Mono ferrous acid citrate ($FeC_6O_7H_2O$) as an iron fortificant

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1. Iron absorption from ferrous citrate (monoferrous acid citrate, $FeC_6H_6O_7H_2O$) was studied in normal healthy male and female volunteers using ferrous citrate labelled with radioactive Fe and whole-body counting. Ferrous citrate was either given alone or with a rice-based meal.

2. Fe absorption from ferrous citrate was satisfactory and was comparable to that from ferrous sulphate.

3. Fortification of crude cooking salt with ferrous citrate was not satisfactory due to colour development on storage. Ferrous citrate can, however, serve as an effective Fe fortificant with sugar or wheat flour.

Iron-deficiency anaemia is a major nutritional problem in several parts of the world. Fortification of foods with Fe has been considered as a practical way of preventing Fe-deficiency anaemia. Several Fe compounds, such as ferrous sulphate, reduced Fe, ferric phosphates have been suggested for fortification of foods. Ferrous sulphate or reduced Fe are unstable when used for fortifying a food item like common salt. Insoluble Fe compounds such as ferric orthophosphate, ferric pyrophosphate and sodium iron pyrophosphate, although stable, are unsatisfactory since availability of Fe from them is extremely low (Narasinga Rao, Surendra Prasad & Apte, 1972). This, however, can be overcome by using either ascorbic acid (Sayers, Lynch, Charlton, Bothwell, Walker & Mayet, 1974) or sodium acid sulphate (Narasinga Rao & Vijayasarathy, 1975) with an insoluble Fe compound like ferric orthophosphate. While seeking an alternative Fe source for fortification of common salt the suitability of ferrous citrate (mono ferrous acid citrate) was investigated. Ferrous citrate is a creamy white powder sparingly soluble in water and stable to oxidation in air (Merck Index, 1968). It is known to be a good source of Fe. Fe absorption from ferrous citrate when given in therapeutic doses has been shown to be about 75% of that from ferrous sulphate (Brise & Hallberg, 1962). There are several forms of ferrous citrate and the exact chemical nature of ferrous citrate employed in the above studies is not clear. The possible use of mono ferrous acid citrate, monohydrate ($FeC_6H_6O_7H_2O$), as a commercial product for fortification of common salt was considered and Fe absorption from this compound in human subjects has, therefore, been investigated.

MATERIALS AND METHODS

Subjects. Twenty-four normal adult men and 11 normal women formed the subjects of this study. All of them had normal haematological status and showed no evidence of achlorhydria or any other gastrointestinal abnormality. The mean haemoglobin concentration (g/dl) was $15{\cdot}2 \pm 1{\cdot}51$ in males and $14{\cdot}0 \pm 0{\cdot}64$ in females. All subjects used for radioactive studies were volunteers who gave informed consent.

Ferrous citrate. An authentic sample of mono ferrous acid citrate monohydrate was obtained as a gift from Scherrer Corporation, USA.

Radioactive iron. [^{59}Fe]ferric citrate was obtained from Bhaba Atomic Research Centre, Trombay, and [^{55}Fe]ferric citrate was obtained from the Radiochemical Centre, Amersham, Bucks.

Labelled ferrous citrate. [^{55}Fe]- or [^{59}Fe]ferrous citrate was prepared by an adaptation of the manufacture procedure (Chemical Abstracts, 1962) to the laboratory scale. A solution of (2·0 ml) of citric acid (47 mg), liquor ammonia (0·24 ml) and ferrous sulphate ($FeSO_4 . 7H_2O$), 40 mg containing about 100 μCi radioactive iron (ferric citrate) at pH 2·9, were sealed in a Pyrex glass ampoule of about 10 ml capacity under nitrogen and heated at 100° in an oven for 48 h. White ferrous citrate which had separated was filtered, washed with distilled water and then with methanol and dried in a vacuum oven. The yield was 50%. This compound was found to be identical with the authentic mono ferrous acid citrate on chemical analysis, microscopic examination and infra-red spectra. This salt after chemical analysis was used for absorption studies.

Labelled ferrous sulphate was prepared according to Steinkamp, Dubach & Moore (1955) as described earlier (Narasinga Rao *et al.* 1972).

Fe absorption from ferrous citrate when given alone was studied in twelve of the male subjects. In the remaining twelve male subjects iron absorption from ferrous citrate when given with a meal was determined. Fe absorption was determined in all the eleven female subjects when ferrous citrate was given alone and in five of them when the Fe was given with a meal. In all the subjects standard Fe absorption was determined with [^{59}Fe]ferrous ascorbate.

For the determination of Fe absorption from ferrous citrate given alone, [^{59}Fe]ferrous citrate containing 3 mg Fe and 5 μCi activity was given to the subjects after an overnight fast. When given with a meal, [^{59}Fe]ferrous citrate containing 7·5 mg Fe and 5 μCi radioactivity was mixed with legume soup which formed part of a rice-based lunch. The composition of the rice lunch was the same as reported earlier (Narasinga Rao & Vijayasarathy, 1975). In five female subjects who were given ferrous citrate both alone and with a meal, Fe absorption was determined by the double isotope method. Ferrous citrate labelled with either ^{59}Fe or ^{55}Fe was given with a meal or alone to the subjects on alternate days. Standard ferrous ascorbate absorption was determined with a dose of 3 mg Fe and 5 μCi 12 d after the last ferrous citrate dose.

Fe retention was measured by whole body counting as described earlier (Narasinga Rao *et al.* 1972). In the double isotope method blood radioactivity of ^{55}Fe and ^{59}Fe was measured by liquid scintillation counting according to a modified procedure of Eakins & Brown (1965).

The possible use of monoferrous citrate for fortifying cooking salt with Fe was studied. Ferrous citrate was mixed uniformly with coarse-ground crude cooking salt purchased locally, at a level to provide 1000 ppm Fe, and the fortified salt was stored in different containers.

RESULTS AND DISCUSSION

The results of absorption study are shown in Table 1. The average Fe absorption from ferrous citrate when given alone was 23·1% in men and 31·1% in women. The corresponding values for ferrous ascorbate absorption were 27 and 42% respectively. The value for ferrous ascorbate absorption in men was similar to that reported earlier (Narasinga Rao *et al.* 1972). Absorption of ferrous citrate relative to ferrous ascorbate was 0·97 in men and 0·66 in women, the over-all ratio being 0·87, slightly higher than the % absorption found by Brise & Hallberg (1962), Wittink, Ybema, Leijuse & Gerbrandy (1966) reported only 9·1% absorption from ferrous citrate. However, these authors had used high levels of Fe dosage, i.e. 30 and 200 mg respectively while in the present study only a 3 mg dose was used. Furthermore, the exact chemical nature of ferrous citrate employed by these authors is not known, as there are several forms of ferrous citrate.

When ferrous citrate was given with a rice-based meal the Fe absorption was reduced to 5·2% in men and 12·3% in women. Absorption in women was significantly ($P < 0·02$)

Table 1. *Iron absorption from ferrous citrate (mono ferrous acid citrate mono hydrate) when given alone or with a rice-based meal*

	Salt alone (3 mg Fe)				Given with a meal*	
Subjects	No. of subjects	Ferrous citrate (%A)	Ferrous ascorbate (%B)	B/A	No. of subjects	Absorption (%)
Male	12	23·0±3·62	27·3±6·40	0·97±0·151	12	5·2±1·43
Female	11	31·1±6·24	42·5±9·75†	0·66±0·114	5‡	12·3±1·63§

* Rice-based meal contained 9·6 mg Fe and ferrous citrate provided 7·5 mg Fe.
† Only 5 subjects studied.
‡ Female subjects ate less rice (100 g) than male subjects (200 g).
§ Significantly higher as compared to absorption in males $P<0.02$.

higher than in men. Absorption of ferrous sulphate given with a rice based meal has also been found to be around 5·0% in males (Narasinga Rao & Vijayasarathy, 1975). It was reported earlier that ferrous sulphate providing 7·5 mg Fe given with a wheat-based meal was found to be only 3·7% in men (Narasinga Rao *et al.* 1972). These results would thus indicate that absorption of Fe from ferrous citrate is satisfactory and is similar to that from ferrous sulphate.

Although ferrous citrate is a good source of Fe from the point of bioavailability, its stability is poor when it is used with a vehicle like crude salt. On storing common salt fortified with ferrous citrate, the mixture developed a yellowish-green colour within 8–10 d. Although ferrous citrate is stable and does not develop colour in the dry state it does so in the presence of moisture. Moisture content of crude salt sold in India is about 5%. This may be the reason for colour development when ferrous citrate is mixed with the crude salt. However, when ferrous citrate is mixed with purified sodium chloride or other dry materials like wheat flour or sugar they can be stored for a longer period without any colour development. Ferrous citrate may therefore be a suitable Fe compound for fortification of dry materials such as cereals and sugar.

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