anderson H, Bingham SA. The effect of bread containing similar amounts of phytattte but different amount of wheat bran on calcium, zinc and iron balance in mam. Br J Nutr 1979, 50:503-510.

Anderson JW, Chen WJL. Plant fiber: carbohydrate and lipid metabolism. Am J Clin Nutr 1979, 32:346-363.

Anderson IH, Levine AS, Levitt MD. Incomplete absorption of carbohydrates in all purpose wheat flour. New Eng J Med 1988, 304:891-892.

Andrieux C, Sacquet E. Effect of amylomaize starch on mineral metabolism in the adult rat: role of the microflora. J Nutr 1986, 116:991-998.

Allen LH. Calcium bioavailability and absorption: a review. Am J Clin Nutr 1982, 35:783-808.

Argenzio RA, Miller M, Engelhardt W von. Effect of volatile fatty acids on water and ion absorption from goat colon. Am J Physiol 1975, 229:997-1002.

Argenzio RA, Southworth M. Sites of organic acid production and absorption in gastrointestinal tract of pig. Am J Physiol 1974, 228:454-460.

Baran DT, Sorensen AM. Rapid action of 1,25 $(OH)_3D_3$ physiologic role. Proc Soc Exp Biol Med 1994, 207:175-179.

Barger-Lux MJ, Heaney RP, Recker RR. Time course of calcium absorption in humans: evidence for a colonic component. Calcif Tissue Int 1989, 44:308-311.

Barltrap D, Mali R, Sutton A. Absorption and endogenous fecal excretion of calcium by low birthweight infants of feedswith varying contents of calcium and phosphorous. Arch Dis Child, 1977, 52:41-49.

Behall KM, Lee K, Wilson A, Prather ES. Effect of purified fibers added to a basic diet on apparent mineral balance of male subjects. Fed Proc 1983, 42:1063.

Behar J, Kerstein MD. Intestinal calcium absorption: differences in transport between duodenum and ileum. Am J Physiol 1976, 230:1255-1260.

Berger D, Weissman G, Bronzo R. Effect of calcium citrate on colonic epithelial cell proliferation and DNA content in patient with ulcerative colitis. Gastroenterol 1991,100:A349.

Berkelhammer CH, Wood RJ, Sitrin MD. Acetate and hypercalciuria during total parenteral nutrition. Am J Clin Nutr 1988, 48:1482-1489.

Binder HJ, Mehta P. Short chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterol 1989, 96:989-996.

Bird RP, Schneider R, Stamp D and Bruce W. Effect of dietary Ca and cholic acid in proliferation indices of mucosal colonic epithelium. Carcinogenesis 1986, 7:1653-1661.

Bird RP and Bruce W. Effect of dietary Ca in toxicity of bile acid and orally administered fat to colonic epithelium. In:Dietary Fat and Cancer, ed C Ip, D Birt, A Rogers and C Mettlin, Alan R. Liss Inc., New York.

Birlouez-Aragon I. Effect of lactose hydrolysis on calcium absorption during duodenal milk perfusion. Reprod Nutr Dev 1988, 28:1465-1472.

Bjorck I, Nyman M, Pedersen B, Siljestrom M, Asp NG, Eggum BO. On the digestibility of starch in wheat bread - studies in vitro and in vivo. J Cereal Sci 1986, 4:1-11.

Bjorn-Rasmussen E. Iron absorption from white bread. Nutr Metab 1974, 16:101-110.
Blackebhorn DH, Hirsch J, Ahrens EH Jr. Transintestinal intubation: technique for measurement of gut length and physiologic sampling at known loci. Proc Exp Biol Med 1955, 88:356-362.

Blau M, Spencer H, Swernov J, Greenberg J, Laszlo D. Effect of intake levels on the utilization and intestinal excretion of calcium in man. J Nutr 1957, 61:507-521.

Branch WJ, Southgate DAT and James WPT. Binding of Ca by dietary fiber; its relationship to unsubstituted uronic acids. Proc Nutr Soc 1975, 34:A120.

Brannan OB, Vergne-Marini P, Pak CYC, Hull HR, Fordtran JS. Magnesium absorption in the human small intestine: reuslts in normal subjects, patients with chronic renal disease and patients with absorptive hypercalciuria. J Clin Invest 1976, 57:1412-1418.

Breuer RI, Christ ML, Lashner BH, Soergel KH, Hanaver SB, Harig JM, Robinson M, Vanagunas A, Keshavarzian A, Wardzala T, Hinkle M, Desai M and Rademaker A. Short chain fatty acids rectal irrigation therapy for left-sided ulcerative colitis: a randomized placebo-controlled clinical trial. Interim report. In: Falk Symposium 73: Short Chain Fatty Acids. Binder HJ, Cummings JH, Soergel KH eds., Kluwer Academic Publishers, Lancaster, UK, 1994;214-220.

Brommage R, Biradua C, Antille S, Carrie A. Intestinal absorption in rats is stimulated by dietary lactulose and other resistant sugars. J Nutr 1993, 123:2186-2194.

Bronner F. Experimental studies of calcium absorption in man. Nutrit et Dieta, 1962, 3:22-31.

Bronner F. Intestinal calcium absorption: mechanisms and applications. J Nutr 1987, 117:1347-1352.

Bronner F. Ca transport across epithelia. Int Rev Cytol, 1991, 131:169-212.

Bronner F, Pansu D, Stein WD. An analysis of intestinal calcium transport across the rat intestine. Am J Physiol 1986, 250:G561-G569.

Buchowski MS, Miller DD. Lactose, calcium source and age affect calcium bioavailability in rats. J Nutr 1991, 121:1746-1754.

Bugaut M. Occurence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp Bioche Physiol B Comp Biochem 1987, 86:439-472.

Callender ST, Warner GT. Iron absorption from bread. Am J Clin Nutr 1968, 21:1170-1176.

Calvo MS, Bell RR, Forbes RM. Effect of protein-induced calciuria on calcium metabolism and bone status in adult rats. J Nutr 1982, 112:1401-1413.

Calloway DH. Respiratory hydrogen and methane as affected by consumption of gas forming foods. Gastroenterol 1966, 51:383-389.

Campbell AK. Symposium on intracellular calcium in the control of metabolism. Proc of Nutr Soc 1990, 49:51-56.

Cannan R. Kirbich A. Complex formation between carboxylic acids and divalent metal cations. J Am Chem Soc 1938, 60:2314-2320.

Carter MJ, Parsons DS. The carbonic anhydrase of some guinea pig tissues. Biochem Biophys Acta 1970, 206:190-192.

Carter MJ, Parsons DS. The isoenzymes of carbonic anhydrase: kinetic property with particular reference to the functions of the intestinal tract. J Physiol 1972, 220:465-478.

Champagne ET. Effects of pH on mineral-phytate, protein-mineral-phytate and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral availability from high fiber foods. J Am Col Nutr 1988, 7(6):499-508.

Chapman RW, Sillery JK, Graham MM, Saunders PR. Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. Am J Clin Nutr 1985,41:1244-1248.

Charney AN, Wagner JD, Birbaum GJ, Johnstone JN. Functional role of carbonic anhydrase in intestinal electrolyte transport. Am J Physiol 1986, 251:G682-687.

Christensen J. Gross and microscopic anatomy of the large intestine. In:The Large Intestine: Physiology, Pathophysiology and Disease. Philipps SF, Pemberton JH, Shorter CG eds., Mayo Foundation, Raven Press Ltd, New York, 1991:13-17.

Clark I. Importance of dietary Ca:PO_4 ratios on skeletal calcium , magnesium and phosphate metabolism. Am J Physiol 1969, 217:865-870.

Clarkson EM, Warren RL, McDonald SJ, De Wardener HE. The effect of high intake of calcium or magnesium metabolism in normal subjects and patients with chronic renal failure. Clin Sci 1967, 132:11-18.

Cochet B, Jung H, Griessen M, Bartholdi P, Schiller P, Donath A. Effects of lactose on intestinal calcium absorption in normal and lactase deficient subjects. Gastroenterol 1983, 84:935-940.

Conrad ME, Schade SG. Ascorbic acid chelates in iron absorption: role of hydrochloric acid and bile. Gastroenterol 1968, 55:35-45.

Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. Am J Clin Nutr 1991, 53:106-111.

Cook JD, Monsen ER. Vitamin C, the common cold and iron absorption. Am J Clin Nutr 1977, 30:235-241.

Cook JD, Monsen ER. Food iron absorption. I. Use of a semisyntheitic diet to study absorption of non-heme iron. Am J Clin Nutr 1974, 28:1289-1295.

Cramer CF. Sites of calcium absorption and calcium concentration of gut contents in the dog. Can J Physiol Pharmacol 1965, 72:192-196.

Cramer CF, Copp RH. Progress and rate of absorption of radiostrontium through intestinal tract of rat. Proc Soc Exptl Biol Med 1960, 102:514-517.

Cummings JH. Quantitating short chain fatty acids production in humans. In: "Falk Symposium 73: Short Chain Fatty Acids. Binder HJ, Cummings JH, Soergel eds., Kluwer Academic Publishers, Lancaster, UK 1994:11-19.

Cummings JH. Dietary fiber and intestinal microflora. In:Nutrition and the Intestinal Flora, Hallgreen B ed, Swedish Nutrition Foundation, 1983:77-86.

Cummings JH. Short chain fatty acids in the human colon. Gut 1981, 22:763-779.

Cummings JH, Banwell JC, Englyst HN, Coleman N, Segal I, Bersolid D. The amount and composition of human large bowel contents. Gastroenterol 1990, 98:A408.

Cummings JH, Englyst HN. Gastrointestinal effects of food carbohydrates. Am J Clin Nutr 1995, 61(suppl):938S-945S.

Cummings JH and MacFarlane GT. The control and consequence of bacterial fermentation in the human colon. J App Bacteriol 1991;70:443-459.

Cummings JH, Pomare EW, Branch WJ, Naylor CPE and MacFarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987, 28:1221-1227.

Cummings JH, Southgate DAT, Branch WJ, Wiggins HS, Houston H and Jenkins DJA. The digestion of pectin in human gut and its effect on Ca absorption and large bowel function. Br J Nutr 1979, 41:477-485.

Cummings JH, Wiggins HS, Jenkins DJA. Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid and fat excretions. J Clin Invest 1978, 61:953-963.

Dahlqvist A, Thomas DL. The digestion and absorption of lactose by the intact rat. Acta Physiol Scand 1964, 61:20-33.

Davies NT, Warrington S. The phytic acid mineral, trace element, protein and moisture content of United Kingdom Asian immigrants foods. Human Nutr Appl Nutr 1986, 40A:49-59.

Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. Proc Soc Expt Biol Med 1964, 117:97-100.

De Boland AR, Nemere AW. Evidence for involvement of protein kinase carbon and cyclic adenosine 3',5' monophosphate dependent protein kinase in the 1, 25 $(OH)_2D_3$-mediated rapid stimulation of intestinal calcium transport (Transcccaltachial). Endocrinology 1990, 127:39-45.

De Gracia J, Ivanovich P, Fellows A, Rich C. A double isotope method for measurement of intestinal absorption of calcium in man. J Lab Clin Med 1965, 66:822-829.

Demigne C, Levrat M, Remesy C. Effect of feeding fermentable carbohydrates on cecal concentration of minerals and their fluxes between the cecum and blood plasma in the rat. J Nutr 1989, 119:1625-1630.

Demigne C, Remesy C, Rayssiguier Y. Effect of fermentable carbohydrates on volatile fatty acid, ammonia and mineral absorption in the rat cecum. Reprod Nutr Develop 1983, 20(4B):1351-1359.

Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB, Mayet F. The effect of tea on iron absorption. Gut 1975, 16:193-200.

Dormanen MC, Bishop E, Hammond MW, Okamura WH, Nemeere I, Norman AW. Non nuclear effects of the steroid hormone, 1, 25 $(OH)_2D_3$: analogs are able to functionally differentiate between nuclear and membrane receptors. Biochem Biophys Res Comm 1994, 201:394-401.

Dostal LA, Tovereid SU. Effects of vitamin D_3 on duodenal calcium absorption in vivo during development. Am J Physiol 1984, 246:G528-G534.

Drasar BS, Jenkins DJA. Bacteria, diet and large bowel cancer. Am J Clin Nutr 1976, 29:1410-1416.

Drasar BS, Jenkins DJA, Cummings JH. The influence of a diet rich in wheat fiber on the human fecal flora. J Med Microbiol 1976, 9:423-431.

Drews LU, Kies C, Fox HM. Effect of dietary fiber on copper, zinc, and magnesium utilization in adolescent boys. Am J Clin Nutr 1979, 32:1893-1897.

Dubois RJ, Schaedler RW. The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. J Exp Med 1962, 115:1161-1171.

Eastwood MA, Kay RM. A hypothsis for the action of fiber along the gastrointestinal tract. Am J Clin Nutr 1979, 32:364-367.

Ehrenkranz R, Ackerman B, Melli C, Janghorbani M. Absorption of calcium in premature infants as measured with a stable isotope ^{46}Ca extrinsic tag. Ped Res 1985, 19: 178-184.

Engelhardt W von. Absorption of short chain fatty acids from the large intestine. In:Physiological and Clinical Aspects of Short Chain Fatty Acids. Cummings JH, Rombeau JL and Sakata T eds. Cambridge University Press, Great Britain, 1995 pp. 149-170.

Engelhardt W von, Rechkemmer G. Segmental differences of short chain fatty acids transport across guinea pig large intestine. Exp Physiol 1992, 77:491-499.

Engelhardt W von , Rechkemmer G. The physiological effects of short chain fatty acids in the hind gut. In: Fiber in Human and Animal Nutrition. Wallace G, Bells eds., Wellington, New Zealand: R Soc. New Zealand 1983; bulletin 20:149-155.

Englyst HN, Cummings JH. Digestion of polysaccharides of some cereal foods in the human small intestine. Am J Clin Nutr 1985, 42:778-787.

Englyst HN, Cummings JH. Digestion of the carbohydrate of banana (Musa paradisiaca sapientum) in the human small intestine. Am J Clin Nutr 1986, 44:42-50.

Englyst HN, Cummings JH. Digestion of polysaccharides of potato in the small intestine of man. Am J Clin Nutr 1987, 45:423-431.

Englyst HN, MacFarlane GT. Breakdown of resistant and readily didgestible starch by human gut bacteria. J Sci Food Agric 1986, 37:699-706.

Farrell HM Jr., Thompson MP. Physical Equilibria: Proteins. In: Fundamental of Dairy Chemistry, 1974, Avi Publishing Co., Wesport, Conn. USA.

Faulks RM, Southon S, Livesey G. Utilization of α-amylase (EC3.21.1) resistant maize and pea (Pisum sativum) in rats. Br J Nutr 1989, 61:291-300.

Favus MJ, Angeid-Backman E. Effects of $1,25(OH)_3D_3$ and calcium channel blockers on cecal calcium transport in the rat. Am J Physiol 1985, 248:G676-G681.

Favus MJ, Langman CB. Effects of 1,25 dihydroxyvitamin D_3 on colonic Ca transport in vitamin D deficient and normal rats. Am J Physiol 1984, 246:G268-G273.

Feher JJ. Facilitated Ca diffusion by intestinal calcium binding protein. Am J Physiol 1983 244:C303-C307.

Finegold SM, Attebery HR, Sutter VL. Effect of diet on human fecal flora: comparison of Japanese and American diets. Am J Clin Nutr 1974, 27:1456-1469.

Finegold AM, Sutter VL, Mathisen GE. Normal indigenous intestinal microflora. In: Human and Intestinal Microflora in Health and Disease, Hentges DJ ed, London:Academic Press, 1983:3-31.

Fituri N, Allawi N, Bentley M, Costello J. Urinary and plasma oxalate during ingestion of pure ascorbic acid: a re-evalution. Eur Urol 1983, 9:312-314.

Fleming SE, Choi SY, Fitch Md. Absorption of short chain fatty acids from rat cecum in vivo. J Nutr 1991, 121:1787-1797.

Flourie B, Florent C, Etanchaud F. Starch absorption by healthy man evaluated by lactulose hydrogen breath test. Am J Clin Nutr 1988, 47:61-66.

Francis RM, Peacock M, Barkworth SA, Marshall DH. A comparison of the effect of sorbitol and glucose on calcium absorption in post menopausal women. Am J Clin Nutr 1986, 43:72-76.

Furda I. Interaction of pectaneceous dietary fiber with some metals and lipids. In: Onglett GE and Falkehag SI eds. Dietary Fibers:Chemistry and New Nutrition, New York Academic Press, 1979;30-48.

Gacs G, Barltrop D. Significance of calcium soap formation for calcium absorption in the rat. Gut 1977, 18:64-68.

Gassaway WC. Volatile fatty acid and metabolizable energy derived from cecal fermentation in the willow ptarmigan. Comp Biochem Physiol A Comp Physiol 1976, 53:115-121.

Gee M, Faulks RM, Johnson IT. Physiological effects of retrograded α-amylase resistant cornstarch in rats. J Nutr 1991, 121:44-49.

Gibaldi M, Perrier D. Pharmokinetics. In Series: Swarbrick J ed. Drugs and the Pharmaceutical Sciences, Marcel Dekker, New York, 1982;1:411.

Gibson RS. Content and bioavailability of trace elements in vegetarian diets. Am J Clin Nutr 1994, 59(suppl):1223S-1232S.

Gorbach SL, Nahas L, LernerDF, Wernstein L. Studies of intestinal microflora. I. Effects of diet, age, periodic sampling on numbers of fecal microorganisms in man. Gastroenterol 1967, 53:845-855.

Gottschalk G (ed). Bacterial Metabolism, 2nd ed. Berlin: Springer Verlag, 1986.

Greenstead WC, Pak CYC, Krejs GJ. Effect of 1,25 dihydroxyvitamin D_3 on Ca absorption in the colon of healthy humans. Am J Physiol 1984, 10:G189-G192.

Griessen M, Cochet B, Infante F, Jung A, Bartholdi P, Donath A, Loizeau E, Courvoisier B. Calcium absorption from milk in lactase-deficient subjects. Am J Clin Nutr 1989, 49:377-384.

Griessen M, Infante F, Speich PV, Bartholdi P, Cochet B, Courvoisier B. Effect of absorbable and unabsorbable sugars on intestinal calcium absorption. Calcif Tissue Int 1986, 39(suppl):128 (abstract).

Guerrant GO, Lambert MA, Moss CW. Analysis of short chain fatty acids from anaerobic bacteria by high performance liquid chromatography. J Clin Microbiol 1982, 16:355-360.

Haboubi NY, Hudson P, Rahman Q, Lee GS, Ross A. Small intestinal transit time in the elderly. Lancet 1988, 1:933.

Hallberg L, Brune M, Erlandsson M, Sandberg A, Hutten L. Calcium: effect pf dietary amounts on non-heme and heme iron absorption in humans. Am J Clin Nutr 1991, 53:112-119.

Hanck AB. The influence of 1000 mg of Vitamin C on renal excretion of electrolyte in healthy human subjects. Int J Vit Nutr Res 1972, 43:34-41.

Hanck AB. The behavior of urine parameters in healthy human subjects dduring intake of 4 g L(+) ascorbic acid. Int J Vit Nutr Res 1974, 44:302-308.

Hardwick LL, Jones MR, Brautbar N, Lee DB. Magnesium absorption: mechanism and influence of vitamin D, calcium and phosphorous. J Nutr 1991, 121:13-23.

Harig JM, Knaup SM, Shosshara J, Dudeja PK, Ramaswamy K, Brasitus TA. Transport of n-butyrate into human colonic luminal membrane vesicles. Gastroenterol 1990, 98:A543.

Harig JM, Soergel KH, Barry JA, Ramaswamy K. Transport of propionate by human ileal brush-border membrane vesicles. Am J Physiol 1991, 260:G776-782.

Harig JM, Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short chain fatty acids irrigation. N Eng J Med 1989;320:23-28.

Harrison HC, Harrison HE. Calcium transport by rat colon in vitro, Am J Physiol 1969, 217:121-125.

Harrison JE, McNeil KG, Wilson DR, Oreopolos DG, Krondl A, Finley JM. An evaluation of Ca absorption tests. Clin Biochem 1973, 6:237-245.

Hatch M. Short chain fatty acid transport and its effect on ion transport by rabbit cecum. Am J Physiol 1987, 253:G171-178.

Heaney RP, Parsons FM. The metabolic effect of high magnesium intake. Clin Sci 1961, 21:273-284.

Heaney RP, Recker RR and Saville PD. Menopausal changes in calcium balance performance. J Lab Clin Med 1978, 92:953-963.

Heaney RP, Smith RT, Recker RR and Hinders SM. Meal effects on calcium absorption. Am J Clin Nutr 1988, 49:372-376.

Heaney RP, Weaver CM. Effect of plant constituents on food calcium availability. J Bone Min Res 1992, 7:S136.

Heaney RP, Weaver CM. Calcium absorption from kale. Am J Clin Nutr 1990, 51:656-657.

Heaney RP, Weaver CM, Fitzsimmons ML. Soybean phytate content: effect on calcium absorption. Am J Clin Nutr 1991, 53:745-747.

Heaney RP, Weaver CM, Fitzsimmons ML, Recker RR. Calcium absorptive consistency. J Bone Min Res 1990, 5(11):1139-1142.

Heaton FW, Oarson FM. The metabolic effect of magnesium intake. Clin Sci 1961, 21:273-284.

Hegsted DM. Calcium and osteoporosis. J Nutr 1986, 116:2316-2319.
Hegsted DM, Moscoso I, Collazos C. A study of minimum calcium requirement of adult man. J Nutr 1952, 46:181-201.

Hegstted DM, Schuette SA, Zemel MB, Linkswiler HM. Dietary calcium and calcium balance in young men as affected by level of protein and phosphorous intake. J Nutr 1981, 111:553-562.

Hendrix JZ, Alcock NW, Archibald RM. Competition betwen calcium, strontium and magnesium for absorption in the isolated rat intestine. Clin Chem 1963, 9:734-744.

Hentges DJ. Fecal flora of volunteers on controlled diet. Am J Clin Nutr 1978, 31:S123-S124.

Hidaka H, Eida T, Takisawa T, Tokunaga T, Tashsiro Y. Effect of fructooligosaccharide in intestinal flora and human health. Bifidobacteria Microflora 1986, 5:37-50.

Holdeman LV, Cato EF, Moore WEC (eds). Anaerobic Laboratory Manual 4th ed., Blacksburg VA, Virginia Polytechnic Institute and State University, 1977.

Holdeman LV, Good IJ Moore WEC. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl Environ Microbiol 1976, 31:359-375.

Hollock MF. Vitamin D biosynthesis, metabolism and mode of action. Endocrinology vol 2, L DeGrootel ed, Saunders Publishing Co., Philadelphia, U.S.A. 1989, pp. 905-926.

Hosoya N, Dhorranintra B, Hidaka H. Utilization of [U-^{14}C] fructooligosaccharide in man as energy resources. J Clin Biochem Nutr 1988, 5:67-74.

Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High- versus low-meat diets: effects on zinc absorption, iron status and calcium, copper, iron magnesium, mangnese, nitrogen, phosphorous and zinc balance in postmenopausal women. Am J Clin Nutr 1995, 62:621-632.

Hurrell RF, Lynch SF, Trinidad TP, Dassenko SA and Cook JD. Influence of endogenous animal protein on iron absorption in humans. Am J Clin Nutr 1988, 47:102-107.

Ink SL. Fiber-mineral and fiber-vitamin interactions. In: Bodwell CE and Erdman JW Jr eds. Nutrient Interactions. New York:Marcel Dekker Inc, 1988:253.

Ismail-Beigi F, Reinhold JG, Faraji B. Effects of cellulose added to diets of low and high fiber content upon metabolism of calcium, magnesium, zinc and phosphorous in man. J Nutr 1977, 107:510-518.

James WPT, Branch WJ and Southgate DAT. Ca binding by dietary fibers. Lancet 1978, 89:638-639.

Jenkins DJA, Wolever TMS, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Albe KGMM. Dietary fibers, fiber analogues and glucose tolerance: importance of viscosity. Br Med J 1978, 1:1392-1394.

Johnson NE, Alcantara EN, Linkswiler H. Effect of level of protein intake on urinary and fecal calcium and calcium retention of young adult males. J Nutr 1979, 100:1425-1430.

Kabayashi A, Kawais S, Ohbe Y, Nagashima Y. Effects of dietary lactose and a lactase preparation on the intestinal absorption of calcium and magnesium on normal infants. Am J Clin Nutr 1975, 28:681-683.

Karbach U, Bridges RJ, Rummel W. The role of paracellular pathway in the net transport of calcium across the colonic mucosa. Naunya-Schmiedeberg's Arch Pharmakol 1986, 334:525-530.

Karbach U, Ewe K. Calcium and magnesium transport and influence of 1,25 dihydroxyvitamin D_3. Digestion 1987, 37:35-42.

Karbach U, Rummel W. Trans and paracellular calcium transport across the colonic mucosa after short and long term treatment with 1, 25 dihydroxyvitamin D_3. Eur J Clin Invest 1986, 16:347-351.

Kelsay JL. A review of research on effects of fiber intake on man. Am J Clin Nutr 1978, 31:142-159.

Kies C. Effect of ditary fat and fiber on calcium bioavailability. In: Kies C ed. Nutritional Bioavailability of Calcium. American Chemical Society: Washington DC 1985;175-187.

Kies C. Mineral utilization of vegetarians: impact of variation in fat intake. Am J Clin Nutr 1988, 48:884-887.

Kim KI, Benevenga NL, Grummer RH. Estimation of the fraction of the lactose in a high lactose diet available for fermentation in the cecum and colon of rat. J Nutr 108:79-89.

Kimberg DV, Schatcher D, Schonker H. Active transport of calcium by intestine: effects of dietary calcium. Am J Physiol 1961, 200:1256-1262.

Kitts DD, Yuan YV, Nagasawa T, Monxama Y. Effect of casein, casein phosphopeptides and calcium intake on ileal ^{45}Ca disappearance and temporal systolic blood pressure in spontaneously hypertensive rats. Brit J Nutr 1992, 68:765-781.

Kivisto B, Andersson A, Cederblad G, Sandberg A, Sandstrom B. Extrusion cooking of a high-fiber cereal product. 2. Effects oon apparent absorption of zinc, iron, calcium, magnesium and phosphorous in humans. Brit J Nutr 1986, 55:255-260.

Kocian J, Skala I, Bakos K. Calcium absorption from milk and lactose-free milk in healthy subjects and patients with lactose intolerance. Digestion 1973, 9:317-324.

Latham MJ, Story JE, Sharpe ME. Effect of low roughage diets on microflora and lipid metabolism in the rumen. Appl Microbiol 1972, 24:871-877.

Lee DBN, Walling MW, Gafter U, Silis V, Coburn JN. Calcium and inorganic phosphate transport in rat colon: dissociated response to $1,25(OH)_3D_3$. J Clin Invest 1980, 65:1326-1331.

Leichsenring JM, Norris LM, Halbert ML. Effect of ascorbic acid and of orange juice on calcium and phosphorous metabolism of women. J Nutr 1957, 63:425-435.

Leichsenring JM, Norris LM, Lameson SA. Magnesium metabolism in college women: observatuions on the effect of calcium and phosphorous intake levels. J Nutr 1951, 45:477-485.

Lengemann FW, Wasserman RH, Comar CL. Studies on the enhancement of radiocalcium and radiostrontium absorption by lactose in the rat. J Nutr 1959, 68:443-445.

Levenson DI, Bockman RS. A review of calcium preparation. Nutr Rev 1994, 52(7):221-232.

Levine AS, Levitt MD, Malabsorpt of starch moiety of oats, corn and potatoes. Gastroenterol 1981, 82:1209.

Levitt MD, Hirsch P, Fetzer CA, Sheahan M, Levine AS. Hydrogen excretion after ingestion of complex carbohydrates. Gastroebterol 1987, 92:383-389.

Lewis LH. Dietary factors - vitamins and minerals. In: Cancer of the Colon, Rectum and Anus. Cohen AM and Winawer SJ eds., McGraw-Hill Inc, New York, USA 1995;41-49.

Lewis NM, Marcus MSK, Behling AP and Greger JL. Calcium supplements and milk: effects on acid-base balance and retention of calcium, magnesium and phosphorous. Am J Clin Nutr 1989, 49:527-533.

Linder CM (ed). Calcium. In: Nutritional Biochemistry and Metabolism, Elsevier, New York, 1991; 202-214.

Linkswiler HM, Joyce CL, Anand CR. Calcium retention of young adult males as afected by level of protein and of calcium intake. Ann NY Acad Sci 1974, 36:333-340.

Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial cell proliferation in subjects at high risk for familial colonic cancer. N Engl J Med 1985, 313:1381-1386.

Livesey G, Davies IR, Brown JC, Faulks RM, Southon S. Energy balance and energy values of α-amylose (EC3.2.1.1) resistant maize and pea (Pisum sativum) starches in rat. Br J Nutr 1990 63:467-480.

Lonnerholm G. Carbonic anhydrase in the intestinal tract of the guinea pig. Acta Physiol Scand 1977, 99:53-61.

Lutwak L. Tracer studies of intestinal calcium absorption in man. Am J Clin Nutr 1969, 22:771-785.

Lutz T, Scharrer E. Effect of short chain fatty acids on calcium absorption by the rat colon. Expt'l Physiol 1991, 76:615-618.

Lutz T, Wurmli R, Scharrer E. Short chain fatty acids stimulate Mg absorption by the colon. In: Lasserre B and Durlach J eds. Magnesium - A relevant Ion. John Libbey and Company Ltd., London, England 1991.

MacFarlane GT, Cummings JH. The colonic flora, fermentation and large bowel digestive function. In:The Large Intestine:Physiology, Pathophysiology and Disease. Phillips SF, Pemberton JH and Shorter RG eds, Mayo Foundation, Raven Press Limited, New York 1991:52-83.

MacFarlane GT, Englyst HN. Starch utilization by human large intestinal microflora. J Appl Bacteriol 1986, 60:195-201.

Macy JM, Probst I. The biology of gastrointestinal bacteroids. Ann Rev Microbiol 1979, 33:561-594.

Madara JL, Pappenheimer JR. Structural basis for physical regulation of paracellular pathways in intestinal epithelia. J Membr Biol 1987, 100:149-164.

Magee DF, Dalley AF. Digestion and the Structural Function of the Gut. Karger, Switzerland, 1986;183.

Malawer SJ, Powell DW. An improved turbidemetric analysis of polyethylene glycol utilizing an emulsifier. Gastroenterol 1967, 53:250-256.

Mallett AK, Bearne CA, Young PJ, Rowland IR. Influence of starches of low digestibility on the rat cecal microflora. Br J Nutr 1988, 60:597-604.

Marcus CS, Lengemann FW. Absorption of ^{45}Ca and ^{85}Sr from solid and liquid food at various levels of the alimentary tract of rat. J Nutr 1962, 77:155-160.

Margen S, Shu JY, Kaufmann NA, Calloway DH. Studies in calcium metabolism. I. The caclciuretic effect of dietary protein. Am J Clin Nutr 1974, 27:584-589.

Marshall DH. Calcium and phosphate kinetics. In: Nordin BEC ed. Calcium, Phosphate and Magnesium Metabolism. Longman Group Ltd, New York 1976;260-266.

Marshall DH, Nordin BEC. A comparison of radioactive calcium absorption tests with net calcium absorption. Clin Sci 1981, 61:477-481.

Mauras N, Haymaond MW, Dormaun D, Vieira NE, Abrams SA, Yergey AL. Calcium and protein kinetics in prepeubertal boys. J Clin Invest 1994, 93:1014-1019.

McBurney MI and Thompson LU. Effect of human fecal innoculum on in vitro fermentation variables. Br J Nutr 1987,58:233-243.

McBurney MI and Thompson LU. In vitro fermentabilities of purified fiber supplements. J Food Sc 1989, 54(2):347-350.

McBurney MI, Thompson LU, Cuff DJ, Jenkins DJA. Comparison of ileal effluents, dietary fibers and whole foods in predicting the physiological importance of colonic fermentation. Am J Gastoenterol 1988, 83:336-340.

McCance RA, Widdowson EM. Mineral balance in dephytinized bread. J Physiol 1942, 101:304-313.

Mehansho H, Kanerva RL, Andepohl GR, Smith KT. Calcium bioavailability and iron-calcium interaction in orange juice. J Am Coll Nutr 1989, 8(1):61-68.

Miller A III, Bronner F. Ca uptake in isolated brush-border vesicles from rat small intestine. Biochem J 1981, 196:391-401.

Miller A III, Li ST, Bronner F. Characterization of calcium binding to brush border membranes from rat duodenum. Biochem J 1982, 208:773-782.

Miller DD. Calcium in the diet: food sources, recommended intakes and nutritional bioavailability. Adv Food Nutr Res 1989, 33:103-156.

Miller DD, Berner LA. Is solubility in vitro reliable predictor of iron bioavailability? Biol Trace Element Res 1989, 19:11-24.

Miller DD, Schricker BR, Rasmussen RR, Van Campen D. An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 1981, 34:2248-2256.

Miller JZ, Smith D, Flora L, Peacock M Jr., Weaver CJ. Ca absorption in children estimated from single and double stable calcium isotope technique. Clin Chim Acta 1989, 183:107-114.

Miller TL, Wolin MJ. Fermentations by sacchardytic intestinal bacteria. Am J Clin Nutr 1979, 32:164-172.

Minghetti PP, Norman AW. 1, 25 $(OH)_2D_3$ receptors: gene regulation and genetic circuitry. FASEB J 1988, 2:3043-3053.

Monner L, Colette C, Aguirre I, Sany C, Mirouza J. Intestinal and renal handling of calcium in human diabetes mellitus: influence of acute glucose loading and diabetic control. Eur J Clin Invest 1978, 3:225-231.

Monsen ER, Cook JD. Food iron absorption in human subjects. V. Effects of the major dietary constituents of a semi-synthetic meal. Am J Clin Nutr 1979, 32:804-808.

Moore WEC, Cato EP, Holdeman LV. Some current concepts in intestinal bacteriology. Am J Clin Nutr 1978, 31:S33-S42.

Moore WEC, Holdeman LV. Discussion of current bacteriological investigations of the relationships between intestinal flora, diet and colon cancer. Cancer Res 1975, 35:3418-3420.

Moore WEC, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl Microbiol 1974, 27:961-979.

Mortensen PB, Holfug K, Rasmussen HS. Short chain fatty acid production from mono- and disaccharides in a fecal intubation system: Implication for colonic fermentation of dietary fiber in humans. J Nutr 1988, 118:321-325.

Munoz JM and Harland B. Overview of the effects of dietary fiber in the utilization of minerals and trace elements. In: Handbook of Dietary Fiber in Human Nutrition. Spiller G ed., CRC Press, Boca Raton 1993;245-252.

Murer H, Biber J, Gmay P, Stieger B. Cellular mechanisms in epithelial transport: advantages and disadvantages of studies with vesicles. Mol Physiol 1984, 6:55-82.

Nancollas GH. Thermodynamics of ion association. Part II. Alkaline earth acetates and formates. J Chem Soc 1956, 744-749.

National Research Council. Diet and Health: implications for reducing chronic disease risk. Washington DC, National Academy Press, 1989.

Nellans HN. Intestinal calcium absorption. Mineral and Elec Metab 1990, 16:101-108.

Nellans HN, Goldsmith RS. Transephithelial calcium transport by rat cecum: high efficiency absorptive site. Am J Physiol 1981, 240:G424-431.

Nellans HN. Contributions of cellular and paracellular pathways to transephithelial intestinal calcium transport. In: Cellular Calcium and Phosphate Transport in Health and Disease, Allan R. Liss Inc., 1988:269-283.

Nellans HN, Kimberg DV. Cellular and paracellular calcium transport in rat ileum: efects of dietary calcium. Am J Physiol 1978, 235:E726-737.

Nemere I, Dormanen MC, Hammon HW, Okamura WH, Morman AW. Identification of a specific binding protein for 1, α-25 $(OH)_2D_3$ in basal-lateral membrane of chick intestinal epithelium and relationship to transcaltachia. J Biol Chem 1994, 269:23750-23756.

Nilsson U, Oste R, Jagerstad M, Birkhed D. Cereal fructans: in vitro and in vivo studies on availability in rats and humans. J Nutr 1988, 118:1325-1330.

Nordin BEC (editor). Calcium, Phosphate and Magnesium Metabolism. London: Churchill Livingston, 1976, p.36

Nordin BEC (editor). Calcium in Human Biology. London:Springer:Verlag 1988, p. 680.

Nordin BEC, Horsman A, Marshall DH, Simpson M, WAterhouse GM. Calcium requirement and calcium therapy. Clin Orth Rel Res 1979, 140:216-239.

Norman DA, Fordtran JS, Brinkley LJ, Zerwekh JE, Nicar MH, Strowig SM, Pak CYC. Jejunal and ileal adaptation to alterations in dietary calcium: changes in calcium and magnesium absorption and pathogenic role of parathyroid hormone and 1, 25 $(OH)_2D_3$. J Clin Invest 1981, 67:1599-1603.

Norman DA, Morawski SG, Fordtran JS. Influence of glucose, fructose and water movement on calcium absorption in the jejunum. Gastroenterol 1980, 28:22-25.

O'Donnell JM, Smith MW. Uptake of calcium and magnesium by rat duodenal mucosa analyzed by means of competing metals. J Physiol 1973, 229:733-749.

Ohta A, Ahtsuki M, Baba J, Adachi T, Sakata T, Sakaguchi E. Calcium and magnesium absorption from colon and rectum are increased in rats fed fructooligosaccharides. J Nutr 1995, 125:2417-2424.

Pansu D, Bellaton C, Bosshard A. Effect of lactose feeding in cell renewal, disaccharide activity and calcium binding protein in the intestinal mucosa of rats. Am Biol Bioch Biophys 1978, 18:127-132.

Pansu D, Bellaton C, Bronner F. Effect of calcium intake on saturable and nonsaturable components of duodenal calcium transport. Am J Physiol 1981, 240:32-37.

Pansu D, Bellaton C, Roche C and Bronner F. Duodenal and ileal Ca absorption in rat and effects of Vitamin D. Am J Physiol 1983, 244:G695-G700.

Pansu D. Chapuy MC. Relation chez l'homme, entro l'action sur la calcemie de divers composes glucidiques et leur utilisation digestive. CR Acad Sci 1970, 270:3103-3106.

Pansu D, Chapuy MC, Milani M, Bellaton C. Transepithelial calcium transport enhance by xylose and glucose in the rat jejunal ligated loop. Calcif Tissue Res 1976, 21:45-52.

Pappernheimer JR. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hampsters. J Mem Biol 1987, 100:137-148.

Pappenheimer JR, Reiss KZ. Contribution of solvent drag through intracellular junction to absorption of nutrient by the small intestine of rats. J Mem Biol 1987, 100:123-136.

Partridge IG. Studies on digestion and absorption in the intestine of growing pigs. 4. Effects of dietary cellulose and sodium levels on mineral absorption. Br J Nutr 1978, 39:539-545.

Patton JS, Carey MC. Watching fat digestion. Science 1979, 204:145-148.

Petith M, Schedl AD. Divalent cation transport by rat cecum and colon in calcium and magnesium deficiency. Proc Soc Expt'l Biol Med 1977, 155:225-229.

Phang J, Berman M, Finerman G, Neir R, Posenberg L, Hahn T, Fisher L, Granger A. Dietary perturbation of calcium metabolism in normal men: comparative analysis. J Clin Invest 1969, 48:67-77.

Pike JW. Vitamin D receptors: structure and function in transcription. Annu Rev Nutr 1991, 11:189-216.

Pittman MS, Kunerth BL. A long time study of nitrogen, calcium and phosphorous metabolism on a medium protein diet. J Nutr 1939, 17:175-185.

Poneros-Schneir AG, Erdman JW Jr. Bioavailability of calcium from sesame seeds, almond powder, whole wheat bread, spinach and nonfat dry milk in rats. J Food Sci 1989, 54:150-153.

Price RI, Kent GN, Rosmen KJB, Gutteridge DH, Reeve J, Allen JP, Strickey BGA, Smith M, Guelfi G, Hickling CJ, Balkeman SC. Kinetics of intestinal calcium absorption in human measured using stable isotope and high precision thermal ionization mass spectrometry. Biomed Environ Mass Spect 1990, 19:353-359.

Rasmussen H, Fontaine D, Max EE, Goodman DP. The effect of alpha hydroxy-D_3 administration on calcium transport in chick intestine brush border membrane vesicles. J Biol Chem 1979, 254:2993-2999.

Rechkemmer G. In vitro studies of short chain fatty acids transport with intact tissue. In: Falk Symposium 73: Short Chain Fatty Acids. Binder HJ, Cummings JH, Soergel KH eds., Kluwer Academic Publishers, Lancaster, UK 1994:83-92.

Rechkemmer G, Engelhardt W von. Concentrations and pH dependence of short chain fatty acids absorption in the proximal and distal colon of guinea pigs (Cavia procellus). Comp Biochem Physiol 1988, 91A:659-663.

Rechkemmer G, Wahl, Kuschinsty W, Engelhardt W von. pH microclimate at the surface of the intestine of guinea pig and rat (Abstract). Pfluegers Arch 1979, 382:R31.

Recker RR, Bammi AM, Barger-Lux MJ and Heaney RP. Calcium absorbability from milk products, an imitation milk and calcium carbonate. Am J Clin Nutr 1988, 47:93-95.

Reeve J, Bijouet DLM, Neer RM, Slovik D, Telley M, Vismans F, Zarelli GD. A comparison between the balance method and radioactive method for measuring calcium absorption in treated and untreated patients with osteoporosis. Metab Bone Dis Rel Res 1980, 2:233-238.

Reichel H, Koeffler P, Norman AW. The role of Vitamin D endocrine system in health and disease. N Eng J Med 1989, 320:980-991.

Reinhold JG, Ismail-Beigi F, Faradji B. Fiber vs phytate as determinant of the availability of calcium, zinc and iron in breadstuffs. Nutr Rep Int 1973a, 12:75-85.

Reinhold JG, Nasr K, Lahimgarzadeh A, Hedayate H. Effect of purified phytate and phytate rich bread upon metabolism of zinc, calcium, phosphorous and nitrogen in man. Lancet 1973b, i:283-288.

Rimmer DW, Wiebe WJ. Fermentative microbial digestion in herbivorous fishes. J Fish Biol 1987, 31:229-236.

Roberfroid M. Dietary fiber, inulin and oligofructose: a review comparing thier physiological effects. Crit Rev Rood Sci Nutr 1993, 33:103-148.

Roberfroid M, Gibson GR, Delzenne N. The biochemistry of oligosaccharides, a nondigestible fiber: an approach to calculate its caloric value. Nutr Rev 1993, 51:137-146.

Roediger WEW. Role of anaerobic bacteria on the metabolic welfare of the colonic mucosa in man. Gut 1980;21:793-798.

Rossander L, Hallberg, Bjorn-Rasmussen E. Absorption of iron from breakfast meal. Am J Clin Nutr 1979, 32:2484-2488.

Rumessen JJ, Bode S, Hamberg O, Hoyer FG. Fructans of Jerusalem antichokes: intestinal transport, absorption, fermentation and influence on blood glucose, insulin and C peptide responses in healthy subjects. Am J Clin Nutr 1990, 52:675-681.

Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG Jr. Absorption of short chain fatty acids by the colon. Gastroenterol 1980, 78:1500-1507.

Sandberg AS, Ahderinne R, Anderson H, Hallgreen B, Hulten L. The effect of citrus pectin in the absorption of nutrients in the small intestine. Human Nutr:Clin Nutr 1983, 37C:171-183.

Sandstrom B, Cederblad A, Kivisto B, Stenquist B, Andersson A. Retention of Zn and Ca from the human colon. Am J Clin Nutr 1986, 44:501-504.

Saunders DR. Absorption of short chain fatty acids in human stomach and rectum. Nutr Res 1991, 11:841-846.

Schatcher D, Dowdle EB, Schenker H. Active transport of calcium by small intestine of the rat. Am J Physiol 1960, 198:263-268.

Schatcher D, Rosen SM. Active transport of ^{45}Ca by the small intestine and its dependence on vitamin D. Am J Physiol 1959, 196:357-362.

Schedl HP, Wilson HD. Calcium uptake by intestinal brush border membrane vesicles: comparison with in vivo calcium transport. J Clin Invest 1985, 76:1871-1878.

Schiller LR, Santa Ana CA, Morawski SG, Fordtran JS. Effect of amiloride on sodium transport in the proximal, distal and entire human colon in vivo. Dig Dis Sci 1988, 33(8):969-976.

Schmidt KH, Haginaier V, Horneg PH, Vuilleumiew P, Rutishauser G. Urinary oxalate excretion after large intake of ascorbic acid in man. Am J Clin Nutr 1981, 34:305-311.

Schuette SA, Knowles JB, Ford HE. Effect of lactose or its component sugars on jejunum calcium absorption in adult man. Am J Clin Nutr 1989, 50:1084-1087.

Schuette SA, Zemel MB, Linkswiler HM. Studies on the mechanism of protein-induced hypercalciuria in older man and woman. J Nutr 1980, 110:305-318.

Schuette SA, Linkswiler HM. Effects of calcium and phosphorous metabolism in humans by adding meat, meat plus milk or purified protein plus calcium and phosphorous to a low protein diet. J Nutr 1982, 112:338-349.

Schultz AGM, Van Amelsvoort JMM, Beynen AC. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. J Nutr 1993, 123:1724-1731.

Scientific Review Committee, Health and Welfare Canada. Nutrition recommendation. Ottawa, Canada. Canadian Government Publishing Center 1990: 34-37.

Sellin JH, DeSoignie R. Short chain fatty acid absorption in rabbit colon in vitro. Gastrenterol 1990, 99:676-683.

Shaafsma G, Vesser WK. Nutritional interelationship between calcium, phosphorous and lactose in rats. J Nutr 1980, 110:1101-1111.

Sheikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS. Gastrointestinal absorption of calcium from milk and calcium salts. N Eng J Med 1987, 317:532-536.

Sheppack W. Short chain fatty acids improve epithelia in ulcerative colitis? Speculations on mechanisms. In: Falk Symposium 73: Short Chain Fatty Acids. Binder HJ, Cummings JH, Soergel KH eds., Kluwer Academic Publishers, Lancaster, UK 1994;206-213.

Slavin JL, Marlett JA. Influence of refined cellulose on human bowel function and calcium and magnesium balance. Am J Clin Nutr 1980, 33:1932-1939.

Smith RH, McAllen AB. Binding of magnesium and calcium in the contents of small intestine of the calf. Brit J Nutr 1966, 20:703-718.

Smith TM, Kolars JC, Savaino DA, Levitt MD. Absorption of calcium from milk and yogurt. Am J Clin Nutr 1985, 42:1197-1200.

Spencer H, Kramer L. Osteoporosis: calcium, fluoride and aluminum interactions. J Am Coll Nutr 1985, 4:121.

Spencer H, Kramer L, De Bartolo M, Norris C, Osis D. Further studies of the effect of a high protein diet as meat on calcium metabolism. Am J Clin Nutr 1983, 37:924-929.

Spencer H, Kramer L, Osis D, Norris C. Effect of phosphorous on the absorpton of calcium and on the calcium balance in man. J Nutr 1978, 108:447-457.

Steggerda FR, Mitchell HH. The calcium balance of adult human subjects on high and low fat (butter) diets. J Nutr 1951, 45:201-211.

Stephen AM, Cummings JH. The microbial contribution to human fecal mass. J Med Microbiol 1980, 13:45-56.

Stephen AM, Haddad AC, Phillips SF. Passage of carbohydrates into the colon: direct measurements of humans. Gastroenterol 1983, 85:589-595.

Stevens CE, Argenzio RA, Roberts MC. Comparative physiology of the mammalian colon and suggestions for animal models of human disorders. Clin Gastroenterol 1986, 15:763-786.

Szymendra J, Heaney R, Saville PD. Intestinal calcium absorption: concurrent use of ion and intravenous tracer and calculation by inverse convolution method. J Lab Clin Med 1972, 79:570-578.

Takito J, Shinki T, Sasaki T, Suda T. Calcium uptake by brush border and basolateral membrane vesicles in chick duodenum. Am J Physiol 1990, 258:G16-G23.

Tannock GW. Effect of dietary and environmental stress on the gastrointestinal microbiota. In:Human Intestinal Microflora in Health and Disease, Hentges DJ ed. London: Academic Press, 1983:517-539.

Tantibhedhyangkul P, Hashim SA. Medium-chain triglycerides feeding in premature infants: effects on calcium and magnesium absorption. Pediatrics 1978, 61:537-545.

Thompson LU, Trinidad TP and Jenkins DJA. Methods for determining minerals available for absorption in the small intestine and colon. Trace Elements in Man and Animals 1991, 7:25-11 - 25-12.

Thompson LU, Trinidad TP, Wolever TMS. Calcium absorption in the colon of man. Trace elements in Man and Animals 1991; 7:30-5.

Titus E, Ahearn GA. Vertebrate gastrointestinal fermentation: transport mechanisms for volatile fatty acids. Am J Physiol 1992, 262 (Regulatory Integrative Comp Physiol 31):R547-553.

Tokunaga T, Oku T, Hosoya N. Utilization and excretion of new sweetener, fructooligossacharides (neosugar) in rats. J Nutr 1989, 119:553-559.

Tolstoi LG and Levin RM. Osteoporosis - the treatment controversy. Nutrition Today 1992, July/August pp 6-12.

Tomlin J, Read NW. The effect of resistant starch on colon function in humans. Br J Nutr 1990, 64:589-595.

Trier JS. Morphology of the epithelium of the small intestine. In:Handbook of Physiology Alimentary Canal. Intestinal Absorption. (code CF., ed), am Physiol Society. Washington DC, USA., 1968, vol III, section 6, pp. 1125-1176.

Trinidad TP. Mineral Availability in the Small intestine and Colon. MSc Thesis, University of Toronto, 1990.

Trinidad TP, Wolever TMS, Thompson LU. Interactive effects of calcium and short chain fatty acids on absorption in the distal colon of man. Nutr Res 1993, 13:417-425.

Trinidad TP, Wolever TMS, Thompson LU. Availability of Ca for absorption in the small intestine and colon from diets containing available and unavailable carbohydrates: an in vitro assessment. Int J Food Sci and Nutr, 1996, 47:83-88.

Trinidad TP, Wolever TMS, Thompson LU. The effect of acetate and/or propionate on calcium absorption in the distal colon of man. Am J Clin Nutr, 1996, 63:574-578.

Tsao OS, Miyashita K, Leung PY. Effect of ascorbic acid in calcium elimination in humans. J Nutr Sci Vitaminol 1986, 32:437-446.

Umesaki Y, Yajima T, Yokohura T, Mutai M. Effect of organic acid absorption on bicarbonate transport in rat colon. Pfluegers Arch 1979, 379:43-47.

Urban E, Pena M. Failure of lactose and glucose to influence in vivo intestinal calcium transport in normal rats. Digestion 1977, 15:18-27.

Van Houte H, Gibbons RJ. Studies of the cultivable flora of normal human feces. Antonie Van Leeuwenhock 1966, 32:212-222.

Vaughan OW, Filer LJ. The enhancing action of certain carbohydrates on the intestinal absorption of calcium in the rat. J Nutr 1960, 71:10-14.

Walser M. Magnesium metabolism. Ergeb Physiol 1967, 59:185-296.

Wasserman RH. The intestinal calbindins: their function, gene expression and modulation in genetic disease. In: Extra- and Intracellular Calcium and Phosphate Regulation, Bronner F and Peterlik M eds. CRC Press, Boca, Rotan, FL, 1992:43-70.

Wasserman RH, Chandler JS, Meyer SA, Smith CH, Brindak MI, Fullmer CS, Penniston JK and Kumar R. Intestinal calcium transport and calcium extrusion processes at the basolateral membrane. J Nutr 1992; 122:662-677.

Wasserman RH, Comar CL. Carbohydrates and gastrointestinal absorption of radiostrontium and radiocalcium in the rat. Proc Soc Exp Biol Med 1959, 101:314-317.

Wargowich MJ, Eng VWS, Newmark H. Calcium inhibits the damaging and compensatory proliferative effect of fatty acids on mouse colon epithelium. Cancer Lett 1984, 23:253-258.

Wasserman RH, Kallfelz EFA. Vitamin D and the unideirectional calcium fluxes across the rachitic chick duodenum. Am J Physiol 1962, 203:221-224.

Wasserman RH, Fullmer CS. Vitamin D and intestinal calcium transport: facts, speculations and hypotheses. J Nutr 1995, 125:1971S-1979S.

Wasserman RH, Lengeman FW. Further observation on lactose stimulation of the gastrointestinal absorption of calcium and strontium in the rat. J Nutr 1960, 70:377-384.

Wasserman RH, Taylor AN. Some aspects of the intestinal absorption of calcium with special reference to vitamin D. In: Mineral Metabolism, an Advanced Treatise. Connar C and Bonner F eds., Academic Press New York, USA, 1969, vol III, pp. 321-403.

Watford M, Lund P, Krebs HA. Isolation and metabolic characteristics of rat and chicken enterocytes. Biochem J 1979, 178:589-596.

Watson AJM, Elliot EJ, Rolston DDK, Borodo MM, Farthing MJG, Fairclough PD. Acetate absorption in the normal and secreting rat jejunum. Gut 1990, 31:170-174.

Weaver GH, Krausse JA, Miller TL, Wolin MJ. Consistency of glucose and starch fermentation by two different fecal microbial community. Gut 1989, 30:19-25.

Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML. Human calcium absorption from whole-wheat products. J Nutr 1991, 121:1769-1775.

Weaver CM, Martin BR, Ebner JS, Krueger CA. Oxalic acid decreases calcium absorption in rats. J Nutr 1987, 117:1903-1906.

Weaver CM, Plawecki KL. Dietary calcium: adequacy of a vegetarian diet. Am J Clin Nutr, 1994, 59(suppl):1238S-1241S.

Wensel RH, Rich C, Brown AC, Volwiler W. Absorption of calcium measured by intubation and perfusion of the intact human small intestine. J Clin Invest 1969, 48:1768-1775.

Wien EM, Schwartz R. Comparison of in vitro and in vivo measurement of dietary calcium exchangeability and bioavailability. J Nutr 1983, 113:388-393.

Wilkinson R. Studies of calcium absorption by small intestine in rat and man. PhD Thesis, University of Leids, 1971.

Williams PI, Warwick R (editors). Gray's Anatomy, 36th edition, New York 1985;1354-1358.

Wolever TMS, Brighenti F, Royall D, Jenkins AL, Jenkins DJA. Effect of rectal infusion of short chain fatty acids in human subjects. Am J Gastroenterol 1989, 84:1027-1033.

Wolever TMS, Cohen C, Thompson LU, Thorne MJ, Jenkins MJA, Prokipchuk KJ and Jenkins DJA. Ileal loss of available carbohydrate in man: comparison of a breath hydrogen method and direct method using a human ileostomy model. Am J Gastroentrol 1986, 81(2):115-121.

Wolever TMS, Trinidad TP, Thompson LU. Short chain fatty acid absorption from the human distal colon: interactions between acetate, propionate and Ca. J Am Coll Nutr 1995,14(4):393-398.

Wong NP, LaCroix DE. Biological availability of calcium in dairy products. Nutr Rep Int 1980, 21(5):673-680.

Wood RJ, Gerhardt A, Rosenberg IH. Effects of glucose and glucose polymers on calcium absorption in helathy subjects. Am J Clin Nutr 1987, 46:699-701.

Wyatt GM, Horn N. Fermentation of resistant food starches by human and rat intestinala bacteria. J Sci Food Agric 1988, 39:281-288.

Yergey AL, Abrams SA, Vieira NE, Aldroubi A, Marini J and Sidbury JB. Determination of fractional absorption of dietary calcium in humans. J Nutr 1994, 124:674-682.

Younoszai MK, Nachan R. Intestinal calcium absorption is enhanced by D-glucose in diabetic and control rats. Gastroenterol 1985, 88:933-938.

Yuan YV, Kitts DD, Nagasawa T. The effect of lactose and fermentation products on paracellular calcium absorption and femur biomechanics in rats. Can Inst Food Sci Technol 1991, 24:74-80.

Zheng JJ, Wood RJ, Rosenberg JH. Enhancement of calcium absorption in rats by coadministration of glucose polymer. Am J Clin Nutr 1985, 41:243-245.

Ziegler EE, Fomon SJ. Lactose enhances mineral absorption in infancy. J Pediatr Gastroenterol Nutr 1983, 2:288-294.

Zornitzer AE, Bronner F. In situ studies of calcium absorption in rats. Am J Physiol 1971, 220:1261-1265.

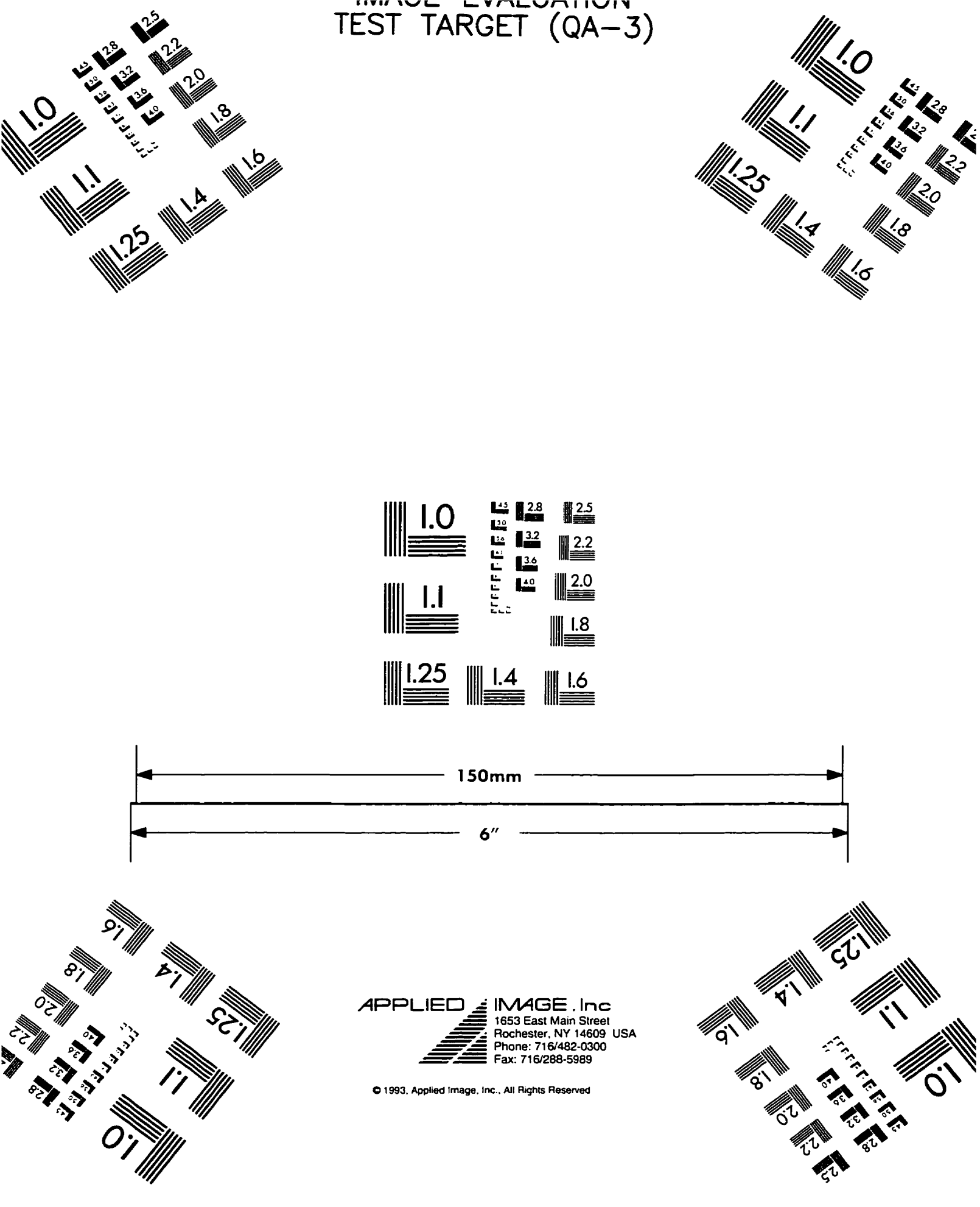


IMAGE EVALUATION
TEST TARGET (QA-3)
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
150mm
6"
APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989
© 1993, Applied Image, Inc., All Rights Reserved