CHAPTER I

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Iron is present in living organisms in only minute amounts but its participation in a number of biological processes that are essential to life, makes it one of the most important trace minerals. In man, the most common derangement of iron metabolism is iron deficiency anemia and it certainly represents a major cause of morbidity and mortality among women and children throughout the world.

Chemically, dietary iron is present in two forms: heme iron and nonheme iron. The portion of heme iron that is usually absorbed by humans from their diet is high (15%) as compared to the nonheme iron absorption, which is highly variable, but it is usually very low (2%) (Monsen et al, 1978). The proportion of heme iron in non-vegetarian, Western type meals is about 10-15% of the total dietary iron, while in the vegetarian meals, heme iron is altogether absent. Thus non heme iron makes a larger contribution to the dietary iron than the heme iron in any type of meal.

Unlike heme iron, the portion of nonheme iron that is absorbed from the diets can be greatly modified by a number of dietary factors ingested concomitantly (Morck and Cook, 1981). These include the enhancers that affect nonheme iron absorption favourably such as ascorbic acid, meat, fish and poultry (MFP) and the inhibitors that affect iron absorption adversely such as tannate, calcium and phosphate.
The enhancing effect of ascorbic acid has been well documented by several investigators (Hallberg et al, 1982; Hallberg and Rossander, 1984). In fact, it has been shown that addition of ascorbic acid (up to 1 g) brings about a marked dose dependent increase in iron absorption in humans (Cook and Monsen, 1977). The role of citrate, a low molecular weight organic acid has not been investigated as much as that of ascorbic acid, although there are indications that citrate may act to enhance iron absorption (Hazell and Johnson, 1987).

The role of polyphenols as inhibitors of iron absorption has been clearly established (Disler et al, 1975; Derman et al, 1977; Narasinga Rao and Prabhavati, 1982). Other food constituents which have been shown to have a marked inhibitory effect on iron absorption include calcium and phosphate salts (Chapman and Campbell, 1957; Monsen and Cook, 1976; Dheer et al, 1990) and phytate (Hallberg et al, 1989).

Oxalate is also believed to have an inhibitory effect on iron absorption (Kojima et al, 1981). However, no direct evidence is available to define the role of oxalate in modifying iron availability (Gordon and Chao, 1984).

Meals that are commonly consumed in India and South Asia are mainly cereal based. As a result the dietary constituents that inhibit iron absorption are present in larger proportion than the enhancers (Narasinga Rao and Prabhavati, 1982). Since the net
availability of iron from a meal is determined by the interaction and proportion of all these factors, it becomes necessary to assess the iron adequacy of the commonly consumed meals, on the basis of the interaction effect of these food constituents. However, no efforts have been made so far in this direction. The only quantitative model available to predict bioavailability of iron, is based on the heme and nonheme iron content of the diet and the amount of enhancers (ascorbic acid and meat, fish or poultry) present in the diet (Monsen and Balintfy, 1982).

In view of the above observations, the present study was planned with the major objective of developing a new regression equation to predict iron availability from vegetarian cereal based meals taking into consideration the inhibitors and enhancers present in such diets, and to use this equation to predict the quantity of enhancers required to achieve a defined level of iron availability.

The hypothesis tested was that an equation derived on the basis of the interaction effect of different enhancers and inhibitors, rather than the equation evolved on the basis of enhancers only would predict with reasonable accuracy, the availability of iron from cereal based Indian meals.

It is extremely difficult, if not impossible, to carry out a study of this kind in the in vivo situation. However, the in vitro methods which have been developed in the last two decades offer an appealing alternative. Of the several in vitro techniques
proposed, the one by Narasinga Rao and Prabhavati (1978) was chosen for this study. The authors have demonstrated a very high correlation ($r=0.94$) between the in vitro availability of iron as estimated by this method and the in vivo iron absorption in 47 human subjects. However the range of in vivo iron absorption in their subjects was narrow (1.6% to 3.8%) and therefore the question of whether this method of in vitro availability will yield a high correlation when compared against actual absorption of wider ranges was addressed first.

Before using this measure of in vitro available iron for developing the prediction equation, studies were undertaken to further validate this method by carrying out in vitro analysis of twelve meals, selected from various in vivo human studies in the literature and to correlate the in vitro available iron with the in vivo iron absorption values reported in the respective studies. These experiments formed the first phase of the study.

In the next two phases, experiments were carried out to develop a regression equation from a pure system containing 3 mg elemental iron in the form of FeCl$_3$ and different levels of enhancers and inhibitors to predict iron availability. Phase II investigated the dose effect of six constituents namely, ascorbic acid, citric acid, tannic acid, phytic acid, oxalic acid and calcium phosphate, and based on this, two levels of each of the six constituents were selected for phase III in which the regression equation was developed.
Phase II and III were designed to test the hypothesis that a regression equation developed from a pure system (equation 1) can predict with reasonable accuracy, the availability of iron from a complex meal system.

A parallel set of experiments were carried out in Phase IV and V using a standard cereal meal, commonly consumed in Gujarat, India, as the source of iron. Dose effect and interaction effect of various enhancers and inhibitors was studied and another regression equation (equation 2) was developed.

The two equations were applied to a set of ten, typical Indian meals, and their predictive powers were evaluated in the last phase of the study.

The specific objectives of the study in each phase were as follows:

Phase I: To establish the correlation between availability of iron as measured by the in vitro method used in the present study and the in vivo bioavailable iron reported for twelve selected meals and thus extend the validity of the in vitro method to a situation that included wider range of iron absorption.

Phase II: To evaluate the dose effect of various enhancers and inhibitors of iron availability from a pure system containing 3 mg of iron as FeCl₃. The purpose of this experimental phase was two-fold - (a) to confirm the in vivo, reported dose dependent enhancement of ascorbic acid in the in vitro system and to establish
the dose effects of other food constituents in the in vitro system and (b) to select two levels of enhancers and inhibitors for the next phase of the study.

Phase III: To study the interaction effect of the enhancers and inhibitors from the pure system and to evolve a regression equation (equation No.1 for predicting iron availability.

Phase IV: To study the dose effect of various enhancers and inhibitors added at similar dose levels as in Phase II, on iron availability from the standard cereal meal. The standard meal was formulated on the basis of the diet surveys carried out in Gujarat by us and diet surveys by others in different regions of India.

Phase V: To study the interaction effect of various enhancers and inhibitors in the standard meal system and to evolve another prediction equation (equation no.2).

Phase VI: To estimate iron availability as well as the content of various enhancers and inhibitors present in ten typical Indian meals.

: To evaluate the predictive powers of equation no.1 (pure system) and equation no.2 (standard meal), as applied to the ten Indian meals, in predicting the availability of iron.

: To select the best predictive model for general use and to suggest the practical implications.