

## From absorption and excretion of minerals . . . to the importance of bioavailability and adaptation

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It was nearly 120 years ago that the physiologist Claude Bernard proposed that for an organism to function optimally the component cells must be surrounded by a medium of closely regulated composition ('La fixité du milieu interne est la condition de la vie libre'). Homeostasis is the term used today to describe this phenomenon, being first coined by Walter B. Cannon in 1929 (Hardy, 1976). This is the underlying physiological principle that explains the relative chemical constancy of the body, including inorganic nutrients such as Fe.

### IRON

#### *The failure of the body to excrete iron*

During the last century and even up until 60 years ago it was assumed that the balance between intake and output of Fe was achieved by changes in the intestinal excretion of Fe. Implicit in this belief was the acceptance that dietary Fe was fully absorbed and that faecal Fe comprised Fe re-excreted for the purposes of maintaining homeostasis, but in 1937 a new theory for the maintenance of Fe balance was put forward by McCance & Widdowson (1937a). Their research into Fe metabolism was stimulated by the finding that when a patient with polycythaemia rubra vera was treated with acetyl phenylhydrazine to break down erythrocytes, none of the liberated Fe was excreted from the body (McCance & Widdowson, 1937b). This observation led to experiments in which Fe was injected intravenously into healthy volunteers (McCance & Widdowson, 1938), or high quantities were taken orally (Widdowson & McCance, 1937), but none of the Fe was excreted. This, plus other contradictory experimental findings, led them to conclude that the quantity of Fe in the body is regulated by absorption rather than excretion (McCance & Widdowson, 1937a).

#### *Iron bioavailability*

Fe balance in adults is only achieved when the quantity of absorbed Fe is equal to Fe losses. However, the regulatory control is still not completely understood (Bothwell, 1995). Over the years, many studies have been performed measuring the absorption of dietary Fe, using Fe isotopes to label the food Fe and hence follow its movement into the body. The development of extrinsic labelling techniques using radioisotopes (Cook *et al.* 1972; Hallberg & Bjorn-Rasmussen, 1972) and stable isotopes (Sandström *et al.* 1993) has greatly aided progress in this field of research. A number of dietary and host-related factors have been identified that influence the efficiency with which the body absorbs and utilizes Fe, namely Fe bioavailability, as illustrated in Table 1 (British Nutrition Foundation, 1995). The realization that haem and non-haem Fe enter a common pool within the gut led to an appreciation of the importance of measuring Fe absorption from whole meals rather

Table 1. *Examples of dietary and humoral factors affecting iron bioavailability*

	Enhancing	Inhibiting
Dietary	Ascorbic acid Animal protein Cysteine-containing peptides Organic acids Ethanol	Phytate Polyphenols Calcium Phosphate Oxalate
Humoral	Iron-deficiency anaemia Low body iron stores High gastric acid secretion Low iron intake Elevated rate of erythropoiesis Growth, pregnancy High altitude, hypoxia	Iron-loading disorders High body iron stores Achlorhydria High iron intake Infection, inflammation

than from individual foods. More recently, it has become apparent that further information is required on Fe bioavailability from whole diets, not only for the derivation of dietary requirements (Food and Agriculture Organization/World Health Organization, 1988; Scientific Committee for Food, 1993), but also in order to understand the long-term control of Fe homeostasis.

#### *Adaptive responses*

Single Fe absorption measurements do not necessarily predict quantitative changes in Fe balance in normal individuals (Finch, 1994). For example, when large doses (2 g/d) of ascorbic acid, a powerful enhancer of Fe absorption, were given with meals to men for 16 weeks there was no change in plasma ferritin concentration, i.e. no increase in body Fe stores (Cook *et al.* 1984). The results of other studies with ascorbic acid are inconsistent, but none confirms the very marked effect observed in single meal absorption tests. The effects of inhibitors of Fe absorption appear to follow the same pattern. Ca reduces Fe absorption from meals, but when given with meals for a period of 6 months, no changes in plasma ferritin are found (Minihane *et al.* 1997). The situation appears to be similar for Fe bioavailability in whole diets. When these are modified to promote or inhibit Fe absorption, single meal measurements produce an exaggerated response compared with longer-term absorption studies (Cook *et al.* 1991; Tidehag *et al.* 1996). Several hypotheses have been put forward to explain the differences between single meals and whole diets, for example (a) the influence of dietary inhibitors and enhancers in a normal diet being diluted by meals that have no overall effect on non-haem Fe absorption, (b) the difference in the time of fasting before breakfast and other meals affecting the efficiency of Fe absorption, and (c) the up- and down-regulation of Fe absorption according to level of previous exposure of mucosal cells to Fe, as demonstrated in animal studies (Fairweather-Tait & Wright, 1984; Fairweather-Tait *et al.* 1985). How the intestinal mucosal cell achieves adaptation is one of the most important unsolved questions in Fe metabolism (Cook, 1990).

#### CALCIUM

##### *Brown v. white flour: effects on calcium balance*

One of the most potent inhibitors of Fe absorption, phytate (myoinositol hexaphosphate), also affects Ca bioavailability. Some of the earliest studies on Ca-phytate interactions were

performed by McCance and Widdowson during the Second World War (McCance & Widdowson, 1942). Because of the national shortage of wheat, investigations were carried out in healthy adults to examine the potential nutritional effects of increasing the extraction rate of flour. The balance studies performed by McCance & Widdowson (1942) demonstrated that Ca was less well absorbed from brown (92 % extraction rate) than white (69 % extraction rate) flour, and that the active ingredient was phytic acid. As a result of this study, in concordance with the recommendations of McCance and Widdowson, the Government adopted a policy of adding CaCO<sub>3</sub> to all flour of less than 100 % extraction rate, and this legislation is still in force today.

### *Calcium-phytate interactions*

The fact that phytate reduces Ca absorption has been demonstrated in balance studies as well as more recent isotope studies (Weaver *et al.* 1991), but the contradictory nature of some of the earlier research on Ca and phytate suggested that adaptation to high-phytate diets might occur over time. An early balance study carried out in South Africa (Walker *et al.* 1948) appeared to confirm the adaptation hypothesis. The proposed mechanism was that phytate complexes were hydrolysed in the digestive tract, thereby releasing Ca for absorption. In rats, intestinal phytase (EC 3.1.3.8) and alkaline phosphatase (EC 3.1.3.1) do not play a role in the adaptive increase in phytate digestibility, and it is suggested that it is in fact related to an increased synthesis of alkaline phosphatase by the gastrointestinal microflora (Moore & Veum, 1983). Significant amounts of Ca can be absorbed in the colon, and the fact that phytic acid has no effect on colonic absorption (Sandström *et al.* 1990) supports the suggestion that the microflora are involved in the breakdown of phytate-mineral complexes. A more recent human study reported no reduction in losses of Ca when high-phytate breads were fed to adults for a period of 98 d (Reinhold *et al.* 1981), which does not lend support to the adaptation theory. Studies with rats show that high and low levels of Ca intake affect the utilization of <sup>14</sup>C-labelled phytate differently (Nahapetian & Young, 1980), which might partly explain the inconsistent results. However, the development of techniques for isolating and quantifying different inositol phosphates should lead to better characterization of the mineral binding properties of phytate (Sandberg & Ahderinne, 1986), since it is known that the lower inositol phosphates do not exhibit mineral binding properties.

## MINERAL BALANCE IN NEONATES

### *Iron, copper and zinc metabolism in newborn infants*

There is a rapid fall in haemoglobin after birth, from 170 g/l to 124 g/l by 4 weeks of age (British Nutrition Foundation, 1995), and the fate of this released Fe was the subject of a number of investigations earlier this century, none of which resolved the question satisfactorily. In most studies a negative Fe balance was found shortly after birth, particularly in infants fed on cows' milk. Stimulated by the reports of negative Fe balance at a time when anabolic needs are greatest, Cavell & Widdowson (1964) carried out a careful study of mineral balance in neonates. They collected meconium passed during the first 24 h of life and analysed it for Fe (1.7 mg/100 g), Cu (1.7 mg/100 g) and Zn (6.5 mg/100 g). Three-day balances in infants of approximately 1 week of age were performed. Balances of Fe and Zn were negative (3.6 and 0.8 mg/d respectively), and it was estimated that infants were losing 1% of the body's total Fe and Zn per day; Cu balances were, however, approximately zero.

*Redistribution and bioavailability*

Following birth, the Fe released from fetal haemoglobin is recycled and used for the production of new erythrocytes and tissue growth. This allows infants to survive on very limited intakes of Fe from breast milk. However, despite the low concentration of Fe in breast milk (0.6–0.9 mg/l; British Nutrition Foundation, 1995), absorption of Fe from breast milk is relatively high, with values ranging from 20–49%. This supply of Fe is usually adequate to meet requirements until the infant is approximately 6 months of age. By comparison, the absorption of Fe from cows' milk formulas is lower, generally accepted to be about 10%. The reason for the difference in bioavailability is not yet established. The suggestion that higher concentrations of lactoferrin in breast milk enhance bioavailability has not been substantiated (Fairweather-Tait *et al.* 1987; Davidsson *et al.* 1993). It has been proposed that the higher content of Ca in cows' milk may be partly responsible for its lower bioavailability (Hallberg *et al.* 1992). Infant formulas are fortified with Fe in quantities considerably in excess of those found in breast milk, in order to compensate for the lower bioavailability.

Approximately 50–60% of Cu in the newborn infant is in the liver, amounting to a total of 8 mg. Serum Cu levels at birth are lower than at any other time in life, and rapidly increase until adult levels are attained at 3–4 months of age, the Cu being provided by the liver store (Mason, 1979). This is due almost entirely to increased synthesis of caeruloplasmin in the liver. Breast milk contains more Cu than cows' milk, and according to results obtained using the sucking rat model, Cu bioavailability is higher from breast than formula milk (Lonnerdal *et al.* 1985). There is, however, no justification for fortifying infant formulas with Cu because the liver stores can be used to make up any dietary deficit. The main excretory pathway for Cu is via bile in the faeces, but whether or not this Cu can be reabsorbed is not yet resolved.

With Zn, unlike Fe and Cu, there are no specific body stores. However, the turnover of the newborn skeleton is relatively rapid, thus much of the Zn initially bound in the bone may become available to the infant for growth and development. Erythrocyte metallothionein concentration (a possible index of Zn status) was observed to decrease steadily from 2 to 9 months of age, and serum Zn concentration was lower at 9 than 6 months (Michaelson *et al.* 1994), suggesting that Zn nutriture may be compromised. Zn bioavailability is higher from breast than cows' milk or infant formula (Sandström *et al.* 1983), but the reason for this is not clear, although it has been subject to a great deal of controversy.

## CONCLUSIONS

McCance and Widdowson were responsible for key observations that form the basis for our current knowledge of mineral homeostasis. They questioned generally accepted beliefs and designed careful experiments to test out new hypotheses. Although our understanding of mineral metabolism is still far from complete, the framework is in place for investigating unresolved questions. New experimental approaches are being employed to study different aspects of mineral metabolism, such as inorganic stable isotopes, kinetic modelling, intestinal cell lines, and molecular biological techniques. It is hoped that these should enable us to establish mechanisms of absorption, bioavailability, and the regulation of homeostasis, thereby providing information needed to determine dietary requirements of minerals for optimal health.

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