ELSEVIER

Food Chemistry 73 (2001) 263–269

Food Chemistry

www.elsevier.com/locate/foodchem

Effects of dietary fibre extracts on calcium absorption in the rat

M.E. Harrington, A. Flynn, K.D. Cashman *

Department of Nutrition, University College, Cork, Ireland

Received 11 July 2000; received in revised form 30 October 2000; accepted 30 October 2000

Abstract

The effects on calcium absorption of several fibre extracts (which differed greatly in their chemical compositions, phytate concentrations, and proportions of soluble and insoluble fibre) and of phytate, as sodium phytate, were investigated in rats. Samples of fibre from apple, orange, pea, sugarbeet, barley and wheat were obtained from Sofalia (France). Calcium absorption (using a ^{47}Ca tracer method) in 9-week-old rats from a purified diet, containing fibre at a level of 50 g/kg, was reduced by wheat and wheat-TDF fibre preparations and was unaffected by apple, orange, pea, sugarbeet, barley, pectin and guar gum, compared to the cellulose-containing diet. Calcium absorption was also reduced by increasing phytate concentration in the range 0 to 30 mmol/kg diet. Overall, the results suggest that calcium absorption is not affected by fibre components per se, (i.e. indigestible polysaccharides) and that the reduction in calcium absorption observed with wheat and wheat-TDF fibres is due to their phytate contents.

Keywords: Dietary fibre; Extracts; Calcium absorption; Rats

1. Introduction

The daily consumption of dietary fibre in Europe is approximately 12–25 g (Cummings, 1993), and strong and continued recommendations have been made to double or even triple the current intake among most populations. The increased awareness of the potential health benefits of dietary fibre among consumers has encouraged food manufacturers to develop fibre-enriched food products which contain plant fibre extracts as ingredients.

However, in spite of the strong evidence for the beneficial effects of dietary fibres, one of the potential nutritional disadvantages of high fibre diets is the adverse effect on the bioavailability of micronutrients, especially minerals and trace elements. With regard to Ca, there is considerable evidence that phytate, which is associated with fibre in many foods, such as cereals and soya products, inhibits Ca absorption in humans (Heaney, Weaver & Fitzsimmons 1991; McCance & Widdowson, 1942a; Morris & Ellis, 1985b) and rats (Kunz & Lönnerdal, 1990; Lönnerdal, Sandberg, Sandström & Kunz, 1989; Taylor & Coleman, 1979). However, there is some doubt as to whether fibres per se have an influence on Ca absorption. For example, while some studies show a negative effect of fibre (predominantly cereal fibres) on Ca absorption (Behall, Scholfield, Lee, Powell & Moser, 1987; Knox et al., 1991; Reinhold, Faradji, Abadi & Ismail-Beigi, 1976; Turnlund, 1987; Weaver, Heaney, Martin & Fitzsimmons, 1991; Weaver, Heaney, Teegarden & Hinders, 1996), other studies have reported no effect (Andersson, Nävert, Bingham, Englyst & Cummings, 1983; O'Brien et al., 1993; Sandberg, Hasselblad, Hasselblad & Hultén, 1982; Van Dokkum, Wesstra & Schippers, 1982). Recently, attention has focused on fibre preparations that are soluble or partly soluble. For example, Hara, Nagata, Ohta and Kasai (1996) reported that ingestion of guar gum hydrolysate, a soluble, highly fermentable dietary fibre, increased apparent Ca absorption in rats. Inulin, a fructo-oligosaccharide, is another soluble fibre which has been shown to enhance Ca absorption in rats (Delzenne, Aertssens, Verplaetse, Roccaro & Roberfroid, 1995; Ohta, Ohtsuki, Baba, Adachi, Sakata &

* Corresponding author. Tel.: +353-21-4902883; fax: +353-21-4270244.

E-mail address: k.cashman@ucc.ie (K.D. Cashman).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00296-X

Sakaguchi, 1995; Levrat, Rémésy & Demigné, 1991) and humans (Coudray, Bellanger, Castiglia-Delavaud, Rémésy, Vermorel & Rayssignuier, 1997). It is apparent that the question of whether fibre adversely affects Ca absorption is far from clear and more research is warranted.

Therefore, the purpose of this study was to investigate the effect on Ca absorption of several fibre extracts which differed greatly in their chemical composition, phytate concentration, and ratio of soluble to insoluble fibre. In addition, we investigated the effect of phytate, as sodium phytate, on Ca absorption. The rat was used in the present study because the absorption mechanisms for Ca are similar in rats and in humans (Bronner, 1987; Norman, 1990) and a number of dietary and physiological factors affect Ca absorption similarly in the two species (e.g. age, pregnancy, lactation, 1, 25-dihydroxycholecalciferol, oxalic acid, phytate; Brommage & Binacua, 1991; Cashman & Flynn, 1996; Mahoney & Hendricks, 1985), although some exceptions to this have also been reported (e.g. lactose, phosphate; Hegsted, Schuette, Zemel & Linkswiler, 1981; Mahoney & Hendricks, 1978; Miller, 1989; Tremaine, Newcomer, Riggs & McGill, 1986).

2. Materials and methods

2.1. Dietary fibres

Dietary fibre preparations produced from apple, orange, pea, sugarbeet, barley and wheat were obtained from Sofalia, Paris, France. The types and selected fibre components of the fibre preparations is shown in Table 1. Pectin (from citrus fruits, Catalogue no. P-9135), guar gum (Catalogue no. G-4129) and sodium phytate were obtained from Sigma Chemical Co Ltd., Poole, Dorset, UK. Cellulose, in the form of Avicel microcrystalline cellulose, was obtained from FMC International, Food and Pharmaceutical Products Division, Little Island, Co. Cork, Ireland.

2.2. Preparation of rat diets

A modified AIN-76 purified diet (American Institute of Nutrition, 1977) was used in the present study. The mineral mix was modified by replacing $CaHPO_4$ with $CaCO_3$ as the sole source of Ca and by including KH_2PO_4 and K_2HPO_4 to supply the P requirement (Table 1). $CaCO_3$ was used in the diets, as the Ca source included in the test meals was $CaCO_3$ uniformly labelled with ^{47}Ca (see later). The Ca content of the fibre preparations was taken into account in formulating the diets to achieve a final Ca concentration of 50 g/kg diet. Cellulose (control) or dietary fibre preparations were included in the diet at a concentration of 50 g/kg. Phytate, as sodium phytate was included with cellulose (50 g/kg diet) in three of the diets at levels of 7.5, 15.0 and 30.0 mmol/kg diet in replacement for sucrose (Table 2).

2.3. Preparation of ^{47}calcium-labelled meals

Labelled $CaCO_3$ was prepared by mixing ^{47}Ca [as $^{47}CaCl_2$ in NaCl (9 g/l), specific activity 7.9 GBq/g; Forskningscenter Riso, 4000 Roskilde, Denmark] with 2 M-$CaCl_2$ addition of a slight molar excess of Na_2CO_3 to precipitate $CaCO_3$, and washing the precipitate on a filter, followed by drying at 100°C. ^{47}Ca-labelled meals, containing 5 g Ca/kg, were prepared by substituting ^{47}Ca-labelled $CaCO_3$ for $CaCO_3$and adding 0.1 g Sc/kg as $ScCl_3$(Aldrich Chemical Co., Milwaukee, WI, USA; as a carrier for ^{47}Sc) in the AIN-76 diet outlined in Table 2.

2.4. ^{47}Calcium absorption study

One hundred and four male rats, 7-weeks-old, Wistar strain (average weight 209 g), were obtained from the Biological Services Unit, University College, Cork, Ireland. Rats were randomized by weight into 13 groups of eight rats each. Rats were housed individually in cages with a grid-floor and a facility for separate collections of faeces and urine. Animals were fed ad libitum on a

Table 1
Fibre types and selected fibre components of the dietary fibre preparations (% of total dietary fibre)[a]

Fibre preparation	(g/kg)	Fibre type		Fibre component		
		Insouble (%)	Soluble (%)	Cellulose (%)	Lignin (%)	Hemicellulose + Pectin (%)
Apple	65	84.6	15.4	37.0	17.0	46.0
Orange	60	58.3	41.7	46.0	4.0	50.0
Pea	85	90.6	9.4	68.0	7.0	25.0
Sugarbeet	75	66.7	33.3	35.0	5.0	60.0
Wheat	55	85.5	14.5	33.0	5.0	62.0
Wheat-TDF[b]	60	NA	NA	NA	NA	NA
Barley	70	88.6	11.4	31.0	5.0	64.0

[a] Manufacturer's data (determined using the AOAC method).
[b] Wheat-TDF, wheat-total dietary fibre; NA, data not available.

purified diet (AIN-76) containing (g/kg): Ca as $CaCO_3$ 5.0, P 4.0, dietary fibres or cellulose (control) 50.0, or cellulose (50.0) plus phytate 13.3, 26.5 or 53.0 (representing 7.5, 15.0 or 30.0 mmol phytate/kg diet) and given distilled water ad libitum for 14 days. Feed was provided at 1700 h each day. On the fifteenth day, after fasting for 10 h (09.00–19.00 h), animals were given overnight (19.00–09.00 h) a radiolabelled meal (10 g AIN-76 diet), containing (per kg) 50 g of the dietary fibres or 13.3, 26.5 or 53.0 phytate, 5.0 g Ca as ^{47}Ca-labelled $CaCO_3$(0.037 MBq ^{47}Ca/10 g meal) and 0.2 g of the food dye Fast Green FCF (Sigma Chemical Co. Ltd). The dye permitted easy identification of the radioactive faeces. On the following morning the radiolabelled meal was removed and any remaining feed was weighed, and after 4 h, the rats were replaced on the AIN-76 diet to which they had been adapted.

Table 2
Composition of the modified AIN-76 diet (American Institute of Nutrition, 1977)[a]

Ingredient[a]	Content (g/kg)
Casein	200.0
DL-Methionine	3.0
Maize starch	150.0
Sucrose	487.5, (474.2), (461.0), (434.5)[b]
Fibre[c]	50.0
Maize oil	50.0
AIN mineral mix[d]	35.0
AIN vitamin mix[e]	10.0
Sodium phytate	0.0, (13.3) (26.5), (53.0)[b]
Calcium carbonate	12.5
Choline bitartrate	2.0

[a] Sources of ingredients: casein (sodium caseinate), Kerrymore Milk Products Ltd., Listowel, Co. Kerry, Ireland; DL-methionine, Rhone Poulenc, Animal Nutrition, Commentry, France; maize starch, Cagill Bergen op Zoom, the Netherlands; sucrose, Irish Sugar plc, Sugar Division, Athy Road, Carlow, Ireland; dietary fibres (see later); maize oil, St. Bernard's brand, Dunnes Stores Ltd., 67 Stephen's Green, Upper Dublin 8, Ireland; choline bitatrate, Brown and Gilmore, Carrigaline East, Co. Cork, Ireland; sodium phytate, Sigma Chemical Co Ltd., Poole, Dorset, UK.

[b] Representing diets containing (per kg) 0, 7.5, 15.0 or 30.0 mmol phytate in replacement for sucrose.

[c] Representing diets containing 50 g/kg of cellulose (Avicel microcrystalline cellulose, N. F., FMC International, Food and Pharmaceutical Products Division, Little Island, Co. Cork, Ireland), or apple, orange, pea, sugarbeet, barley, wheat, wheat-TDF (Sofalia, Paris, France), pectin or guar gum (Sigma Chemical Co Ltd., Poole, Dorset, UK).

[d] Contained (g/kg): potassium dihydrogen phosphate 376, dipotassium hydrogen phosphate 160, sodium chloride 74, magnesium oxide 24, manganous carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, chromium potassium sulphate 0.55, sucrose 354.

[e] Contained (per kg): nicotinic acid 3 g, calcium pantothenate 1.6 g, riboflavin 600 mg, thiamin-HCl 600 mg, pyridoxine-HCl 700 mg, folic acid 200 μg, biotin 20 μg, cyanocobalamin 1 mg, cholecalciferol 2.5 mg, menaquinone 5.0 mg, retinyl palmitate 120 mg, DL-α tocopheryl acetate 5000 mg.

Ca absorption was determined by modification of the ^{47}Sc:^{47}Ca ratio method of Brommage and Binacua (1991) and McCredie, Troehler and Bonjour (1984). This method is based on the fact that ^{47}Ca decays to ^{47}Sc, which is not absorbed by the adult rat intestine and thus acts as an inert marker for estimation of the intestinal absorption of ^{47}Ca. In this ^{47}Sc:^{47}Ca ratio method, quantitative collections of green-coloured faeces were carried out daily for 3 days immediately after administration of the radiolabelled meals. ^{47}Ca and ^{47}Sc in the daily faecal samples and in radiolabelled meal samples were determined in a well γ-counter (Compugamma, LKB Wallac, LKB Instruments Ltd, South Croydon, Surrey, UK) using energy ranges of 144–178 and 1144–1295 keV for ^{47}Sc and ^{47}Ca, respectively. Using these counting windows, neither isotope contributes to the counts for the other and complete discrimination between the isotopes is achieved.

Fractional absorption of ^{47}Ca was calculated using the following equation:

$$\text{Fractional absorption (\%)} = \frac{(^{47}\text{Sc}:^{47}\text{Ca})_{\text{faeces}} - (^{47}\text{Sc}:^{47}\text{Ca})_{\text{meal}}}{(^{47}\text{Sc}:^{47}\text{Ca})_{\text{faeces}} - (^{47}\text{Sc}:^{47}\text{Ca})_{\text{meal}} + (^{47}\text{Sc}:^{47}\text{Ca})_{\text{meal}} \times e^{a}_{t=0}}$$

where $e^{a} = e^{-(\lambda^{47}Sc - \lambda^{47}Ca)\times t}$ [t is the time elapsed after initial administration of isotope ($t=0$), $\lambda = \log 2/t_{0.5}$, $t_{0.5}$ is the radioactive half-life of the element ($t_{0.5}$ for ^{47}Sc and ^{47}Ca are 3.43 and 4.53 days, respectively)].

Endogenous loss of ^{47}Ca in faeces was calculated from the slope of the plot of log ^{47}Ca retention versus time, from day 3 to day 7 after feeding the radiolabelled meal, estimated from the loss of ^{47}Ca in quantitative faecal collections.

2.5. Experimental techniques

2.5.1. Calcium content of fibre preparations

Portions of the dietary fibre preparations were dry ashed according to the method of the Association of Official Analytical Chemists (AOAC, 1984). The ashed samples were reconstituted in 1 N-HNO_3 and Ca was analysed in triplicate by atomic absorption spectrophotometry (Pye-Unicam Atomic Absorption Spectrophotometer, Model SP9; Pye-Unicam, Cambridge, Cambs., UK) after appropriate dilution with $LaCl_3$ solution (5 g/l, BDH Ltd, Poole, Dorset, UK). A range of Ca standards was used to obtain a Ca calibration curve. The intra- and inter-assay CV for Ca was 2.8 and 7.8%, respectively.

2.5.2. Phytate content of fibre preparations

The phytate content of the fibre preparations was determined using the extraction and anion-exchange procedure of Harland and Oberleas (1986), followed by

digestion and P determination by the method of the AOAC (1984).

2.5.3. Statistical methods

Data are presented as means with their standard errors. ^{47}Ca absorption data was subjected to one-way analysis of variance (ANOVA) with variation attributed to fibre preparation (Snedecor & Cochran, 1967). To follow up the ANOVA, all pairs of means were compared by the method of least significant difference (Snedecor & Cochran).

3. Results and disscussion

Except for cellulose, pectin and guar gum, the dietary fibre preparations used in the present study were heterogeneous in composition and, in addition to fibre (Table 1), they also contained varying amounts of protein, fat, available carbohydrate, and ash (data not shown). The phytate content of the fibre preparations varied over a wide range (0.8–48.0 mmol/kg fibre) and was highest in the cereal fibres (15.0, 48.0 and 37.2 mmol phytate/kg in barley, wheat and wheat-TDF fibre preparations, respectively) and thus, in the meals containing the cereal fibres (Table 3).

In the present study, fractional absorption of Ca from a meal in 9-week-old rats was significantly ($P < 0.001$) reduced by wheat and wheat-TDF fibre extracts (by 16 and 17%, respectively), but was unaffected by the other fibre extracts, compared to the cellulose control (Table 3). These findings are similar to those obtained in a study by Behall et al. (1987) on the effects on Ca balance of wheat bran compared to purified dietary fibres in human subjects. They found that, while apparent Ca absorption was reduced by a daily supplement of 30 g of wheat bran, it was unaffected by a daily supplement of 24 g of purified fibres, such as cellulose, carboxymethylcellulose, locust bean gum or Karay gum. The authors suggested that a possible explanation for this was the high phytate content of the wheat bran compared to that of the purified fibres. Recently, Kennefick and Cashman (2000), using Caco-2 cell monolayers as a model for studying Ca absorption in humans, reported that the rate of transepithelial ^{45}Ca transport was unaffected by cellulose or barley fibre extract, whereas inclusion of wheat fibre extract (extracts which were identical to those used in the present study) in the Ca transport buffer (50 g fibre/l) significantly reduced the rate of ^{45}Ca transport (by 19%) relative to a control fibre (cellulose) transport buffer.

Table 3
Effect of dietary fibre preparations and phytate on fractional ^{47}calcium absorption in 9-week-old male rats[a] (mean values with their standard errors)

Fibre preparation[b]	n	Phytate (mmol/kg meal)	^{47}Ca absorption (%)	
			Mean[c]	S.E.
Cellulose (control)	8	0.00	54.5e	1.9
Guar gum	8	0.00	54.6e	2.3
Pectin	8	0.00	52.7e	2.9
Apple	8	0.11	48.4e	1.6
Orange	8	0.05	49.2e	1.1
Pea	8	0.08	52.9e	3.6
Sugarbeet	8	0.04	48.1e	3.3
Barley	8	0.75	53.1e	2.7
Wheat	8	2.40	45.3f	2.4
Wheat-TDF[d]	8	1.86	45.6f	2.6
Cellulose + phytate	8	7.50	45.9f	2.1
Cellulose + phytate	8	15.00	43.6f	1.7
Cellulose + phytate	8	30.00	40.8f	1.3
ANOVA (one-way), P value			< 0.001	

[a] For details of diets and procedures, see Table 1 and pp. 5–9.
[b] Fibre preparation included in the meal at a concentration of 50 g / kg meal.
[c] Mean values within the column with different letters are significantly different, $P < 0.05$ (ANOVA followed by least significant difference test).
[d] Wheat-TDF, Wheat-total dietary fibre.

There is considerable evidence that wheat bran is inhibitory to Ca absorption in humans and rats. As long ago as 1942, McCance and Widdowson reported that apparent Ca absorption in human subjects was lower from diets containing bread made from brown flour (92% extraction rate) than from diets containing bread made from white flour (69% extraction rate, and thus containing less fibre than the brown bread; McCance & Widdowson, 1942a). These findings are supported by a number of other more recent studies. For example, human subjects have been reported to go into negative Ca balance when fed diets containing 50% of calories from whole meal bread (Reinhold et al., 1976) or a daily supplement of 31 g wheat fibre (Cummings, Hill, Jivraj, Houston, Branch & Jenkins, 1979). Weaver et al. (1991) found that wheat bran (which is high in phytate), but not other wheat flour products, interferes with the absorption of co-ingested Ca in humans. Bagheri and Guéguen (1981) reported that diets containing wheat bran at levels of 50, 100 and 150 g wheat bran per kg diet reduced Ca balance in weanling rats, while Donangelo and Eggum (1986) reported that inclusion of 80 g wheat fibre/kg diet significantly reduced Ca absorption in 5-week-old rats.

The component(s) of wheat fibre responsible for the impairment of Ca absorption has not been identified with certainty, but fibre and/or phytate are most likely to be the major inhibitory factors. However, it has been suggested that it is difficult to separate clearly the effects of the two factors because their concentrations in cereal products are correlated (Miller, 1989). Recently, Kennefick and Cashman (2000) reported that dephytinization of a wheat fibre extract negated its inhibitory effects on the rate of transepithelial Ca transport and Ca

uptake by Caco-2 cells, which led the authors to conclude that their findings were strongly suggestive of the fact that phytate and not the fibre components per se was responsible for the impaired Ca bioavailability. In addition, McCance and Widdowson (1942b) found that reduction of the phytate content of brown bread resulted in increased apparent Ca absorption in human subjects. Morris and Ellis (1985a) found that apparent Ca absorption in human volunteers was significantly greater from dephytinized bran muffins than from whole-wheat bran muffins. There is also evidence from animal studies, which agrees with the findings in humans. Larsen (1991) reported that phytate reduction of a high phytate diet by phytase, resulted in a significant increase in Ca balance in weaned rats.

In the present study, wheat and wheat-TDF fibres contained much higher levels of phytate (2.40 and 1.86 mmol/kg diet, respectively) than the other fibre preparations (0.05–0.75 mmol/kg diet), and thus might explain the differential effects of these different dietary fibre extracts on Ca absorption. In addition, the findings of the present study showed that fractional absorption of meal Ca was significantly reduced by phytate, as sodium phytate, at levels present in some human foods. Increasing the phytate concentration of the diet from 0 to 7.5 mmol/kg reduced the fractional absorption of Ca by 16%. However, increasing the phytate content further had no further significant effect. This apparent plateau in the effect of phytate on calcium absorption has been noted in some studies (Cashman & Flynn, 1993; Kennefick & Cashman 2000) but not in others (McCance & Widdowson, 1942a; Morris & Ellis, 1985a,b), and thus warrants further investigation. Overall, the findings of the present study in rats agree with those from human studies that demonstrated an inhibition of Ca absorption by phytate (Heaney et al., 1991; McCance & Widdowson, 1942a; Morris & Ellis, 1985b). Furthermore, Kennefick and Cashman (2000) showed that transepithelial Ca transport and Ca uptake by Caco-2 cells was significantly reduced by phytate, as sodium phytate, when added at levels present in wheat fibre extract (the same fibre preparation that was used in the present study).

The mechanism by which phytate reduces Ca absorption is believed to be through the formation of unabsorbable phytate-Ca complexes in the small intestine, which is the principle site of Ca absorption (Erdman, 1979; Forbes, Weingartner, Parker, Bell & Erdman, 1979). Lönnerdal et al. (1989) showed that the inhibitory effect of the inositol phosphates on Ca absorption in suckling rats was confined to the penta- and hexaphosphates (phytate) and that the tri- and tetra-phosphates have no effect.

The observation in the present study in 9-week-old rats that dietary fibres other than those from wheat, i.e. those with a low content of phytate associated with them, have little or no effect on Ca absorption is supported by evidence from other studies. For example, pectin does not appear to affect Ca absorption in humans (Behall et al., 1987; Cummings et al., 1979) or rats (Bagheri & Guéguen, 1981). Cummings et al. (1979) observed that increasing pectin intake to 36 g/day had no overall effect on Ca balance in male subjects. Guar gum has been reported to have no effect on Ca absorption in humans (Behall et al) or rats (Tulung, Rémésy & Demigné, 1987).

The fibre extracts used in the present study had variable ratios of soluble to insoluble fibre content (Table 1). This could, to various degrees, increase the viscosity and reduce the rate of migration of minerals, which in addition to reduced transit time could result in changes in the bioavailability of Ca (FrØlich, 1995). For example, Coudray et al. (1997) found that addition of inulin (40/day), a highly soluble fibre, to a control diet (containing 18 g dietary fibres/day) for 28 days significantly increased apparent absorption of Ca in young adult men compared to the control diet, whereas addition of sugarbeet fibre (40 g/day), a partly soluble fibre, had no effect on Ca absorption. However, in the present study, Ca absorption in young adult rats was unaffected by the addition of soluble (guar gum, pectin), partly soluble (orange, sugarbeet) or relatively insoluble fibres (apple, pea, wheat, barley) to the diets for 2 weeks.

In conclusion, the results from the present study suggest that wheat fibre extracts significantly reduced Ca absorption. This may have been due to the phytate in the wheat fibre extract rather than the fibre components per se. Other cereal- and fruit and vegetable-based fibre extracts, which had relatively lower phytate contents, and were heterogeneous in fibre composition (i.e.% cellulose, lignan, hemicellulose and pectin) and in the ratio of soluble to insoluble fibre, had no effect on Ca absorption. Therefore, our findings suggest that only fibre extracts with a high phytate content adversely affect Ca bioavailability. Dephytinization of such high-phytate fibre extracts, before adding them to products, could negate this nutritional disadvantage of the otherwise beneficial food ingredients.

Acknowledgements

This research was partly supported by an EU FLAIR Concerted Action Project (AGRT-CT-90-0038C).

References

American Institute of Nutrition (1977). Report of the American Institute *ad hoc* committee on standards for nutritional studies. *Journal of Nutrition, 107*, 1340–1348.

Andersson, H., Nävert, B., Bingham, S. A., Englyst, H. N., & Cummings, J. H. (1983). The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. *British Journal of Nutrition, 50*, 503–510.

Association of Official Analytical Chemists (AOAC). (1984) In S. Williams *Official methods of analysis of the AOAC Williams S ed.* Virginia: The Association of Official Analytical Chemists Inc.

Bagheri, M., & Guéguen, L. (1981). Influence of wheat bran diets containing unequal amounts of calcium magnesium phosphorus and zinc upon the absorption of these minerals in rats. *Nutr. Reports. Int., 32*, 271–274.

Behall, K. M., Scholfield, D. J., Lee, K., Powell, A. S., & Moser, P. B. (1987). Mineral balance in adult men: effect of four refined fibres. *American Journal of Clinical Nutrition, 46*, 307–314.

Brommage, R., & Binacua, C. (1991). Intestinal calcium absorption varies during the rat estrous cycle. In P. Burckhardt, & R. P. Heaney, *Nutritonal aspects of osteoporosis* (pp. 147–155). New York: Raven Press.

Bronner, F. (1987). Intestinal calcium absorption: Mechanisms and applications. *Journal of Nutrition, 117*, 1347–1352.

Cashman, K., & Flynn, A. (1993). Calcium absorption from cow's milk and soya milks in young rats. *Proc. Nutr. Soc., 52*, 273A.

Cashman, K., & Flynn, A. (1996). Effect of dietary calcium intake and meal calcium content on calcium absorption in the rat. *British Journal of Nutrition, 76*, 463–470.

Coudray, C., Bellanger, J., Castiglia-Delavaud, C., Rémésy, C., Vermorel, M., & Rayssignuier, Y. (1997). Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *European Journal of Clinical Nutrition, 51*, 375–380.

Cummings, J. H. (1993). Dietary fibre intakes in Europe: an overview. In J. Cummings, & W. Frolich, *Food technology, dietary intake in Europe — a survey of the Cost 92 concerted action on 'metabolic and physiological aspects of dietary fibre in food' commission of the European communities* (Vol. 200) (pp. 11–19). Brussels: DG XII, B-1049 Rue de la Loi.

Cummings, J. H., Hill, M. J., Jivraj, T., Houston, H., Branch, W. J., & Jenkins, D. J. A. (1979). The effect of meat protein and dietary fibre on colonic function and metabolism I. Changes in bowel habit bile acid excretion and calcium absorption. *American Journal of Clinical Nutrition, 32*, 2086–2093.

Delzenne, N., Aertssens, J., Verplaetse, H., Roccaro, M., & Roberfroid, M. (1995). Effect of fermentable fructo-oligosaccharides on mineral, nitrogen and energy digestive balance in the rat. *Life Science, 57*, 1579–1587.

Donangelo, C. M., & Eggum, B. O. (1986). Comparitve effects of wheat bran and barley husk on nutrient utilization in rats II. zinc, calcium and phosphorus. *British Journal of Nutrition, 56*, 269–280.

Erdman Jr., J. W. (1979). Oilseed phytates: nutritional implictions. *Journal of the American Oil Chemists' Society, 56*, 736–740.

Forbes, R. M., Weingartner, K. E., Parker, H. M., Bell, R. R., & Erdman, J. W. (1979). Bioavailability to rats of zinc magnesium and calcium in casein- egg- and soy protein-containing diets. *Journal of Nutrition, 109*, 1652–1660.

FrØlich, W. (1995). Bioavailability of micronutrients in a fibre-rich diet, especially related to minerals. *European Journal of Clinical Nutrition, 49*(3), S116–S122.

Hara, H., Nagata, M., Ohta, A., & Kasai, T. (1996). Increases in calcium absorption with ingestion of soluble dietary fibre, guar-gum hydrolysate, depend on the caecum in partially nephrectomized and normal rats. *British Journal of Nutrition, 76*, 773–784.

Harland, B., & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: Collaborative Study. *J. AOAC, 69*, 667–670.

Heaney, R. P., Weaver, C. M., & Fitzsimmons, M. C. (1991). Soybean phytate content: effect on calcium absorption. *American Journal of Clinical of Nutrition, 53*, 745–747.

Hegsted, M., Schuette, S. A., Zemel, M. B., & Linskwiler, H. M. (1981). Urinary calcium and calcium balance in young men as affected by level of protein and phosphorus intake. *Journal of Nutrition, 111*, 553–562.

Kennefick, S., & Cashman, K. D. (2000). Inhibitory effect of wheat fibre extract on calcium absorption in Caco-2 cells: evidence for a role of associated phytate rather than fibre per se. *European Journal of Nutrition, 39*, 12–17.

Knox, T. A., Kassarjian, Z., Dawson-Hughes, B., Golner, B. B., Dallal, G. E., Arora, S., & Russell, R. M. (1991). Calcium absorption in elderly subjects on high- and low-fiber diets: effect of gastric acidity. *American Journal of Clinical Nutrition, 53*, 1480–1486.

Kunz, C., & Lönnerdal, B. (1990). Calcium retention from human milk infant formula and preterm formula. *FASEB Journal, 4*, A521.

Larsen, T. (1991). Dephytinization of a rat diet. Consequences for mineral and trace element absorption. *Biological Trace Element Research, 39*, 55–71.

Levrat, M. A., Remesy, C., & Demigne, C. (1991). High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. *Journal of Nutrition, 121*, 1730–1737.

Lönnerdal, B., Sandberg, A. S., Sandström, B., & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *Journal of Nutrition, 119*, 211–214.

Mahoney, A. W., & Hendricks, D. G. (1978). Some effects of different compounds on iron and calcium absorption. *Journal of Food Science, 43*, 1473–1476.

Mahoney, A. W., & Hendricks, D. G. (1985). Assaying calcium bioavailability in foods (applicability of the rat as a model). In C. Kies, *Nutritional bioavailability of calcium* (pp. 17–27). Washington DC: American Chemical Society.

McCance, R. A., & Widdowson, E. M. (1942a). Mineral metabolism of healthy adults on white and brown dietaries. *Journal of Physiology, 10*, 44–85.

McCance, R. A., & Widdowson, E. M. (1942b). Mineral metabolism on dephytinised bread. *Journal of Physiology, 101*, 304–306.

McCredie, D. A., Troehler, U., & Bonjour, J. P. (1984). In vivo determination of intestinal calcium absorption, with scandium-47 used as a marker. *J. Lab. Clin. Med., 103*, 354–362.

Miller, D. D. (1989). Calcium in the diet: Food sources recommened intakes and nutritional bioavailability. *Adv. Food Nutr. Res., 33*, 103–156.

Morris, E. R., & Ellis, R. (1985a). Bioavailability of dietary calcium: effect of phytate on adult men consuming nonvegetarian diets. In C. Kied, *Nutritional Bioavailability of Calcium Symposium Series, 275* (pp. 63–72). Washington DC: American Chemical Society.

Morris, E. R., & Ellis, R. (1985b). Trace element nutriture of adult men consuming three levels of phytate. In C. F. Hills, I. Bremner, & J. K. Chester, *Trace elements in man and animals, 5* (pp. 443–452). Slough, England: Commonwealth Agricultural Bureaux.

Norman, A. W. (1990). Intestinal calcium absorption: a vitamin D-hormone mediated adaptive response. *American Journal of Clinical Nutrition, 51*, 290–300.

O'Brien, K. O., Allen, L. H., Quatromoni, P., Sui Caldera, M. L., Vieira, N. E., Perez, A., Holick, M. F., & Yergey, A. L. (1993). High fiber diets slow bone turnover in young men but have no effect on efficiency of intestinal calcium absorption. *Journal of Nutrition, 123*, 2122–2128.

Ohta, A., Ohtsuki, M., Baba, S., Adachi, T., Sakata, T., & Sakaguchi, E. (1995). Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. *Journal of Nutrition, 125*, 2417–2424.

Reinhold, J. G., Faradji, B., Abadi, P., & Ismail-Beigi, F. (1976). Decreased absorption of calcium, magnesium, zinc and phospohorus by humans due to increased fibre and phorphorus consumption as wheat bread. *Journal of Nutrition, 106*, 493–503.

Sandberg, A. S., Hasselblad, C., Hasselblad, K., & Hultén, L. (1982). The effect of wheat bran on the absorption of minerals in the small intestine. *British Journal of Nutrition, 48*, 185–191.

Snedecor, G. W., & Cochran, W. G. (1967). *Statistical methods Iowa state*. Ames, Iowa: University Press.

Taylor, T. G., & Coleman, J. W. (1979). A comparitive study of the absorption of calcium and the availability of phytate-phosphorus in

the golden hamster (Mesorcricetus auratus) and the laboratory rat. *British Journal of Nutrition, 42*, 113–119.

Tremaine, W. J., Newcomer, A. D., Riggs, B. L., & McGill, B. D. (1986). Calcium absorption from milk in lactase-deficient and lactase-sufficient adults. *Digestive Diseases and Sciences, 31*, 376–378.

Tulung, B., Rémésy, C., & Demigné, C. (1987). Specific effect of guar gum or gum arabic on adaptation of cecal digestion to high fiber diets in the rat. *Journal of Nutrition, 117*, 1556–1561.

Turnlund, J. R. (1987). Carbohydrate, fiber and mineral interactions. In O. A. Levander, *Nutrition '87* (pp. 37–40). Bethesda, MD: American Institute of Nutrition.

Van Dokkum, W., Wesstra, A., & Schippers, F. A. (1982). Physiological effect of fibre-rich types of bread I. The effect of dietary fibre from bread on the mineral balance of young men. *British Journal of Nutrition, 47*, 451–460.

Weaver, C. M., Heaney, R. P., Martin, B. R., & Fitzsimmons, M. L. (1991). Human calcium absorption from whole-wheat products. *Journal of Nutrition, 121*, 1769–1775.

Weaver, C. M., Heaney, R. P., Teegarden, D., & Hinders, S. M. (1996). Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *Journal of Nutrition, 126*, 303–307.