The effect of calcium on iron absorption

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The experimental and epidemiological evidence demonstrating that Ca inhibits Fe absorption was reviewed, with the objectives of estimating the potential impact of variations in Ca intake on dietary Fe bioavailability and of providing some guidelines for predicting the effects on Fe status of recent recommendations for higher dietary Ca intake. In animal models Ca salts reduced both haem- and non-haem-Fe absorption, the effect being dependent on the amount of Ca administered rather than the Ca:Fe molar ratio; dairy products had a variable effect; factors other than Ca may have been important. In single-meal human absorption studies, both haem- and non-haem-Fe absorption was inhibited by Ca supplements and by dairy products, the effect depending on the simultaneous presence of Ca and Fe in the lumen of the upper small intestine and also occurring when Ca and Fe were given in the fasting state. The quantitative effect, although dose dependent, was modified by the form in which Ca was administered and by other dietary constituents (such as phosphate, phytate and ascorbic acid) known to affect Fe bioavailability. The mechanism by which Ca influences Fe absorption has not been elucidated. The effects of factors that modulate Fe bioavailability are known to be exaggerated in single-meal studies, and measurements based on several meals are more likely to reflect the true nutritional impact. The results of most multiple-meal human studies suggest that Ca supplementation will have only a small effect on Fe absorption unless habitual Ca consumption is very low. Outcome analyses showed that Ca supplements had no effect on Fe status in infants fed Fe-fortified formula, lactating women, adolescent girls and adult men and women. However it should be noted that the subjects studied had adequate intakes of bioavailable Fe and, except in one study, had relatively high habitual Ca intakes. Although cross-sectional analyses in Europe have shown a significant inverse correlation between Ca intake (derived primarily from dairy foods) and Fe stores, the quantitative effect was relatively small. The general conclusion is that dietary Ca supplements are unlikely to have a biologically significant impact on Fe balance in Western societies unless Ca consumption is habitually very low; however, increased consumption of dairy products may have a small negative effect that could be functionally important in pregnancy if Fe

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Abbreviation: SU.VI.MAX, Supplementation des Vitamines et Mineraux Antioxydants.

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supplements are not taken. It is uncertain whether the inverse relationship between consumption of dairy products and Fe status is due entirely to increased Ca intake; substitution of milk proteins for meat may also have negative effects on Fe balance.

Iron: Absorption: Calcium: Supplementation

Introduction

Worldwide, Fe deficiency is the commonest nutritional deficiency. As many as two-thirds of women and young children may be affected in many developing countries (DeMaeyer & Adiels-Tegman, 1985). The prevalence is much lower in industrialized societies where meat consumption is high and many foods are fortified with inorganic Fe. Nevertheless, a small but significant proportion of women and young children still suffer from Fe deficiency in Western societies. In the USA, 9% of toddlers aged 1–2 years and 9–11% of adolescent girls and women of child-bearing age are Fe deficient (Looker *et al.* 1997).

Dietary Fe intake always exceeds the body's requirements by a significant margin. Fe deficiency occurs because the human diet contains compounds that limit the absorption of the food Fe when physiological requirements are increased by growth, pregnancy or blood loss (Bothwell *et al.* 1979; Baynes, 1994). Ca salts and dairy products have been shown, in various experimental studies, to have this property. The importance of an adequate Ca intake for bone formation and the recent strong advocacy of Ca supplementation (NIH Consensus Development Panel on Optimal Calcium Intake, 1994) have raised concern in the nutritional community about possible deleterious effects on Fe nutrition (Whiting, 1995; Whiting & Wood, 1997; Hallberg, 1998).

Physiology of iron balance

Fe excretion is unregulated in man (Bothwell *et al.* 1979). In the absence of bleeding or pregnancy, adults lose only a small quantity of Fe each day (Green *et al.* 1968); body Fe is therefore highly conserved. Men need to absorb only 1 mg each day to remain in Fe balance. Absorption is limited to 5–10% of the dietary intake. Menstrual blood loss increases the requirement in women. The average menstruating woman must absorb 1·4 mg/d (approximately 15% of intake; Bothwell *et al.* 1979; Hallberg & Rossander-Hulten, 1991). Requirements are even higher during pregnancy; a woman may need to absorb 4–5 mg/d to maintain Fe balance in the last trimester. Requirements are also increased significantly in young children and adolescents because of rapid growth (Dallman, 1992).

The preservation of Fe homoeostasis when requirements are high depends on the ability of the digestive and absorptive processes to extract additional Fe from the food. For example, more than 50% of the Fe in a high-bioavailability diet can be absorbed during the last trimester of pregnancy (Barrett *et al.* 1994). Dietary factors that reduce bioavailability by even a relatively small margin may have a significant impact on Fe balance.

There are two pathways for the absorption of Fe in human subjects (Baynes, 1994); one mediates the uptake of the small quantity of haem-Fe derived primarily from haemoglobin and myoglobin in meat; the other regulates the absorption of elemental Fe that can be extracted

from food and rendered soluble in the lumen of the stomach and duodenum (non-haem-Fe). Most of the Fe eaten by human subjects is in the latter non-haem form.

Haem-Fe is highly bioavailable. Absorption is little affected by most dietary factors. The haem moiety enters the mucosal cell intact. After cleavage of the haem ring by haem oxygenase, the Fe is delivered to an intracellular pool shared with non-haem-Fe absorbed from the diet (Grasbeck *et al.* 1979, 1982; Parmley *et al.* 1981; Wheby & Spyker, 1981). The subsequent fate of Fe in the common intracellular pool is discussed below.

Non-haem-Fe absorption commences with the solubilization of predominantly Fe^{3+} food Fe in the acid milieu of the stomach and its reduction to Fe^{2+} by dietary components such as ascorbic acid (Dorey *et al.* 1993; Han *et al.* 1995) or ferrireductase present at the mucosal surfaces of cells in the duodenum (Jordan & Kaplan, 1994; Han *et al.* 1995). This bioavailable Fe is then absorbed in a three-step process: it is taken up by the enterocytes found on the tips of duodenal villi, crossing the cellular apical membrane by an energy-dependent carrier-mediated process (Muir & Hopfer, 1985; Baynes, 1994); it is transported intracellularly; finally, it is transferred across the basolateral membrane, where it is bound to transferrin.

A series of studies reported recently by Gunshin *et al.* (1997) and Canonne-Hergaux *et al.* (1999) strongly suggest that divalent metal transporter-1 (Nramp 2) mediates the uptake of elemental Fe into duodenal cells. The protein is expressed on the apical brush-border surfaces of duodenal mucosal villous tip cells. Expression is related to the body's Fe requirements and is markedly up-regulated in animals placed on a low-Fe diet.

The mechanisms involved in the transport of Fe to the basolateral surface of the enterocyte and its release to circulating transferrin are poorly understood (Baynes, 1994). Excess Fe taken up by mucosal cells that is not transferred to the plasma is stored as ferritin. Only about 10% of this storage Fe re-enters the transfer pathway (Wheby *et al.* 1964); most of it is eventually lost with exfoliation of the cell.

Non-haem-Fe absorption is regulated both during its entry into enterocytes and at the time of its exit to the circulation. Kinetic modelling in dogs indicates that the rate-limiting step is uptake of the metal from the duodenal lumen across the apical brush-border membrane (Nathanson & McLaren, 1987). Although the absorption pathway for elemental Fe is relatively specific, it may be shared partially by several other metals including Co, Mn, Pb and Zn, but not Ca. Ca absorption is not increased in Fe-deficient rats (Pollack *et al.* 1965). Furthermore, Ca does not appear to be a substrate for divalent metal transporter-1 (Gunshin *et al.* 1997).

In contrast to Fe, Ca absorption can occur throughout the intestine (Bronner & Pansu, 1999). When the intake is low, transcellular transport in the duodenum is up-regulated. Entry into duodenal cells occurs across the brush borders down an electrochemical gradient via Ca channels. Transfer across the cell to the basolateral pole is the rate-limiting step. It is mediated by the vitamin D-dependent Ca-binding protein calbindin (Feher *et al.* 1992; Stein 1992). Transfer out of the cell at the basolateral surface is mediated by Ca ATPase (Carafoli, 1994). When the Ca intake is high (> 800 mg/d), absorption occurs primarily by passive paracellular diffusion throughout the small intestine. Absorption also occurs in the caecum and ascending colon. Active transport in the duodenum is down-regulated.

It appears, therefore, that Fe and Ca are absorbed by independent cellular mechanisms. An interaction between Ca and food components that affect Fe bioavailability, or an effect of Ca on the luminal surface receptors that mediate Fe uptake into enterocytes might provide more plausible explanations for the inhibitory effect of Ca than competition for an intracellular transport mechanism.

Calcium intake and sources of calcium

Dairy products are the primary sources of dietary Ca. Of the Ca in the food supply of the USA, 73% is derived from dairy products, 9% from fruits and vegetables, 5% from grain products and the remainder from all other sources (Center for Nutrition Policy and Promotion, US Department of Agriculture, 1996). The estimated median daily intakes for male and female subjects aged 9 years and older are 925 mg and 657 mg respectively (Cleveland *et al.* 1996). Approximately 25% of American women take Ca supplements (Moss *et al.* 1989), while the corresponding percentages for men and children aged 2–6 years are 14 and 7·5 respectively. The median dose (mg/d) is also highest in women, 248 compared with 160 and 88 for men and children respectively.

Effect of calcium salts and dairy products on iron absorption

The effect of several Ca salts and dairy products on Fe absorption has been tested in both experimental animals and human volunteers.

Studies in experimental animals

Kletzein (1935, 1938) first demonstrated that $CaCO_3$ inhibits Fe absorption in experimental animals. Subsequently, several sources of Ca (including $CaCO_3$, $CaCl_2$, calcium lactate, calcium phosphate and bone meal) have been shown to reduce Fe retention and the rate of haemoglobin regeneration in animal models (Anderson *et al.* 1940; Kletzein, 1940; Freeman & Ivy, 1942; Chapman & Campbell, 1957*a*,*b*; Dunn, 1968). Fe-deficiency anaemia occurred in weanling rats given large amounts of Ca in their diets (Fuhr & Steenbock, 1943). Young female mice and their litters became anaemic when the dams were fed high doses of $CaCO_3$ (Greig, 1952). Although the majority of experiments carried out in experimental animals have demonstrated an inhibitory effect of Ca, the finding has not been universal. Wauben & Atkinson (1999) recently reported that Ca did not inhibit Fe absorption or alter Fe status in infant piglets. The quantities of Ca and Fe used by Wauben & Atkinson (1999) were chosen to reflect current feeding practices for premature human infants.

Radioisotope-labelling techniques have been used in experimental animals to confirm the inhibitory effect of Ca on elemental Fe absorption and to elucidate the mechanism by which it exerts this effect (Manis & Schachter, 1962*a*,*b*; Greenberger *et al.* 1969; Amine & Hegsted, 1971; Barton *et al.* 1983). Ca reduced the uptake of Fe by duodenal brush borders isolated from rats and piglets (Greenberger *et al.* 1969; Wauben & Atkinson, 1999). It also decreased and delayed the uptake of Fe from a $FeCl_2$ solution introduced into isolated gastrointestinal loops *in vivo* in rats (Barton *et al.* 1983). The predominant effect appeared to be related to the initial uptake of Fe into mucosal cells and to depend primarily on the absolute quantity of Ca present in the duodenal lumen, not the Ca:Fe molar ratio.

Prather & Miller (1992) examined the effect of $CaCO_3$, $CaSO_4$, Na_2CO_3 and Na_2SO_4 on Fe absorption using the rat haemoglobin repletion model in an attempt to differentiate between the effects of the cations and anions in these salts. $CaCO_3$ had the greatest inhibitory effect, but $CaSO_4$ and Na_2CO_3 also reduced the rate of haemoglobin repletion. Prather & Miller (1992) concluded that both the cation and the anion reduced Fe absorption. Their studies suggest complex luminal interactions in which changes in pH and Ca content, as well as Fe solubility

and binding to low-molecular-weight complexes in the food in the intestinal lumen, may be involved.

While the experiments described earlier suggest that Ca affects elemental Fe solubility in the intestinal lumen or its initial uptake by duodenal enterocytes, Ca has also been shown to inhibit haem-Fe absorption in experimental animals (Amine & Hegsted, 1971). Since the initial uptake of elemental (non-haem-) and haem-Fe by enterocytes is thought to involve different receptor-mediated pathways, the finding of an inhibitory effect on both forms makes it necessary to consider the possibility of an interaction between Ca and Fe within the enterocyte or at the point of transfer out of the cell. Non-haem- and haem-Fe share a putative common pathway at these stages of absorption.

The effects of dairy products on Fe availability have been reviewed in depth recently (Hazell, 1985; Jackson & Lee, 1992); the information related to experimental animals is not re-evaluated here. It is important to note that dairy products contain other constituents that affect Fe bioavailability. Jackson & Lee (1992) concluded that some reports described substantial reductions in Fe availability, whereas other reports indicated little effect. They ascribed the conflicting data to differences in techniques employed to measure Fe bioavailability, the species of experimental animal used, the form of Fe in the diet and food composition.

Effects of calcium salts on iron absorption from single meals and iron supplements in human subjects

Non-haem-iron absorption Single-dose studies in human subjects have also generally demonstrated inhibition of Fe absorption, although the findings have not been consistent in all settings and with all sources of Ca. The most reliable information has been obtained by using radioisotopes or stable isotopes to tag the common Fe pools in food or an Fe supplement given either in the fasting state or with food. Several Ca salts that are recommended as supplements have been evaluated (Tables 1 and 2).

Monsen & Cook (1976) demonstrated that the addition of 178 mg Ca as $CaCl_2$ to a meal composed of semi-purified ingredients and containing 24 mg native Ca reduced absorption by

Table 1. Effect of calcium on the absorption of non-haem-iron in single-meal studies

Ca source	Dose (mg)	Meal	Ca content of meal (mg)	Absorption ratio*	Reference
$CaCl_2$	178	Semi-synthetic	24	0·71	Monsen & Cook (1976)
	178	Semi-synthetic†	24	0·30	Monsen & Cook (1976)
	40– 600‡	Wheat rolls	10	0·61–0·23	Hallberg *et al.* (1991)
	40– 600§	Wheat rolls	10	1·0–0·41	Hallberg *et al.* (1991)
$CaCO_3$	500	Breakfast	227	0·43	Dawson-Hughes *et al.* (1986)
	600	Hamburger	141	0·68	Cook *et al.* (1991*b*)
	600	Breakfast	597	0·58	Cook *et al.* (1991*b*)
Calcium phosphate	178	Semi-synthetic	24	0·50	Monsen & Cook (1976)
	182	Semi-synthetic//	20	0·46	Monsen & Cook (1976)
	600	Hamburger	141	0·61	Cook *et al.* (1991*b*)
	600	Breakfast	597	0·37	Cook *et al.* (1991*b*)
Calcium citrate–malate	500	Breakfast	238	0·72	Deehr *et al.* (1990)
Calcium citrate	600	Hamburger	141	0·89	Cook *et al.* (1991*b*)
	600	Breakfast	597	0·43	Cook *et al.* (1991*b*)
Hydroxyapatite	500	Breakfast	227	0·46	Dawson-Hughes *et al.* (1986)

* Absorption with Ca supplement:absorption without Ca supplement.
† Semi-synthetic meal with K_3PO_4.
‡ $CaCl_2$ added to dough before baking.
§ $CaCl_2$ added after baking.
// Semi-synthetic meal with beef.

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Table 2. Effect of calcium on the absorption of supplementary iron (data from Cook *et al.* 1991*b*)

Ca source	Dose (mg)	Fe supplement dose (mg)*	Meal	Ca content of meal (mg)	Absorption ratio†
$CaCO_3$	300	37	None	0	0·85
	300	37	Hamburger	141	0·76
	600	18	None	0	1·20, 0·91‡
	600	18	Hamburger	141	0·56, 0·84‡
Calcium phosphate	600	18	None	0	0·38
	600	18	Hamburger	141	0·43
Calcium citrate	600	18	None	0	0·51
	600	18	Hamburger	141	0·60

* Dose of Fe given as $FeSO_4$.
† Absorption with Ca supplement:absorption without Ca supplement.
‡ Measured in subjects with reduced Fe stores and normal Fe stores respectively.

29 %, a value that was not statistically significant. Hallberg *et al.* (1991) measured the effect of adding $CaCl_2$ to a meal comprising two wheat rolls made from low-extraction flour in forty-five men and eighty-one women. The whole meal contained 20 mg Ca, 0·3 mg intrinsic Fe and 3·5 mg fortification Fe added as $FeSO_4$. The supplemental Ca doses were between 40 and 600 mg. When the supplements were mixed into the dough there was a clear dose-related inhibition of Fe absorption for Ca doses between 40 mg (39 % inhibition; $P < 0{\cdot}001$) and 300 mg (74 % inhibition; $P < 0{\cdot}001$). The difference in percentage Fe absorption between meals containing 300 and 600 mg Ca (77 % inhibition) was not significant, suggesting that the maximal inhibitory effect had been attained with the 300 mg dose.

A similar dose-related pattern of inhibition was noted when the $CaCl_2$ was added after baking, but the effect was smaller. There was no reduction in absorption with 40 mg; with the higher doses, absorption values again decreased progressively; they were 56 and 59 % lower with the 300 and 600 mg doses respectively ($P < 0{\cdot}001$). Hallberg *et al.* (1991) noted that the addition of $CaCl_2$ to the dough before baking reduced phytate degradation during fermentation and baking. Although the phytate content of the wheat rolls was low, they concluded that sufficient phytate was retained to influence Fe absorption and account for the observed difference in absorption.

Dawson-Hughes *et al.* (1986) reported that the ingestion of a $CaCO_3$ supplement containing 500 mg Ca with a breakfast meal reduced the intrinsic food Fe absorption to about 43 % of the control value ($P = 0{\cdot}002$). Cook *et al.* (1991*b*) confirmed and extended their findings. First, they measured the effect of a 600 mg $CaCO_3$ supplement on intrinsic food Fe absorption from two meals with markedly different Fe bioavailabilities. The $CaCO_3$ reduced the absorption of non-haem-Fe by 32 % ($P < 0{\cdot}001$) from a meat-containing high-bioavailability meal (5·1 mg Fe, 3·7 mg non-haem-Fe) and by 42 % ($P < 0{\cdot}05$) from a meal with low bioavailability containing an egg, bran flakes and coffee (4·7 mg non-haem-Fe).

$CaCO_3$ also reduced the absorption of Fe from Fe supplements (Table 2) when the Ca and Fe were taken together in the fasting state (Seligman *et al.* 1983; Cook *et al.* 1991*b*) or with a meal (Cook *et al.* 1991*b*). When 37 mg Fe as $FeSO_4$ was taken with 300 mg Ca after an overnight fast, absorption was inhibited by 15 %. Although 600 mg Ca taken with 18 mg Fe as $FeSO_4$ caused a 9 % decrease in Fe absorption in volunteers with normal Fe stores, it had no effect in a group of individuals with low Fe stores. The inhibitory effect of Ca was, however, not statistically significant in these studies. $CaCO_3$ was somewhat more inhibitory when the supplements were taken together with a hamburger meal. Absorption was reduced by 24 % when 300 mg Ca was taken with 37 mg Fe as $FeSO_4$ ($P = 0{\cdot}043$). With 600 mg Ca and 18 mg Fe, absorption was 44 % lower in subjects with reduced Fe stores ($P < 0{\cdot}001$), but not sig-

nificantly different in those subjects with normal Fe stores. The variable effects of $CaCO_3$ may have resulted from the poor solubility of Ca taken as $CaCO_3$ in the fasting state (Recker, 1985; Heaney *et al.* 1989; Barger-Lux, 1991).

Monsen & Cook (1976) first demonstrated that calcium phosphate reduced Fe absorption by adding either $CaCl_2$ (178 mg Ca) and K_2HPO_4 (374 mg P) or $CaHPO_4$ (178 mg P) to a meal comprising semipurified ingredients and containing 24 mg native Ca. Absorption values were reduced by 70% ($P < 0{\cdot}005$) and 50% ($P < 0{\cdot}001$) respectively. The substitution of beef for egg albumen as the protein source did not reduce the inhibitory effect of Ca significantly. In a subsequent experiment, Cook *et al.* (1991*b*) found calcium phosphate to be more uniformly inhibitory to Fe absorption than was the case for $CaCO_3$. The reduction in absorption of food Fe from the high- and low-bioavailability meals described earlier was 39% ($P = 0{\cdot}03$) and 63% ($P < 0{\cdot}01$) respectively. When Ca and Fe supplements were taken in the fasting state (600 mg Ca, 18 mg Fe), absorption was reduced by 62% ($P < 0{\cdot}001$); when taken with the hamburger meal, absorption was decreased by 57% ($P = 0{\cdot}015$).

Deehr *et al.* (1990) studied the effect of a calcium citrate–malate supplement containing 500 mg Ca given with a breakfast meal (3·18 mg Fe, 238 mg Ca) on the absorption of the food Fe using an extrinsic tag and whole-body counting in nineteen Fe-replete post-menopausal women. Absorption was reduced by 28% ($P < 0{\cdot}05$). The inhibitory effect was reversed by adding 450 ml orange juice containing 193 mg ascorbic acid to the meal. In the series of studies performed by Cook *et al.* (1991*b*), a 600 mg supplement of calcium citrate reduced the absorption of food Fe from the low-bioavailability meal described earlier by 57% ($P < 0{\cdot}01$), but had no significant effect in the higher-bioavailability meat-containing meal. Calcium citrate (600 mg Ca) also reduced the absorption of an 18 mg Fe supplement, given after an overnight fast, by 49% ($P = 0{\cdot}013$). When both supplements were given with the high-bioavailability meat-containing meal, Fe absorption was reduced by 40% (NS, $P = 0{\cdot}058$).

Haem-Fe absorption There are two reports dealing with the effect of Ca salts on haem-Fe absorption. In the first, Hallberg *et al.* (1991) found a 24% reduction in haem-Fe absorption ($P < 0{\cdot}01$) when 165 mg Ca was added to a hamburger meal. The second (Hallberg *et al.* 1992*c*) was designed to determine whether Ca influences haem-Fe absorption by suppressing the enhancing effect of meat (Layrisse *et al.* 1973; Hallberg *et al.* 1979). Absorption was measured from a hamburger meal and from two wheat rolls eaten with and without 165 mg Ca as $CaCl_2$: Ca reduced absorption to the same extent (approximately 55% of the control value) in both meals, indicating that Ca is a direct inhibitor of haem-Fe absorption; its effect is independent of the meat effect.

Effects of dairy products on iron absorption from single meals in human subjects

A chemical-balance technique was used to measure Fe absorption from a cereal-based meal in the earliest study carried out in India (Apte & Venkatachalam, 1964). Three levels of Ca (ranging between 400 and 1600 mg/d) were added in the form of skimmed-milk powder and Fe absorption from the meals with the highest amount of Ca was increased; however, subsequent studies suggested that milk was inhibitory to non-haem-Fe absorption. Other dietary manipulations were also performed in the earlier experiments, making it difficult to define the specific effect of milk (Rossander *et al.* 1979; Jackson & Lee, 1992). Several recent reports describe experiments in which the effect of dairy products was evaluated more definitively. Milk and cheese reduced non-haem-Fe absorption from meals that did not contain meat, to about 50% of

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the control value (Deehr *et al.* 1990; Hallberg *et al.* 1991, 1992*a*). Formulas based on cows' milk are not suitable vehicles for Fe fortification unless vitamin C is added to improve bioavailability (Stekel *et al.* 1986). In meals containing meat the effect, if any, was much smaller. Hallberg & Rossander (1982) reported a 13% reduction (not statistically significant) from a composite hamburger meal, while Galan *et al.* (1991) found absorption of non-haem-Fe from a typical French meal containing meat to be unaffected when skimmed milk or plain yogurt were added.

It is important to note that the effect of dairy products may not be entirely attributable to Ca. Milk proteins have also been shown to have an inhibitory effect on Fe absorption (Cook & Monsen, 1976; Hurrell *et al.* 1989; Jackson & Lee, 1992; Lonnerdal, 1997). Nevertheless, a study carried out by Hallberg *et al.* (1992*b*) suggests that Ca is the most important inhibitor. They compared the absorption of Fe from human and cows' milk in adult volunteers. As reported previously by other investigators (McMillan *et al.* 1976, 1977; Saarinen *et al.* 1977), absorption from cows' milk was approximately 50% that from human milk (which contains very little Ca). In the study by Hallberg *et al.* (1992*b*), the Ca concentrations in the human and cows' milk were 190 and 960 mg/l respectively. The addition of Ca to human milk to yield a concentration equal to that in the cows' milk reduced Fe absorption significantly, accounting for approximately 70% of the difference in bioavailability between human and cows' milk.

The single-meal studies demonstrate that, with few exceptions, Ca derived from Ca salts and dairy products is inhibitory to non-haem-Fe absorption. Milk and calcium phosphate were the most inhibitory and $CaCO_3$ the least. The effects on non-haem food Fe, Fe supplements given in the fasting state and Fe supplements given with meals were similar. The importance of dose was evaluated rigorously in only one study that suggested the presence of a maximal effect at approximately 300 mg Ca per meal (Hallberg *et al.* 1991). The absolute quantity of Ca ingested appeared to be more important than the Ca:Fe molar ratio confirming the earlier observations made in experimental animals by Barton *et al.* (1983). Haem-Fe absorption was also inhibited by Ca salts.

It is important to note that the experimental designs chosen for most of the single-meal studies would ensure a maximal inhibitory effect. The control meals contained very little Ca. The Ca supplement in the majority of cases approximated or exceeded the maximal-effect threshold. There is one striking exception to this generalization; Cook *et al.* (1991*b*) found a more marked inhibitory effect in a low-bioavailability breakfast meal than in a higher-bioavailability hamburger meal. The Ca contents of the two meals were 597 and 141 mg respectively. On the basis of the expected maximal threshold effect of meal Ca content, very little inhibition would have been expected to result from the additional Ca in the low-bioavailability meal. Nevertheless, a significant decrease in percentage absorption was observed in three different experiments using $CaCO_3$, calcium phosphate and calcium citrate respectively. It is difficult to know how much weight to give this single set of observations; the meal used may have had some unrecognized effect on the interaction between Fe and Ca. More importantly, as the investigators themselves pointed out, percentage Fe absorption from the meal was very low (1·2% without Ca), making it difficult to quantify an inhibitory effect precisely.

Two additional experimental observations are important, both for our understanding of the mechanisms by which Ca exerts its inhibitory effect and for attempts to predict the nutritional impact of increased Ca consumption. The inhibitory effect was seen only when the Ca and Fe were given at the same time (Gleerup *et al.* 1995). Meal composition was important. The inhibitory effect could be overcome by the addition of a known enhancer of Fe absorption, such as ascorbic acid, in both experimental animals (Mehansho *et al.* 1989) and human volunteers (Deehr *et al.* 1990).

Mechanisms responsible for the inhibitory effects of calcium

The mechanisms by which Ca reduces Fe absorption remain undetermined. Hallberg *et al.* (1992*c*) have suggested that the process of transfer of Fe from the enterocyte to the plasma may be inhibited. They based this assertion on the fact that both non-haem-Fe and haem-Fe absorption are affected by Ca (the two different forms of dietary Fe enter duodenal enterocytes via separate pathways, but are thought to form a common cellular pool before transfer to the plasma). Furthermore, Hallberg & Hulten (2000) recently analysed the reported relationship between the absorption ratio for Fe (absorption with Ca : that without Ca) and the amount of Ca in a meal. They concluded that the relationship has a sigmoid form, suggesting one-site competitive binding at a receptor. Although their postulate is intriguing, there is as yet no direct evidence to indicate that Fe and Ca share a common absorptive pathway.

Recent experimental evidence suggests that high Ca levels may affect the function of divalent metal transporter-1, the putative brush-border transporter for elemental Fe in duodenal enterocytes. However, if this is the mechanism by which Ca limits non-haem-Fe absorption, it would be necessary to postulate a concomitant effect on the luminal membrane transporter for the haem moiety. It is also theoretically possible that high concentrations of Ca alter the rheological properties of the mucus layer in the upper small intestine (Crowther *et al.* 1984). A series of experiments carried out by Conrad and his colleagues suggest that mucins may have a role in Fe absorption (Conrad & Umbreit, 1993).

Finally, consideration should be given to mechanisms involving other food components that directly or indirectly affect the solubility of Fe in the bowel lumen. Some of the effects of Ca salts may be related to the accompanying anion (Prather & Miller, 1992). Studies by Hallberg *et al.* (1991) suggest that interactions with phytate may be important in meals containing bread. Phytates are powerful inhibitors of non-haem-Fe absorption (Hallberg *et al.* 1989; Hurrell *et al.* 1992). Poorly-soluble calcium phytate complexes formed in the dough during fermentation and baking may prevent phytate degradation by natural phytases in bread (Zhou & Erdman, 1995).

In conclusion, it seems likely that Ca influences Fe absorption by more than one mechanism. Cellular effects are clearly important, but interactions with other meal components may also have a role.

Effect of calcium supplements on dietary iron absorption (multiple-meal studies)

Six multiple-meal dietary studies in which the consequences of modifying Ca intake were evaluated over periods of 1–10 d were reviewed (Table 3). Dietary Ca was adjusted by supplementation with a Ca salt in one of the studies and by varying the intake of dairy products in the other five.

Ames *et al.* (1999) examined the effect of a high-Ca diet, achieved through the increased consumption of dairy products, on Fe absorption from a single day's diet in eleven children aged 3–5 years. They used stable isotopes and measured erythrocyte incorporation of the stable-isotope Fe tracer. Increasing the dietary Ca intake from an average of 502–1180 mg/d had no effect on Fe absorption.

Turnland *et al.* (1990) measured Fe absorption from a whole-day's cereal-based diet in eight young women by faecal monitoring after the ingestion of meals tagged with stable radio Fe isotopes. The lunch and dinner meals were eaten on consecutive days with 150 g milk or 150 g water. The consumption of milk had no effect on Fe absorption.

Table 3. Effect of calcium on dietary iron absorption: multiple-meal studies

Study population	Meals	Ca content of control diet (mg/d)	Form of supplement and dose	Result	Reference
Children	Mixed diet	502	Dairy foods (total daily intake 1180 mg)	NE	Ames *et al.* (1999)
Young women	Cereal-based diet	NA	Milk (300 ml)	NE	Turnland *et al.* (1990)
Adult ileostomy subjects	Mixed diet	160	Milk (total daily intake 1400 mg)	NE	Tidehag *et al.* (1995)
Adults	Mixed diet	684*	Dairy foods (total daily intake 1281 mg)	NE	Reddy & Cook (1997)
Adults	Mixed diet	280†	Dairy foods (total daily intake 1281 mg)	0·81‡	Reddy & Cook (1997)
Adults	Mixed diet	250, 310	$CaCO_3$ (1200 mg/d with meals)	0·30§	Minihane & Fairweather-Tait (1998)

NA, not available; NE, no effect.
* Self-selected diet.
† Low-calcium diet.
‡ Absorption ratio, high-calcium : low-calcium diet.
§ Absorption ratio, high-calcium : control diet.

The effect of milk was also measured by Tidehag *et al.* (1995), using a chemical-balance method in nine ileostomy subjects. Meat was eaten with each of the three main meals during the test periods. The chemical-balance method employed would be expected to detect the composite effect on both haem- and non-haem-Fe absorption. The control diet provided 160 mg Ca/d. When this diet was consumed with milk or fermented milk, the daily Ca intake was 1380 and 1430 mg respectively. Fe absorption was unaffected by milk consumption.

Reddy & Cook (1997) used an extrinsic radioactive Fe tag to measure absorption from the non-haem-Fe pool in fourteen healthy volunteers over each of three 5 d periods. The diets were freely chosen during one of the periods and modified to increase or decrease Ca content maximally during the other two periods. The mean Ca intake was 684 mg/d when the volunteers were eating their self-selected diets, and was increased and reduced to 1281 (range 664–1957) and 280 (range 147–697) mg/d when the diet was modified to raise and lower the Ca intake respectively. The mean absorption from the high-Ca diet was 19 % less than that from the low-Ca diet, but the difference was not significant, leading the investigators to conclude that dietary Ca content does not influence Fe absorption from a varied diet.

Minihane & Fairweather-Tait (1998) used stable-isotope extrinsic tags to measure the effect of $CaCO_3$ on the absorption of non-haem-Fe from meals containing between 67 and 165 mg Ca. The breakfast, lunch and dinner meals were all labelled with the stable isotope. On the following day the same meals were consumed with two tablets of $CaCO_3$ providing 400 mg Ca. The mean absorption values differed significantly (15·8 % and 4·7 % without and with the Ca supplement respectively ($P < 0·001$).

Another dietary experiment also demonstrated an effect on non-haem-Fe absorption measured from the whole diet, although there was no true control group (Gleerup *et al.* 1995). Twenty-one healthy women were given a mixed diet providing 937 mg Ca/d, derived primarily from dairy products. Fe absorption was measured during two 10 d periods by using an extrinsic radio Fe tag to label the dietary common-pool Fe to a uniform specific activity. The distribution of the Ca with respect to the main meals of the day was varied. During one experimental period

an attempt was made to reduce the inhibitory effect of Ca by serving no milk or cheese with the lunch and dinner meals that provided most of the dietary Fe, and during the other period the dairy products were provided more evenly throughout the day's diet. Fe absorption was 32% higher when dairy products were eliminated from the main Fe-containing meals.

In summary, all but one of the multiple-meal studies indicate that Ca is likely to have a far smaller influence on Fe absorption than would be predicted from the single-meal experiments. The exception is the experiment reported by Minihane & Fairweather-Tait (1998). The reason for the much larger inhibitory effect of Ca observed in this study is uncertain; the selection of control meals with low Ca contents may have been a contributory factor.

There are several possible explanations for the differences between single- and multiple-meal studies. In most cases single-meal experiments were designed to maximise sensitivity for detecting inhibition. With few exceptions, control meals contained very little Ca. Since there appears to be a dose-related response, the impact of additional Ca might have been less if the control meals had been more representative of the subjects' habitual Ca consumptions. Furthermore, Cook *et al.* (1991*a*) have shown that single-meal studies exaggerate the influence of factors that affect Fe bioavailability.

Effect of calcium intake on iron status

Direct measurements of dietary Fe bioavailability (multiple-meal studies) provide information about the short-term effects of adjustment in meal composition, but may not accurately reflect long-term adaptive consequences. Meals in Western countries are highly varied. The evaluation of the effect of Ca on specific diets or even on short-term self-selected diets may not be representative of the scope of an individual's food choices over longer time periods. Seasonal change in availability of specific food items may also have a role. Absorption measurements, although very important, provide little more than a broad set of guidelines for predicting the consequences of changes in dietary patterns. Epidemiological studies of the relationship between Fe status and Ca intake should furnish the most reliable information.

The careful evaluation and standardisation of a selected set of laboratory tests have made it possible to characterise Fe status precisely in epidemiological surveys carried out in Western countries (Expert Scientific Working Group, 1985). They have been used in cohort supplementation trials and cross-sectional epidemiological surveys. However, it is important to note that the precision and specificity of the information provided by each of these approaches is limited. Cohort studies designed to evaluate Fe status must be conducted over long periods. Changes in Fe status occur very slowly because of the body's adaptive capacity (Cook, 1990); observations that are limited to a few months of follow-up may be misleading because of their failure to detect a small negative balance that will over a period of years lead to Fe deficiency. Cross-sectional surveys that correlate Ca intake with Fe status may also be misleading, because it is difficult to isolate Ca as a single dietary factor; other unidentified constituents of Ca-rich foods may be equally or more important. Furthermore, reported dietary adjustments involving Ca sources do not always reveal concomitant changes in other dietary components that may have substantial independent effects.

Cohort studies The impact of Ca supplementation on Fe status has been evaluated in infants, adolescent girls and adults.

Dalton *et al.* (1997) randomly assigned 103 healthy full-term infants aged 2·5–5 months to receive a Fe-fortified cows'-milk-based infant formula containing 465 mg Ca/l or the same

formula with added calcium glycerophosphate (1800 mg Ca/l). No effect on serum ferritin, total Fe-binding capacity, erythrocyte protoporphyrin or packed cell volume was observed after 9 months.

Yan *et al.* (1996) found no effect on serum ferritin levels among sixty Gambian women given a dose of 1000 mg Ca as $CaCO_3$, 5 d/week, despite the fact that they were accustomed to a low dietary Ca intake (283 mg/d). The study was, however, designed to minimize any inhibitory effect of Ca. The supplementary Ca was provided in the form of two tablets of $CaCO_3$ that were taken in the early evening at least 2 h after lunch and 1 h before dinner. Kalkwarf & Harrast (1998) evaluated the effect on serum ferritin concentrations of a 500 mg $CaCO_3$ supplement given twice daily with meals for a period of 6 months in 158 women (seventy-eight supplemented, eighty controls) during the postpartum period. Their mean daily dietary Ca and Fe intakes were 721 and 13·2 mg respectively. Supplementation occurred between month 6 and month 12 after delivery. Seventy-six of these subjects were lactating at the start of the study, but weaned their infants about 2 months later. Serum ferritin values were higher in lactating women than in non-lactating women at the start of the study; geometric mean values were 47·7 and 31·5 μg/l respectively ($P < 0{\cdot}001$). By the end of the study there was no longer a statistically significant difference in serum ferritin levels: the geometric mean values were 30·5 and 25·5 μg/l for the previously lactating and non-lactating women respectively. Ca supplementation had no effect in either group.

Adolescence is a period during which Fe requirements are increased because of rapid growth and the onset of menstruation. An adequate supply of bioavailable dietary Fe is essential to prevent Fe deficiency. Ilich-Ernst *et al.* (1998) enrolled 354 girls aged 8–13 years in a randomized double-blind placebo-controlled intervention trial to assess the effects of Ca supplementation on bone mass acquisition. The girls received tablets containing either 250 mg Ca in the form of calcium citrate–malate or a placebo. They were instructed to take two of the tablets in the morning after breakfast and in the evening before bedtime. The daily mean dietary Ca and Fe intakes of these girls was between 798 and 878 mg and 12·1 and 14·3 mg respectively. There were no differences between the two groups in serum ferritin values, haemoglobin concentrations or erythrocyte indices during the 4-year follow-up period.

The effect of Ca supplements on Fe-storage status was examined in adult volunteers in two reports. In the first report, fifty-seven premenopausal women took 500 mg Ca as $CaCO_3$ with each of two daily meals for 12 weeks (Sokoll & Dawson-Hughes, 1992). The fifty-two women in the control group were not given a placebo. The habitual Ca intake of the group was approximately 600 mg; their dietary Fe and ascorbic acid intakes were relatively high (mean values, 15·1 mg and 227 mg respectively). The Ca supplement had no effect on plasma ferritin concentration, serum Fe concentration, total Fe-binding capacity, transferrin saturation, haemoglobin level or packed cell volume. Finally, Minihane & Fairweather-Tait (1998) followed a small number of adults (seven women, four men) given 400 mg Ca as $CaCO_3$ with each of three daily meals for 6 months; a control group (ten women, three men) had no dietary intervention. The mean habitual Ca intake of these individuals was approximately 1000 mg. Once again, there was no change in plasma ferritin, Zn protoporphyrin, haemoglobin level or packed cell volume as the result of Ca supplementation.

The cohort studies are consistent. They indicate that Ca supplementation is unlikely to have a significant effect on Fe status. However, it is important to note that the habitual Ca intake was high in all but one of the studies; in the latter study, the inhibitory effect may have been reduced, as the supplements were taken between meals.

Cross-sectional studies Several investigators have examined the relationship between Ca intake and laboratory measurements of Fe status in selected population groups. Takkunen

(1976) found a negative correlation between Fe status and the consumption of dairy products in Finnish adults. An inverse correlation between Ca intake and serum ferritin was also observed in four French surveys. Dietary information was obtained by the dietary history method in three surveys and by the analysis of a reported 24 h dietary record collected every 2 months over a 1-year period in the fourth (Supplementation des Vitamines et Mineraux Antioxydants (SU.VI.MAX) Study). The primary source of Ca was dairy products in all of them. Galan *et al.* (1985) surveyed 476 French female students whose mean daily Fe intake was 10·9 mg; only 1·3 % were anaemic but 16 % were judged to be Fe deficient on the basis of a serum ferritin level below 12 μg/l. Tea and dairy-product intakes were negatively correlated with serum ferritin values ($P < 0{\cdot}05$). Serum ferritin, haemoglobin, serum Fe and total Fe-binding capacity were measured in 203 menstruating women and dietary intakes were evaluated in 127 of the women (Soustre *et al.* 1986). Anaemia was present in 2·9 % and 20·7 % were Fe deficient (serum ferritin < 12 μg/l). Their mean daily Fe consumption was 11·6 mg. Serum ferritin was positively correlated with meat intake ($P = 0{\cdot}04$) and negatively correlated with the consumption of dairy products ($P = 0{\cdot}05$). A cross-sectional nutritional survey was conducted in 1988 in the Val-de-Marne district of France (Preziosi *et al.* 1994). Dietary and biochemical data were obtained from 1108 subjects of both sexes aged between 6 months and 97 years; both serum ferritin values ($P < 0{\cdot}001$) and haemoglobin concentrations ($P < 0{\cdot}001$) were negatively correlated with Ca intake. Finally, the Fe status of a national sample of adults living in France and participating in the SU.VI.MAX Study was assessed by measuring serum ferritin and haemoglobin concentrations (Galan *et al.* 1998). Both laboratory data and dietary intakes were recorded in 3111 women and 2337 men aged 45–60 years. The mean daily Fe intakes for men and women were 16·7 and 12·3 mg respectively. Fe deficiency was very rare among the men, but 22·7 % of menstruating women and 5·3 % of post-menopausal women were Fe deficient (ferritin < 15 μg/l). The consumption of dairy products showed a negative correlation with serum ferritin levels ($P = 0{\cdot}0001$), but not with haemoglobin concentrations.

The observations from Finland and France are supported by a recent survey conducted in six European countries (van de Vijver *et al.* 1999). A 3 d food record was used to estimate Ca and Fe intakes in 1080 girls (mean age 13·5 years) and 524 women (mean age 22 years). Serum ferritin, serum Fe, transferrin concentration and percentage saturation of transferrin were used to characterise Fe status. Mean Ca and Fe intakes for the girls were 992 and 10·8 mg respectively; corresponding values for the women were 988 and 10·3 mg. Dietary Ca intake was weakly inversely associated with serum ferritin concentration ($P < 0{\cdot}05$ for the data, $P < 0{\cdot}01$ after adjustment for Fe intake, age, menarche, protein, tea and vitamin C consumption, and country), with a relatively small quantitative effect confirming the earlier French observations. An inverse relationship with transferrin saturation was also recorded for the girls, but not for the women. It is important to note that there was a considerable variation in Ca intake in these subjects. Nevertheless, the predicted effect on Fe status (as determined by serum ferritin values) was small. Mean Ca consumption for girls in the lowest quartile of intake was 462·0 mg/d. In the highest quartile the value was 1686·9 mg/d. Mean ferritin values were 34 and 37·8 μg/l respectively. A similar range for Ca intake was evident among the women, 482·9–1597·9 mg/d. The mean serum ferritin values for the lowest and highest quartiles were also not significantly different (41·2 and 36·4 μg/l respectively. van de Vijver *et al.* (1999) calculated that the serum ferritin would be reduced by a factor of 1·6 % for every 100 mg/d increase in Ca intake in the girls; a slightly higher value of 3·3 % was computed for the women. Thus, even at the extremes of intake the effect would be modest. This analysis did not detect a threshold effect for dose, and the temporal relationship between the Fe and Ca ingestion appeared to be unimportant.

Robinson *et al.* (1998) also reported a negative effect of milk consumption on serum ferritin values in 576 women in the second trimester of pregnancy ($P < 0{\cdot}0001$). The difference in the mean serum ferritin concentrations for women in the lowest and highest quartiles for Ca consumption (< 945 and > 1518 mg/d respectively) was only 11 μg/l. However, most of these women were not taking Fe supplements, and there was a significant difference in the percentage of individuals in the lowest and highest quartiles for Ca intake, with serum ferritin values of < 12 μg/l (9 and 17% for primipara; 19 and 43% for multipara respectively). Haemoglobin concentrations were not affected.

Two recent epidemiological surveys failed to detect a relationship between Ca intake and Fe status. In the first survey the consumption of dietary factors known to influence non-haem-Fe absorption was evaluated in 634 (254 men and 380 women) free-living elderly individuals (age range 67–93 years) who were participants of the Framingham Heart Study (Fleming *et al.* 1998). Dietary intakes for the previous year were assessed by a food-frequency questionnaire. The intake of mineral supplements was also recorded. Serum ferritin concentration was used to determine Fe status. The mean total dietary intakes of Fe and Ca were 17·8 and 807 mg respectively. Neither dietary nor supplemental Ca intake was correlated with serum ferritin concentrations.

The second epidemiological survey was a cross-sectional study of about 405 women aged between 32 and 66 years, living in five countries in rural China (Root *et al.* 1999). Dietary intakes were estimated from 3 d surveys, and haemoglobin, plasma ferritin and plasma Fe were measured to characterise Fe status. Their Fe intakes (15–28 mg/d) were relatively high. There was no relationship between Ca intake and Fe status.

In summary, a statistically significant inverse relationship between Ca intake and Fe status was reported in seven of the nine cross-sectional studies described earlier. However, the quantitative effect was small and, with the exception of the study carried out in pregnant women, of doubtful biological significance. Since dairy products were the primary sources of Ca in all of these studies, it is difficult to define the role of Ca with certainty. The inhibitory effect of milk proteins or the substitution of dairy products for animal tissues as a source of protein may have played a part.

Conclusions

Although the experimental and epidemiological data are not wholly consistent, most of the information indicates that changes in the Ca content of Western diets is likely to have only a small influence on Fe absorption. An increase in Ca consumption is unlikely to have a biologically significant effect on Fe status in most individuals, although one report suggests that Fe supplementation may be necessary during pregnancy to prevent Fe deficiency in women with high milk intakes. The putative benefits of a higher Ca intake are likely to outweigh any negative consequences for Fe balance. It has been suggested that the effect of Ca supplements on Fe absorption can be minimised by recommending that they are not taken with the meals that provide most of the dietary Fe. The need for this precaution has not been established clearly. However, Ca and Fe supplements should, if possible, be taken at different times of the day. Cows' milk and cows'-milk-based infant formulas are not suitable vehicles for fortification with Fe unless steps are taken to improve bioavailability.

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