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In vitro availability of calcium from sources of cellulose, methylcellulose, and psyllium

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Abstract

Certain types of dietary fibres may decrease the amount of dietary calcium available for absorption. The objective of this study was to determine the in vitro calcium availability from sources of cellulose, methylcellulose, and psyllium, such as cereals, fibre supplements, and purified fibres. Total dietary fibre, soluble and insoluble dietary fibre, acid detergent fibre, neutral detergent fibre, cellulose, hemicellulose, lignin, protein, uronic acid, phytate, water holding capacity, and total endogenous calcium content were determined. Results from the chemical analyses of the fibre sources varied significantly. Free endogenous calcium, at pH 3–8, was determined via gel filtration. Calcium was added to each fibre source and free exogenous calcium was determined via gel filtration at pH 1 and pH 8. The analysis of variance procedure was used to determine differences in binding capacities of the fibres. There was virtually no binding of exogenous calcium by sources of cellulose, methylcellulose, or psyllium.

Keywords: Calcium; Fibre; Cellulose; Methylcellulose; Psyllium; Calcium binding; Calcium availability

1. Introduction

Many in vitro studies suggest that certain types of dietary fibre decrease the amount of dietary calcium available for absorption. The binding capacity of dietary fibres for calcium is further impacted by the presence of other factors, such as oxalates and tannins (Harland, 1989). Blaney, Zee, Mongeau, and Marin (1996) found that ionic calcium was significantly reduced by oat bran, wheat bran, and lignin in the presence of protein, while pectin and cellulose had no effect on ionic calcium under the same conditions. Similarly, Torre, Rodriguez, and Saura-Calixto (1992) found a strong association between lignin and calcium ions in solution, and weak associations between cellulose and calcium, as well as between pectin and calcium.

Calcium-binding to dietary fibres may be pH-dependent. In vitro changes in pH, which parallel physiological conditions, impact mineral-binding capacities of various fibres (Thompson & Weber, 1979). The amount of calcium bound to soluble fibre-containing hydrocolloids decreased *in vitro* with a decrease in pH below six (Ha, Thomas, Dyck, & Kunkel, 1989). Heynck, Krampitz, and Hesse, (1995) found that soluble bran fibres weakly bound calcium at pH 2 and that adjusting the pH to six and eight significantly increased calcium binding. The authors suggested that most of the acid groups, such as the phosphate ester of phytic acid and the carboxyl ester of uronic acids, were undissociated at the lower pH and thus had fewer free binding sites for calcium. Wheat bran was found to bind significantly more calcium at pH 7 in vitro than rice bran or oat fibre and a significant amount of the calcium was released at pH 1. Wheat bran may either have more specific binding sites for calcium than rice bran or oat bran, or may have binding sites with a higher affinity for calcium. Furthermore, these three fibres bound significantly less calcium when added to the fibres in combination with other minerals (Idouraine, Khan, & Weber, 1996b). On the other hand, Weber, Kohlhepp, Idouraine, and Ochae (1993) found no significant relationship between insoluble fibre concentrations and calcium binding in vitro. Kelly and Potter (1990) found that acidification to pH 4, pasteurization, and boiling did not affect the per

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cent of calcium liberated from non-fat dry milk combined with soluble-fibre containing gums and thickeners.

Clearly, many factors impact the availability of calcium from soluble and insoluble dietary fibres. The objective of this study was to determine the effects of sources of three types of dietary fibre on calcium availability in vitro. The fibres studied included cellulose, methylcellulose, and psyllium. Cellulose is the most common component of the plant cell wall and therefore the most abundant organic substance in nature. Cellulose is a linear polymer of up to 10,000 β-1, 4-linked glucose units which strongly favours the formation of hydrogen-bonding between glucose units in the chain and between adjacent chains. Cellulose is an insoluble fibre and a common component of many cereal grains. Methylcellulose is the methyl ether of cellulose made by reacting cellulose with sodium hydroxide to produce alkali cellulose, which is then reacted with methyl chloride. As a soluble fibre and food additive, methylcellulose is used as a dispersing and thickening agent and is included in several common fibre supplements. Psyllium, or *Plantago psyllium*, is also known as fleawort and is native to the Mediterranean region, North Africa and India. Its laxative properties have been known for many centuries and are primarily due to its composition of β-1, 4-linked xylose units with various residues of arabinose, galacturonic acid, rhamnose, and galactose. Recent health claims of the beneficial effects of psyllium (Food and Drug Administration, 1998) have led to the inclusion of this soluble fibre in cereals and fibre supplements.

2. Materials and methods

2.1. Samples

Methylcellulose samples were Citrucel® brand fibre supplement (SmithKline Beecham, Pittsburgh, PA) purchased at a local drugstore and purified methylcellulose (Sigma, St. Louis, MO). Cellulose samples were Fiber One® cereal (General Mills, Minneapolis, MN), purchased from a local supermarket, and purified microcrystalline cellulose (Sigma, St. Louis, MO). Psyllium samples were All-Bran® Bran Buds® cereal (Kellogg's, Battle Creek, MI) purchased from a local supermarket, Metamucil® brand fibre supplement (Procter & Gamble, Cincinnati, OH) purchased from a local drugstore, and purified powdered psyllium seed husk (Frontier, Norway, IA) purchased from a local grocery store. Consistent lot numbers were purchased of samples which needed to be obtained in multiple boxes of cereal or containers of fibre supplement. These samples were considered representative of what could be purchased by a consumer. All samples were ground to pass a 1-mm screen.

2.2. Methods

2.2.1. Fibre analysis

Total, soluble, and insoluble dietary fibre contents of the samples were determined according to the AOAC Method 991.43 (1992). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by modifications of the method described by Robertson and Van Soest (1981) using an ANKOM$^{200/220}$ Fibre Analyzer (ANKOM Technology, Fairport, NY). To determine ADF, duplicate samples were agitated under pressure with hot acid detergent solution for 60 min, rinsed in hot water and dried. To determine lignin content, duplicate samples were digested in 72% (v/v) sulfuric acid, following ADF analysis. Cellulose content of samples was calculated from ADF minus the lignin content. To determine NDF, duplicate samples were agitated with neutral detergent solution and heat-stable α-amylase for 60 min, rinsed and dried. Hemicellulose content of samples was calculated as NDF minus ADF.

2.2.2. Chemical analysis

Nitrogen content of the samples was determined using the micro-Kjeldahl technique-modified from the AOAC Method 47.021 (1975). Protein content was calculated using 6.25 as the nitrogen to protein conversion factor. Uronic acid presence in 0.1 and 0.5% (w/v) fibre solutions was determined according to the method of Dische (1947). Phytate concentrations were determined according to the AOAC Method 986.11 (1990). Water-holding capacity was determined according to the AACC Method 88-04 (1991).

2.2.3. Calcium analysis

To determine endogenous calcium, triplicate 100-mg samples were wet-ashed with nitric acid and perchloric acid according to the method of Neidermeier, Griggs, and Johnson (1971). To determine endogenous calcium binding, all fibre solutions were made at 0.5% (w/v) in 0.5% (w/v) lanthanum chloride. Duplicate samples were adjusted to pH 3, 4, 5, 6, 7, and 8, with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. Solutions were mixed for approximately 2.5 h. After stirring, 0.5 ml of sample was applied to a Sephadex G-25M Column PD-10 (Pharmacia, Uppsala, Sweden). Samples were eluted to 100 ml in 10 ml increments, following the collection of a 5 ml void volume (as determined with Blue Dextran) with 0.5% (w/v) lanthanum chloride adjusted to pH 3, 4, 5, 6, 7, or 8 with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide.

To determine exogenous calcium-binding, all fibre solutions were made at 0.5% (w/v) in 0.5% (w/v) lanthanum chloride. The following amounts of calcium were added to duplicate fibre samples: 63.4 μg calcium to Citrucel®, 1000 μg calcium to methylcellulose, 71.5 μg calcium to Fiber One®, 1000 μg calcium to cellulose,

165 μg calcium to All-Bran® Bran Buds®, 180 μg calcium to Metamucil®, and 48 μg calcium to psyllium. The amount of calcium added to each sample was based on amount of total endogenous calcium detected. Samples were stirred for approximately 2.5 h. After stirring, 0.5 ml of sample was applied to a Sephadex G-25M Column PD-10 (Pharmacia, Uppsala, Sweden). Samples were eluted in a total of 50 ml, in 10 ml increments, following the collection of a 5 ml void volume (as determined with Blue Dextran) with 0.5% (w/v) lanthanum chloride.

To determine the effects of extremes in pH on exogenous calcium, duplicate fibre solutions were prepared with calcium using the same method as for the determination of exogenous calcium-binding. The pH was adjusted to one with 0.1N hydrochloric acid or to eight with 0.1 N sodium hydroxide. Samples were stirred for approximately 2.5 h. After stirring, 0.5 ml of sample was applied to a Sephadex G-25M Column PD-10 (Pharmacia, Uppsala, Sweden). Samples were eluted in a total of 50 ml, in 10 ml increments, following the collection of a 5 ml void volume (as determined with Blue Dextran) with 0.5% (w/v) lanthanum chloride, adjusted to the corresponding pH with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide.

Calcium atomic absorption reference solution (Fisher Scientific, Norcross, GA) was diluted to 0.5, 1.0, 1.5, 2.0, 4.0, and 5.0 μg/ml with 0.5% (w/v) lanthanum chloride for use as standards. Total endogenous calcium, free endogenous calcium, and free exogenous calcium content were analyzed with a Hitachi Model 170-50 Atomic Absorption Spectrophotometer with an air-acetylene flame and a hollow Ca-Mg cathode lamp at 422.7 nm.

2.2.4. Statistical analysis

All statistical analyses were performed using the Statistical Software System (SAS, Cary, NC). The analysis of variance procedure was performed to determine whether there were any differences among fibres in cellulose content, hemicellulose content, protein content, phytic acid content, uronic acid content, total endogenous calcium concentrations or water-holding capacity. A correlation analysis was performed to determine whether there were any relationships between water-holding capacity and concentrations of TDF, SDF, or insoluble dietary fibre (IDF). The general linear models procedure was performed to determine whether there were any relationships between pH and free calcium concentrations. The significance of differences were determined with the probability of a type I error set at 5%.

3. Results and discussion

3.1. Fibre analysis

Dietary fibre composition of the samples is listed in Table 1. Citrucel® and purified methylcellulose samples had significantly different contents of total dietary fibre (TDF) and soluble dietary fibre (SDF). These results are similar to the information that appears on the label of Citrucel® (SmithKline Beecham, Pittsburgh, PA). As expected, purified methylcellulose was nearly all dietary fibre. When expressed as a percentage of the respective mean TDF value, the mean SDF content of Citrucel® was 44.6% and the mean SDF content of methylcellulose was 26.5%.

Fiber One® and purified cellulose samples were significantly different in TDF and IDF content. These results agree with the information reported on the Nutrition Facts panel of Fiber One® (General Mills, Minneapolis, MN). The present data are much higher than results reported by Dreher (1987), in that Fiber One® was 38.0% total dietary fibre. As expected, purified cellulose was nearly all dietary fibre. The present data are higher than, but similar to, those reported by Weber et al. (1993), who reported that cellulose contained 91.4% total dietary fibre and 87.0% insoluble dietary fibre. When expressed as a percentage of TDF,

Table 1
Fibre analysis

Fibre Source	Components (g/100g)							
	Total dietary Fibre[a]	Soluble dietary fibre	Insoluble dietary fibre	Acid detergent fibre	Neutral detergent fibre	Lignin	Cellulose	Hemicellulose
Citrucel®	26.5±1.6 d	18.9±0.4 e	–	3.5±0.6 d	13.6±0.7 f	ND[b]	3.5±0.5 d	10.1±0.1 d
Methylcellulose	98.1±2.4 a	92.3±0.1 a	–	30.2±2.3 b	65.8±5.2 c	ND	30.2±2.3 b	35.6±2.9 b
Fiber One®	50.1±0.6 c	–	44.9±1.1 b	8.9±0.2 c	44.4±2.5 d	0.8±1.1 b	8.1±0.1 c	35.6±2.3 b
Cellulose	96.3±0.1 a	–	96.8±0.3 a	64.2±0.8 a	72.8±2.1 b	ND	64.2±0.8 a	8.6±1.2 d
All-Bran® Bran Buds®	51.5±2.1 c	27.1±0.5 d	–	8.7±0.5 c	36.5±1.3 e	1.4±0.1 a	7.3±0.4 c	27.9±0.8 c
Metamucil®	68.3±5.9 b	32.5±2.1 c	–	2.0±2.1 d	37.1±1.8 e	0.2±0.1 c	1.8±2.0 d	35.2±0.2 b
Psyllium	90.7±4.2 a	85.2±3.9 b	–	4.0±1.2 d	97.2±0.6 a	0.2±0.1 c	3.8±1.1 d	93.3±0.6 a

[a] Means ±S.E.M. in a column with the same letter are not significantly different ($P > 0.05$).
[b] None detected.

the IDF fraction of Fiber One® was 89.6% and the IDF fraction of cellulose was 100.5%.

All-Bran® Bran Buds®, Metamucil®, and purified psyllium were all significantly different from each other in their TDF and SDF contents. These data are much higher than results reported by Dreher (1987), in that All-Bran® was 30.1% total dietary fibre. These results are also higher than information provided on the Nutrition Facts panel of All-Bran® Bran Buds® (Kellogg, Battle Creek, MI) and lower than the information that appears on the label of Metamucil®. The present data are higher than, but similar to Robertson (1993), who reported that Metamucil® contained 52.6% total dietary fibre and 44.3% soluble dietary fibre. As expected, purified psyllium was mostly dietary fibre. Of the TDF content, 52.6% of All-Bran® Bran Buds®, 47.6% of Metamucil®, and 93.9% of psyllium was SDF. The present data for psyllium-containing samples may be higher than data reported elsewhere because there were difficulties in filtering the samples.

It has been suggested that the AOAC enzymatic–gravimetric method for determining dietary fibre results in higher values than those obtained by the Englyst chemical method (Englyst, Quigley, & Hudson, 1995). However, a collaborative study has concluded that the AOAC enzymatic–gravimetric method is the most effective method for determining dietary fibre for quality control and nutrition labelling purposes (Lee, 1995).

The methods of analysis of TDF and its components are continuously being scrutinized and improved (Asp, Johnasson, Hallmer, & Siljeström, 1983; Brillouet, Rouau, Hoebler, Barry, Carré, & Lorta, 1988; Halvarson & Alstin, 1984; Jung, 1997; Lee & Prosky, 1992; Mongeau & Brassard, 1990; Theander, Åman, Westerlund, Andersson, & Pettersson, 1995; Van Soest, Robertson, & Lewis, 1991). For example, the proportion of TDF extracted as SDF is dependent on the method of analysis (Marlett, Chesters, Longacre, & Bogdanske, 1989). SDF such as psyllium becomes highly viscous in aqueous solutions. Increased viscosity of the sources of psyllium impeded the filtering step and this may have resulted in artificially high TDF and SDF values in this study. Other researchers have also reported problems in the filtering step of the AOAC method with psyllium-containing fibre sources (Prosky, Asp, Schweizer, DeVries, & Furdu, 1988). Lee, Rodriguez, Storey, Farmakalidis, and Prosky (1995) have proposed a method for determining SDF and IDF from psyllium-containing foods, which includes sonication to reduce the viscosity of the sample. Using this technique the researchers found that purified psyllium contained 86.2% total dietary fibre and 71.1% soluble dietary fibre (Lee et al., 1995), lower values than what are reported in the present study.

Purified methylcellulose had significantly higher ADF and NDF contents than Citrucel® (Table 1). Purified cellulose had significantly higher ADF and NDF contents than Fiber One®. Nearly all of the dietary fibre in the 3 psyllium samples was NDF; purified psyllium had significantly higher NDF content than All-Bran® Bran Buds® or Metamucil®.

Lignin has a high affinity for calcium ions in solution (Torre et al., 1992). The lignin content of the fibres studied ranged from none detected (ND) to 1.4% (Table 1). Lignin may have been partially solubilized at the ADF determination step (Jung, 1997). Consequently, the cellulose content of the samples was calculated to be very similar to the ADF content. The hemicellulose content of the samples was determined by calculating the difference between ADF and NDF.

3.2. Chemical analysis

The protein contents of fibres studied are listed in Table 2. Citrucel® and purified methylcellulose did not have significantly different endogenous protein contents. Fiber One® had more than nine times the endogenous protein content of purified cellulose. The present figures are much higher than those provided on the Nutrition Facts panel of Fiber One® (General Mills, Minneapolis, MN), that a 30 g serving of the cereal contains 2 g, or 7%, protein. The present figures are similar to those of others (Blaney et al., 1996; Sosulski & Cadden, 1982; Thompson & Weber, 1979; Weber et al., 1993) who reported that cellulose was 0.0% crude protein. Metamucil® and purified psyllium did not have significantly

Table 2
Chemical analysis

Fibre source	Protein (g/100g)[a]	Water holding capacity (ml/g)	Phytic acid (mg/g)	Uronic acid
Citrucel®	6.3±1.4 bc	0.8±0.3 f	0.92±0.34 c	No
Methylcellulose	3.7±0.2 cd	8.8±0.5 c	ND[b]	No
Fiber One®	25.5±1.3 a	2.9±0.2 e	10.24±0.52 a	Yes
Cellulose	2.8±1.3 d	3.8±0.1 d	ND	Yes
All-Bran® Bran Buds®	25.9±0.4 a	2.8±0.1 e	8.54±1.66 b	Yes
Metamucil®	7.3±0.4 b	10.1±0.6 b	0.29±0.04 d	Yes
Psyllium	6.8±1.9 b	11.8±0.5 a	0.58±0.08 cd	Yes

[a] Means ±S.E.M. in a column with the same letter are not significantly different ($P > 0.05$).
[b] None detected.

different endogenous protein concentrations, but All-Bran® Bran Buds® had significantly more protein than either Metamucil® or purified psyllium. According to the Nutrition Facts panel of All-Bran® Bran Buds® (Kellogg, Battle Creek, MI), each 30 g serving of the cereal contains 3 g, or 10% protein, which is much lower than the present findings. The present data for Metamucil® and psyllium are lower than other findings of 16.6% crude protein in psyllium seeds (Sosulski & Cadden, 1982).

Fiber One® and All-Bran® Bran Buds® may have had higher than expected nitrogen concentrations because of nitrogen-containing compounds added during processing, or the occurrence of Maillard products, which may have formed during the processing of cereals. Maillard products are insoluble in 72% sulfuric acid and would have been recovered in the lignin fraction (Theander, Åman, Westerlund, & Graham, 1990). However, Rendleman (1982) concluded that the affinity of protein for calcium is very low and probably not a factor in the binding of calcium to cereal complexes, so any protein in these samples may not contribute to calcium-binding.

Water-holding capacities were significantly different for each fibre within groups (Table 2). Purified methylcellulose, Metamucil®, and purified psyllium, all of which are primarily soluble fibres, had significantly higher water-holding capacities than the cereal samples and Citrucel® fibre supplement. These results generally agree with data cited in the literature. Psyllium hydrocolloid has a water-holding capacity of 8.6–10.1 g water/g organic matter (McBurney, 1991; Sosulski & Cadden, 1982) and cellulose binds 2.5–3.6 g water/g sample (Dreher, 1987; Weber et al., 1993).

Figs. 1 and 2 illustrate the relationship between water-holding capacity and TDF and SDF, respectively. Although the correlations between water-holding capacity and TDF and SDF were not significant, there does appear to be a trend towards increased water-holding capacity with high TDF and SDF content of a fibre source.

Under physiological pH conditions phytic acid is ionized and able to bind with metal ions such as calcium (Nolan, Duffin, & McWeeny, 1987). Thus, the solubility of metal cations can be affected by phytate at physiological pH (Valiente, Mollá, Martin-Cabrejas, López-Andréu, & Esteban, 1996). The insolubility of the mineral-phytate complex leads to its negative impact on mineral bioavailability. In general, the solubility of the mineral-phytate complex is dependent on pH, the specific mineral, mineral and phytate concentrations, as well as the presence of other minerals (Champagne, 1988).

Phytate concentrations differed significantly among fibre samples (Table 2) and this may be because phytate can exist in many bound as well as free forms (Maga, 1982). Phytic acid is found in significant quantities in cereal grains (Frølich, 1990) so, as expected, the Fiber One® and All-Bran® Bran Buds® cereal samples had significantly higher phytate concentrations compared with the other fibre samples.

The presence or absence of uronic acid in the fibres is listed in Table 2. No uronic acid was detected in Citrucel® and purified methylcellulose samples. The cellulose and psyllium samples tested positive for uronic acid,

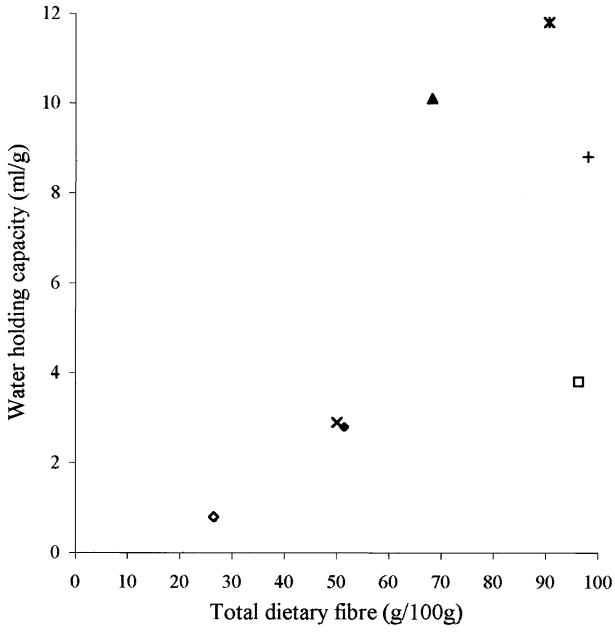

Fig. 1. Effect of total dietary fibre of fibre sources on water holding capacity. Fibre sources were: (◇) Citrucel®; (+) purified methylcellulose; (×) Fiber One®; (□) purified cellulose; (◆) All-Bran® Bran Buds®; (σ) Metamucil®; and (⋆) purified psyllium.

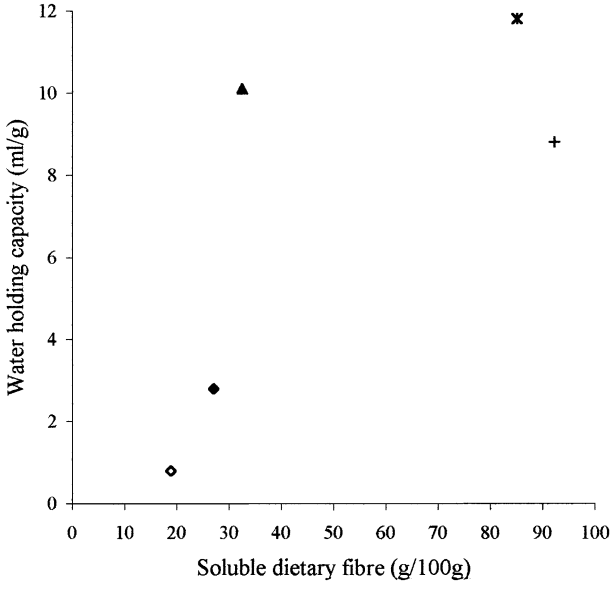

Fig. 2. Effect of soluble dietary fibre of fibre sources on water holding capacity. Fibre sources were: (◇) Citrucel®; (+) purified methylcellulose; (◆) All-Bran® Bran Buds®; (σ) Metamucil®; and (⋆) purified psyllium.

although no attempt was made to quantify the amount detected.

3.2.1. Calcium analysis

The amount of total endogenous calcium detected in the fibre samples is listed in Table 3. According to the label on Citrucel® (SmithKline Beecham, Pittsburgh, PA), dibasic calcium phosphate was added to the product, which may have contributed to the amount of free endogenous calcium detected. The present data generally agree with the information provided on the Nutrition Facts panels of Fiber One® (General Mills, Minneapolis, MN) and All-Bran® Bran Buds® (Kellogg, Battle Creek, MI). Metamucil® and purified psyllium contained less than 1.0% of endogenous calcium. Calcium was not detected in purified methylcellulose and purified cellulose samples. This generally agrees with findings that cellulose contains less than 1% endogenous calcium (Blaney et al., 1996).

It has been suggested that in vitro studies examining the interaction of minerals with fibre-rich foods be performed under sequential pH treatment, simulating gastrointestinal pH conditions, since many reactions are pH-dependent (Platt & Clydesdale, 1987). Therefore, free endogenous calcium was determined over a pH range of 3–8. No free endogenous calcium was detected for purified methylcellulose, purified cellulose, All-Bran® Bran Buds®, Metamucil®, or purified psyllium, at any pH tested. Citrucel® had 7.1 μg free calcium/mg sample at pH 3, 3.2 μg free calcium/mg sample at pH 4, and 3.5 μg free calcium/mg sample at pH 5. No free calcium was detected at pH 6, 7, or 8 from Citrucel®. Fiber One® had 1.5 μg free calcium/mg sample at pH 3 and no free calcium was detected at pH 4–8.

Thus, for Citrucel® and Fiber One®, more endogenous calcium was free at a lower pH compared with a higher pH. These results confirm that endogenous minerals in different fibre sources are free at acidic pH and bound when pH is raised (Thompson & Weber, 1979). A reason for this might be that most of the acid groups of fibre sources act as cation-exchangers, with high hydrogen ion density at low pH.

Table 3
Total endogenous calcium of fibre sources

Fibre	Calcium (μg/mg sample)[a]
Citrucel®	6.34±0.78 [a]
Methylcellulose	ND[b]
Fiber One®	1.43±0.01 b
Cellulose	ND
All-Bran® Bran Buds®	0.33±0.00 cd
Metamucil®	0.36±0.01 cd
Psyllium	0.96±0.06 bc

[a] Means ±S.E.M. in a column with the same letter are not significantly different (*P*bma0.05).
[b] None detected.

Calcium was added to each fibre solution at 5–1000 times the total endogenous calcium content at pH 1 and 8. The maximum amount of calcium added to each fibre source and the amounts bound and recovered are listed in Table 4. There was significantly more free calcium at pH 1 than at pH 8 from methylcellulose, All-Bran® Bran Buds®, and Metamucil®.

However, the present data show virtually no binding of exogenous calcium by sources of cellulose, methylcellulose, or psyllium. Torre et al. (1992) found that cellulose had little to no affinity for calcium over a pH range of 3–7. These researchers concluded that the binding ability of cellulose was not related to the hydroxyl group of its polymeric chains but rather a result of an adsorption phenomenon. Similarly, other researchers have reported extremely little interaction between calcium and cellulose (Chiang, Thomas, & Kunkel, 1994; Rendleman, 1982) as well as between calcium and hemicellulose at physiological pH (Rendleman, 1982). Other fibres that typically bind calcium ions have consistently been observed to release bound calcium at low pH (Ha et al., 1989; Kolb & Kunkel, 1994; Weber et al., 1993).

It is unlikely that the phytic acid content of the fibre samples contributed to any calcium binding in vitro. When expressed as a percentage, the mean phytic acid content of the fibre sources was 0.06% for purified

Table 4
Free and bound exogenous calcium from fibre sources at pH 1 and pH 8

Fibre	Calcium added (μg/mg sample)[a]	pH	Per cent free[b]	Per cent bound[c]
Citrucel®	63.4	1	90.3±3.0 a	9.7
	63.4	8	95.0±4.2 a	5.0
Methylcellulose	100	1	103±0.6 a	0
	100	8	109.6±1.9 b	0
Fiber One®	71.5	1	112±5.2 a	0
	71.5	8	98.0±2.7 b	2.0
Cellulose	100	1	105±1.1 a	0
	100	8	109±6.6 a	0
All-Bran® Bran Buds®	165	1	94.1±0.6 a	5.9
	165	8	100±4.0 b	0
Metamucil®	180	1	105±0.1 a	0
	180	8	123±1.1 b	0
Psyllium	48	1	104±7.1 a	0
	48	8	106±1.0 a	0

[a] Means ±S.E.M. in a column with the same letter are not significantly different ($P > 0.05$).
[b] Per cent free = amount recovered − free endogenous calcium.
[c] Per cent bound = 100 − per cent free.

psyllium, 0.03% for Metamucil®, and 0.85% for All-Bran® Bran Buds®. Shah, Malcolm, Belonje, Trick, Brassard, and Mongeau (1990) concluded that phytate levels from 0.1 to 1.1% of dietary fibre did not affect calcium bioavailability. It is possible that iron or copper formed highly insoluble complexes with phytic acid to reduce the number of binding sites available for calcium binding.

4. Conclusions

In vitro studies are appealing in bioavailability research because they allow for greater control over the subject matter, and are less expensive and typically faster than in vivo research. However, experimental variables are not always well defined in in vitro studies (Johnson, 1989) and the binding capacity values of soluble and insoluble fibres for calcium by different methods can vary significantly (Idouraine, Khan, Kohlhepp, & Weber, 1996a). Enzymatically digesting the fibre samples prior to calcium-binding experiments may have resulted in calcium-binding by exposing binding sites to the cation. It is also possible that calcium-binding was reduced due to competitive interactions between calcium and other minerals in the fibre samples. This may be the case, especially, for the cereal samples, which were fortified with minerals other than calcium.

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