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Methodological considerations in measuring human calcium absorption: relevance to study the effects of inulin-type fructans

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During the last 50 years, a variety of methods have been developed to estimate Ca absorption in man. Mass balances were initially used, but these were unable to accurately measure fractional Ca absorption because they cannot distinguish unabsorbed dietary Ca from endogenous faecal Ca excretion (excretion of previously absorbed Ca back into the gut). A number of isotopic methods have been developed that can measure true fractional Ca absorption, employing radioisotopes, stable isotopes, or both. Different methods involve collection of urine, faecal or plasma samples. Of the currently available methods, the dual isotope tracer method with a timed urine collection is probably the most precise and reliable. It is also relatively straightforward to carry out and avoids the need for a faecal collection. The purpose of the present paper is to discuss the general advantages and disadvantages of the different methods of Ca absorption. In addition, the limitations the different methods have in examining the possible effects of non-digestible oligosaccharides on Ca absorption will be discussed.

Calcium absorption: Stable isotopes: Non-digestible oligosaccharides

Maintenance of good Ca nutrition is vital throughout the life cycle, both to achieve optimum peak bone mineral mass and to reduce subsequent declines in peak bone mineral mass (IOM, 1997). Ca is absorbed by two main mechanisms – an active saturable transcellular process and a passive non-saturable paracellular process (Weaver & Heaney, 1999). The paracellular process becomes increasingly important as Ca intake increases and the transcellular pathway becomes saturated (Weaver & Heaney, 1999).

Active transport appears to be calbindin-dependent and in turn dependent on vitamin D status as $1,25(OH)_2$ vitamin D increases calbindin mRNA transcription. After binding Ca^{2+} ions at the luminal surface of the enterocytes, calbindin is internalized via endocytosis. Fusion with lysozymes cause a pH-dependent release of Ca^{2+} ions and calbindin is recycled to the enterocyte luminal membrane. Ca^{2+} ions are exported across the basolateral surface by an ATP-dependent Ca pump, an Na/Ca exchanger or by endocytosis (Weaver & Heaney, 1999). Paracellular Ca transport occurs via the enterocyte tight junctions and is increased as the tightness of the junctions falls (Weaver & Heaney, 1999).

Although most Ca absorption occurs in the small intestine, about 5 % occurs in the colon (Barger-Lux *et al.* 1989). Although this contribution is relatively small, it may be particularly important when examining the effect of non-digestible oligosaccharides on Ca absorption, as we shall discuss later.

In practical terms, Ca behaves as a threshold nutrient (Matkovic & Heaney, 1992). When Ca intakes are low, an increase in Ca intake leads to significantly higher Ca absorption. However, at a certain Ca intake, a threshold is reached and increasing intake further has relatively little effect on total Ca absorption (Matkovic & Heaney, 1992).

Carbohydrates and calcium absorption

In recent years, there has been an increasing understanding of the role of disaccharides, oligosaccharides, soluble fibre and other carbohydrates as enhancers of Ca absorption. Animal or human studies support a beneficial effect of lactose (Demigné *et al.* 1989; Brommage *et al.* 1993; Abrams *et al.* 2002), lactulose (Brommage *et al.* 1993; van den Heuvel *et al.* 1999*a*; Beynen *et al.* 2001), mannitol (Fukahori *et al.* 1998), pectin (Demigné *et al.* 1989), polydextran (Hara *et al.* 2000), starches (Demigné *et al.* 1989), difructose anhydrides (Mitamura *et al.* 2002), galactooligosaccharides (Chonan & Watanuki, 1995; Chonan *et al.* 1995; van den Heuvel *et al.* 2000) and inulin-type fructans (Delzenne *et al.* 1995; Ohta *et al.* 1995*a*, 1999; Coudray *et al.* 1997; Griffin *et al.* 2002, 2003; Uenshi *et al.* 2002) on Ca absorption. Of these, the most widely studied in man, are the inulin-type fructans and, to a lesser extent, the galactooligosaccharides. However, before considering the human literature, it is first important to review the possible methods by which Ca absorption can be measured in man.

Methods to measure calcium absorption in man

Mass balance

The most obvious method to measure Ca absorption is using a mass balance. All dietary Ca consumed over a given period is calculated and Ca losses in the feces and urine over the corresponding period measured. Ca absorption is given by

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$$\text{Calcium absorption} = \text{Calcium intake} - \text{Urinary calcium excretion} - \text{Faecal calcium.}$$

However, this method has major limitations. Faecal Ca comprises both dietary Ca that has been unabsorbed, and absorbed Ca that has been re-secreted back into the gastrointestinal tract (endogenous faecal Ca excretion). As such, it measures net Ca balance rather than true fractional Ca absorption (Heaney, 2001). Although this may be useful in certain circumstances, it is not the optimal method for assessing the effect of oligosaccharides on Ca absorption. Oligosaccharides are likely to change Ca absorption but have little if any direct effect on endogenous faecal Ca absorption. Therefore, measurement of fractional Ca absorption is much more helpful in understanding the effects of oligosaccharides on Ca bioavailability.

Faecal isotope balance

Radioisotopes had been used since 1940s to study Ca metabolism in animals, but it was not until the mid-1950s that they were used in man. The first isotope used was ^{45}Ca, which decays by β-emission. The radioisotope was administered orally and fractional Ca excretion measured from the appearance of the isotope in faeces over the next several days (Blau *et al.* 1954; Bronner & Harris, 1954, 1956). As the dose of radioisotope given was low, absorbed radioisotope made a minor contribution to the exchangeable Ca pool. Also, as the faecal collection was of relatively short duration, little absorbed radioisotope was excreted in the gut and the confounding effect of re-secretion of previously absorbed isotope (endogenous faecal Ca excretion) was minimized. The method, therefore, gave a good estimation of fractional Ca excretion. Due to its relatively long half-life, orally administered ^{45}Ca could be allowed to equilibrate with the rapidly exchangeable Ca pool and its subsequent excretion could be used to estimate endogenous faecal Ca excretion (Blau *et al.* 1957). Similar studies could be carried out with intravenously administered ^{45}Ca (Bronner & Harris, 1956). The main problems of this method were the exposure to radioactivity and the need for a careful faecal collection. Although using the shorter half-life γ-emitter ^{47}Ca reduced the radiation exposure (Jaworski *et al.* 1963), a faecal collection was still required.

Serum isotope appearance

When a Ca isotope is given orally, its peak absorption can occur as quickly as 15 min later, after which the serum concentration of the isotope declines exponentially (Szymendera *et al.* 1972). Several investigators have tried to use the plasma appearance of an oral isotope as a measure of Ca absorption. For example, Bhandarkar *et al.* (1961) used the specific activity of ^{47}Ca 2 h after oral dosing as a measure of Ca absorption, and Jaworski *et al.* (1963) used the peak specific activity of ^{47}Ca as an index of Ca absorption and compared it to the faecal balance method. They studied eight healthy adults whose Ca absorption by faecal isotope balance was between 30·9 and 43·6 %; however, the peak specific activity was much more variable ranging from 1·2 to 2·3 % of the oral dose (Jaworski *et al.* 1963). The authors concluded, 'the plasma radioactivity peak…was not found to be a reliable index of intestinal calcium absorption' (Jaworski *et al.* 1963). This is not surprising as the peak specific activity depends on a number of factors in addition to the amount of isotope absorbed. Differences exist, for example, in the size of the exchangeable Ca pool that oral absorbed Ca is distributed across (presumably largely a reflection of body size) and this would change specific activity, independent of the amount of isotope absorbed. Furthermore, the specific activity at a given time-point is the integration of Ca entry to that pool (absorption) and clearance from that pool (a combination of excretion and uptake by tissues). As clearance is likely to vary between individuals, a further source of variability is introduced. This factor should vary little from day-to-day, so paired measurements in the same subject may reflect *relative* changes in Ca absorption; however, this method does not allow measurement of *absolute* fractional Ca absorption. There are a number of methods to try and correct for these between-subject differences in clearance and pool size. Lekkerkerker *et al.* (1971) used the clearance of an intravenous dose of ^{45}Ca to correct the plasma appearance curve of a subsequent dose of oral ^{45}Ca and estimate true fractional absorption. Szymendera *et al.* (1972) administered ^{47}Ca orally and ^{45}Ca intravenously and used the plasma appearance of ^{47}Ca to correct the appearance data for ^{45}Ca and estimate true Ca absorption. This method gave estimates of true Ca absorption very similar to those obtained simultaneously from faecal isotope balance or the ^{47}Ca:^{45}Ca equilibrium ratio in urine and plasma (Szymendera *et al.* 1972).

A much more appealing correction has been described by Heaney & Recker (1985) initially in adult women. In this method, the serum specific activity of a radioisotope is measured 5 h after oral dosing and it is corrected for differences in pool size using a body surface area correction. These investigators measured Ca absorption by the current gold standard, the dual stable isotope method (see later), and correlated this with the specific activity of an oral isotope in plasma 5 h later. Together, true Ca absorption (by the dual isotope tracer method) and body surface area explained almost 92 % of the variance in the 5 h specific activity of the oral Ca isotope. Ca absorption could be accurately estimated by correcting the 5 h specific activity for differences in body surface area using the equations (Heaney & Recker, 1985)

$$\text{Calcium absorption} = (2{\cdot}305 \times 5\,\text{h specific activity}) + (0{\cdot}1084 \times \text{body surface area}) - 0{\cdot}1735$$

or

$$\text{Calcium absorption} = (2{\cdot}305 \times 5\,\text{h specific activity}) + (0{\cdot}0220 \times (\text{weight in kilograms})^{0{\cdot}425}) \times (\text{height in centimetres})^{0{\cdot}725}) - 0{\cdot}1735.$$

Subsequently, similar work has been carried out to produce similar equations for adult men (Heaney *et al.* 2002). Although this method has typically been carried out using radioisotope, there are no theoretical reasons why it could not be modified to use stable isotopes. Currently, the method is only validated in adults and it is inappropriate to extend its use beyond that age range, say to children or adolescents, without additional validation studies.

Dual isotope methods

The dual isotope tracer method is probably the most commonly used method to measure Ca absorption and it is widely felt to

be the optimum method (Heaney, 2001). Two different isotopes are administered – one orally and one intravenously. Ca absorption is measured from the relative recovery of the oral and intravenous isotopes in a urine sample (Abrams, 1999). Several versions of this method have been described differing principally in the timing of the urine collection and whether stable isotopes (Hillman *et al.* 1988; Abrams, 1999), radioisotopes (Griessen *et al.* 1985) or both (Beck *et al.* 2003) are used. The method was first described by Bronner (1962) and subsequently by DeGrazia *et al.* (1965).

In the early description of the method, urine was collected at least 24 h after isotope administration and was shown to correlate well with faecal isotope balance (DeGrazia *et al.* 1965). Subsequently, a complete 24 h urine collection has been preferred (Hillman *et al.* 1988; Eastell *et al.* 1989). This method correlates well with the data from faecal balance (Eastell *et al.* 1989; Abrams *et al.* 1994) although the variability is less for the dual isotope tracer method than for faecal balance (Abrams *et al.* 1994). The dual isotope tracer method also correlated well with estimates made by deconvolution analysis (Yergey *et al.* 1994) and whole body counting (Beck *et al.* 2003). However, measurement made on a random 'spot' urine sample significantly overestimate Ca absorption compared to the dual isotope tracer method (Yergey *et al.* 1994).

This method is easy to carry out, requiring only a single intravenous infusion and a urine collection (Abrams, 1999). It is more precise than other methods with a CV for repeated measures in the same subject of about 10 %, most of which is true biological variation (Heaney *et al.* 1988). It is less affected by homeostatic control of plasma Ca concentrations than some other methods (Heaney, 2001) and the use of tracers increases signal to noise ratio and improves precision (Heaney, 2001). It avoids the need for a prolonged faecal collection, is less variable than faecal balance methods and is generally considered the optimum method of measuring Ca absorption (Heaney, 2001).

We have used such methods to measure Ca absorption across the entire life cycle (Abrams *et al.* 1994, 1995, 2002; Abrams, 1999; Ames *et al.* 1999; Griffin *et al.* 2002, 2003). A stable isotope is given orally with breakfast and a second isotope given by slow intravenous infusion. As soon as isotopes are administered a 24-h urine collection (occasionally longer) is started. Ca absorption is calculated using the equation (Abrams, 1999)

$$\begin{aligned}&\text{Calcium absorption}\\&= (\text{Urinary excretion of oral tracer}/\text{Dose of oral tracer})/\\&\quad(\text{Urinary excretion of intravenous tracer}/\\&\qquad\text{Dose of intravenous tracer}).\end{aligned}$$

Serum calcium appearance

Several investigators have attempted to estimate Ca absorption by the increase in serum Ca after a relatively large Ca dose. This has all the disadvantages inherent in the serum isotope appearance method. In addition, a relatively large change in serum Ca concentration is required. However, serum Ca concentration is under homeostatic control and this serves to damp down the changes in serum Ca and reduce signal to noise ratio (Heaney, 2001). Heaney (2003) compared the 5 h specific activity method to the serum Ca absorption method and showed that there was a statistically significant correlation between the two. The area under the curve for serum Ca appearance was calculated for the first 5, 7, 9 or 12 h after the test meal. The correlation between the 5 h specific activity method and the serum Ca absorption method was best when the area under the curve for the first 9 h was used. However, even in that case, the area under the curve explained less than 50 % of the variability in true fractional Ca absorption (Heaney, 2003). Although this method may be useful for comparing different groups, it is far less precise than other methods. This is partly because the calcaemia that is required to produce leads to homeostatic changes that serve to blunt the risk in total serum Ca. As isotopic methods require a far smaller rise in total serum Ca in order to produce measurable changes in serum tracer concentration, these homeostatic effects are greatly reduced.

Urine calcium excretion

This method uses the urinary excretion of Ca after a large Ca load as a measure of Ca absorption. Therefore, it relies on producing calcaemia proportional to the amount of Ca absorbed and that this then spills over into the urine. As such it has all the limitations of the serum Ca appearance method, as well as the additional variability introduced by between-subject differences in renal handling of Ca. This method would be expected to be even more variable and less precise than the serum Ca appearance method (Heaney, 2001).

Special considerations when studying the effects of non-digestible oligosaccharides on calcium absorption

As mentioned already, oral Ca is absorbed very rapidly, with maximum absorption occurring within 15–20 min (Szymendera *et al.* 1972). Once specific activity peaks, it falls exponentially over the next several days (Szymendera *et al.* 1972). As the amount of Ca absorbed distally in the gastrointestinal tract is normally small, measuring absorption 5 h after ingestion of the isotope can produce good estimates of Ca absorption (Heaney & Recker, 1985; Heaney *et al.* 2002). Under normal circumstances less than 5 % of Ca absorption occurs in the colon (Barger-Lux *et al.* 1989). This contribution to total Ca absorption will be 'missed' by measurements that occur before the ingested Ca has reached the colon, as these measurements cannot anticipate what will occur in the colon. Although this error is usually small, it may be very important when studying the effects of non-digestible oligosaccharides on Ca absorption.

The mechanisms by which non-digestible oligosaccharides affect Ca absorption are not entirely clear. In human patients with a permanent ileostomy, almost 90 % of ingested inulin-type fructans pass through the ileum undigested. Upon entering the large intestine they may be fermented to SCFA (Van Loo *et al.* 1999) and lower the pH of the luminal contents (Greger, 1999), increase the amount of Ca in the soluble phase and so may increase colonic absorption of Ca. An alternative hypothesis is that non-digestible oligosaccharides have an overall trophic effect on the gut that leads to increased Ca absorption along the length of the gastrointestinal tract (Greger, 1999). A third possibility is that they may have effects on tight junctions leading to increased paracellular absorption (Mineo *et al.* 2002). A better understanding of the mechanism(s) by which non-digestible oligosaccharides affect Ca absorption is urgently needed. Until the potential site at which non-digestible oligosaccharides enhance Ca absorption is known, the use of methods that are capable of including colonic Ca absorption should be used.

Experiments in rats tend to support the first hypothesis and have suggested that non-digestible oligosaccharides may increase Ca absorption most in the distal parts of the gut (Ohta *et al.* 1995*a*). Non-digestible oligosaccharides increase the amount of soluble Ca in the caecal lumen (Younes *et al.* 1993; Ohta *et al.* 1995*b*) and increase caecal Ca absorption (Ohta *et al.* 1995*a*). This enhancement of Ca absorption is lost in rats following a caecectomy (Ohta *et al.* 1994). Although the rat is a relatively poor model for human mineral absorption, a similar mechanism may occur in man. In order to fully assess the effect of non-digestible oligosaccharides on Ca absorption, the selected methods should be able to 'capture' any enhancement of colonic Ca absorption. The use of methods such as the 5 h specific activity (Heaney & Recker, 1985; Heaney *et al.* 2002) or urine excretion methods carried out a few hours after administration of the test meal would clearly be inadequate for this purpose.

Non-digestible oligosaccharides and Ca absorption

A number of studies have been carried out on the effect on non-digestible oligosaccharides on Ca absorption (Table 1). The most studied compounds are the inulin-type fructans such as oligofructose and inulin. The most widely used method has been the dual isotope tracer technique, although mass balances have also been used. More recently, shorter-term urinary methods have been employed (Ohta *et al.* 1999; Uenshi *et al.* 2002) that would fail to capture any colonic component to absorption.

A variety of inulin-type fructans have been studied. Oligofructose is a mixture of linear fructans with low degrees of polymerization and short chain length. In contrast, the high-molecular-weight inulins have much longer chain lengths and a higher degree of polymerization. A third type is oligofructose-enriched inulin that contains both short chain length (oligofructose) and long chain length (inulin) fructans. Two studies have been carried out using galactooligosaccharides that like oligofructose have relatively short chain lengths.

van den Heuvel *et al.* (1998) studied the effect of three different non-digestible oligosaccharides in twelve men using the dual isotope tracer technique. Neither high-molecular-weight inulin, oligofructose nor galactooligosaccharide had any effect on Ca absorption. These results contradicted earlier work by Coudray *et al.* (1997) that had shown that high-molecular-weight inulin significantly increased Ca absorption in adult men using an 8 d mass balance (Coudray *et al.* 1997). One possible reason for this discrepancy may have been the length of the urine collection in the van den Heuvel study. It was speculated that the 24 h collection period used may have been inadequate to capture any enhancing effect of the oligosaccharides on colonic Ca absorption (Coudray & Fairweather-Tait, 1998). This criticism appeared to be confirmed when further studies by the same investigator showed that both oligofructose (van den Heuvel *et al.* 1999*b*) and *trans*-galactooligosaccharides (van den Heuvel *et al.* 2000) significantly increased Ca absorption when the dual isotope tracer method was modified to include a 36 h urine collection. However, since then additional studies have made the effect of oligofructose less, rather than more, clear. Two studies have failed to show any benefit of oligofructose on Ca absorption using dual isotope tracer methods with a 48 h urine collection (Griffin *et al.* 2002) or a 5–7 d mass balance (Tahiri *et al.* 2003). Recently, two studies have shown a beneficial effect of a different type of oligofructose (Meioligo-P; Meiji Seika, Chiyoda, Japan) on Ca absorption (Ohta *et al.* 1999; Uenshi *et al.* 2002). However, these studies are methodologically limited as neither could assess the colonic phase of Ca absorption. Ohta *et al.* (1999) estimated Ca absorption from Ca-fortified candies with and without added oligofructose from the urinary excretion of Ca 2, 4, 6 and 8 h after ingestion of the candies. At each time-point, urinary Ca excretion was highest from the oligofructose-containing candy (Ohta *et al.* 1999). These results are difficult to interpret, as the design would have missed any colonic component of Ca absorption. Furthermore, no adaptation period was allowed before the start of the study. In other studies, variable periods of adaptation have occurred to the oligosaccharide diet. The reason for this is that the proposed mechanism of increased mineral absorption – a change in colonic pH or an overall trophic effect on the gut – would be expected to take several days to reach a steady state. The results of the Ohta study show, instead, very short-term effects as no adaptation period was allowed (Ohta *et al.* 1999). This raises the possibility that the addition of oligofructose had a physical effect on the candy leading to increased solubility or bioavailability shortly after consumption. Similar problems exist with the study of Uenshi *et al.* (2002) who measured Ca absorption from a malt beverage with or without oligofructose.

Table 1. Summary of the studies examining the effects of non-digestible oligosaccharides on calcium absorption in man

Authors	Oligosaccharide studied	Subjects	Method	Results
van den Heuvel *et al.* (1998)	Oligofructose	12 men (20–30 years)	Dual isotope (24 h collection)	No change
van den Heuvel *et al.* (1999)	Oligofructose	12 males (14–16 years)	Dual isotope (36 h collection)	Increase
Ohta *et al.* (1999)	Oligofructose	10 males	Urinary Ca (2, 4, 6, 8 h samples)	Increase
Griffin *et al.* (2002)	Oligofructose	30 girls (11–14 years)	Dual isotope (48 h collection)	No change
Uenshi *et al.* (2002)	Oligofructose	8 women (20–22 years)	Urinary Ca, urinary ^{44}Ca:^{43}Ca (12 h collection)	Increase
Tahiri *et al.* (2003)	Oligofructose	12 postmenopausal women	Isotope balance (5–7 d)	No change*
Coudray *et al.* (1997)	High-molecular-weight inulin	9 men	Balance (8 d)	Increase
van den Heuvel *et al.* (1998)	High-molecular-weight inulin	12 men (20–30 years)	Dual isotope (24 h collection)	No change
Griffin *et al.* (2002)	Oligofructose-enriched inulin	30 girls (11–14 years)	Dual isotope (48 h collection)	Increase
van den Heuvel *et al.* (1998)	Galactooligosaccharides	12 men (20–30 years)	Dual isotope (24 h collection)	No change
van den Heuvel *et al.* (2000)	Galactooligosaccharides	12 postmenopausal women	Dual isotope (36 h collection)	Increase

* Trend for increased Ca absorption in women >6 years post-menopause.

Once again, the method of estimating Ca absorption (urinary Ca excretion and urinary ^{43}Ca:^{44}Ca ratio within 12 h of taking the drink) would have missed any colonic component and no adaptation period allowed (Uenshi *et al.* 2002). Once again, this raises the possibility that these inulin-type fructans may be working by a different mechanism than in other studies.

Although the data for short-chain length inulin-type fructans such as oligofructose are contradictory, the data for inulin are more consistent. Except for the study by van den Heuvel *et al.* (1998) with a 24 h urine collection discussed earlier, the two other studies using an 8 d balance (Coudray *et al.* 1997) or a dual isotope tracer method with 48 h urine collection (Griffin *et al.* 2002) show significant effects on Ca absorption. One of these studies has shown a beneficial effect with a propriety inulin preparation consisting of a combination of high-molecular-weight inulin and oligofructose (i.e. oligofructose-enriched inulin), but not oligofructose alone (Griffin *et al.* 2002).

There are relatively few data concerning the effect of galactooligosaccharides on Ca absorption. One study by van den Heuvel *et al.* (1998) using a dual isotope tracer method with a 24 h urine collection showed no effect on Ca absorption, while a second study by the same investigators using 36 h urine collection did show a beneficial effect on Ca absorption (van den Heuvel *et al.* 2000).

We have recently pooled our data on the effect of oligofructose-enriched inulin on Ca absorption (Griffin *et al.* 2002) with investigators from Omaha Nebraska who used a very similar protocol to study the effects of a proprietary oligofructose-enriched inulin (Synergy-1; Orafti, Thienen, Belgium; Griffin *et al.* 2003). Overall oligofructose-enriched inulin significantly increased Ca absorption (36·1 (SD 9·8) % *v.* 33·1 (SD 9·2) %, $P=0·027$). However, this effect was seen in the Houston cohort (38·2 (SD 9·8) % *v.* 32·3 (SD 9·8) %, $P=0·007$) but not in the Omaha cohort (33·9 (SD 8·4) % *v.* 33·6 (SD 9·4) %, $P=0·87$). Weight, height, age and pubertal stage were not associated with a beneficial response to oligofructose-enriched inulin. The response did differ between different ethnic groups (African–Americans +8·9 (SD 12·2) %, Caucasians +1·0 (SD 8·4) %, Hispanics +13·6 (SD 9·1) %, Others −1·1 (SD 9·3) %; $P=0·003$). The increase in fractional Ca absorption when consuming oligofructose-enriched inulin was significantly greater in non-Caucasians (+8·7 (SD 11·0) %) than in Caucasians (+1·0 (SD 8·4) %; $P=0·008$). When studied by multiple regression analysis, the only factor related to a beneficial effect of oligofructose-enriched inulin was Ca absorption on placebo. The largest benefit was seen in subjects with the poorest Ca absorption on placebo. Some of this effect was due to regression to the mean, but a significant proportion was a true biological effect (Griffin *et al.* 2003). Variability amongst results from different studies may, therefore, be related to intrinsic differences between study populations, as well as differences in methodology and in the exact chemical nature of the oligosaccharides studied. How do the effects of these prebiotics on Ca absorption compare with other types of fermentable fibre? Is it a fermentation effect or a true prebiotic effect?

Summary and conclusion

Many methods exist to measure Ca absorption in man. The preferred method remains the dual isotope tracer method. It is simple to carry out, avoids the need for a prolonged faecal collection and does not involve the use of ionizing radiation. It is also more precise than other methods. In the specific case of non-digestible oligosaccharide, the dual isotope tracer method has a further advantage – the urine collection can be prolonged to capture any colonic component of Ca absorption.

In order to fully understand the effect of non-digestible oligosaccharides on Ca absorption, a better understanding of their mechanism of action is required. Do they enhance Ca absorption along the length of the gastrointestinal tract or selectively increase colonic absorption? To answer this, a more sophisticated approach will be required to examine the kinetics and time course of Ca absorption before and after adaptation to non-digestible oligosaccharides.

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