Potential Iron Bioavailability in Usual Diets of the Imbo Region of Burundi

J. Nkunzimana, John A. Zee,* H. Turgeon-O'Brien, and Johanne Marin

Groupe de Recherche en Nutrition Humaine, Département des Sciences des Aliments et de Nutrition, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Sainte-Foy, Québec G1K 7P4, Canada

The purpose of this study was to assess the potential iron bioavailability of eight meals consumed in the Imbo region of Burundi. These meals were based on cassava flour and rice and contained legumes, fish, and vegetables. After an *in vitro* digestion, total and soluble iron concentrations were read from the atomic absorption spectrophotometer. Available iron was also estimated with the method of Monsen et al. Results showed that one-half of the studied meals contained only non-heme iron. Expressed as the ratio of soluble to total iron, potential iron bioavailability varied from 7.8% to 24%. Soluble iron and estimated absorbable iron were highly correlated ($r^2 = 0.93$; $p = 0.0001$), ranging from 0.76 to 5.08 and from 0.80 to 4.2 mg/person/day, respectively. It was concluded that the lack of heme iron and ascorbic acid in diets should be the main determinant of the low potential iron bioavailability in the Imbo region of Burundi.

Keywords: *Iron bioavailability; absorbable iron; in vitro digestion; soluble iron*

INTRODUCTION

Iron deficiency is a major health problem in developing countries (Hercberg, 1988). In tropical regions, it is a prevalent disorder with adverse consequences (Hercberg et al., 1987; Fairbanks, 1994). In Africa, epidemiological surveys have shown that iron deficiency is highly prevalent in menstruating and pregnant women, adolescents, and young children (Hercberg et al., 1988; DeMaeyer, 1989a). Although it is particularly widespread in that area, few studies on dietary causal factors have been carried out. Although reports associate iron deficiency with parasitic infestation (Fleming, 1980, 1990; Jackson and Jackson, 1987), there is increasing evidence that both inadequate diets and impaired iron absorption also play an important role (Hercberg and Galan, 1992). Therefore, while the amount of dietary iron intake is obviously important in the determination of iron status, an even greater factor is the varying bioavailability of food iron.

Parasitic disease control and iron supplementation are significant strategies for reducing iron deficiency anemia in developing countries, but problems exist with the effectiveness of large scale programs (Gillepsie et al., 1991). Moreover, little attention is focused on the possibility of enhancing iron bioavailability from local foods thus promoting iron absorption (DeMaeyer, 1989b; Tandu Umba, 1995).

As a biological concept, bioavailability should be determined, strictly speaking, by measurements *in vivo*. Human *in vivo* studies, however, are time-consuming and very expensive. Comparatively, *in vitro* methods are simple, rapid, and inexpensive, so they offer an appealing alternative even though it is not possible to simulate all physiological conditions *in vitro*. The model developed by Monsen et al. (1978) and refined by Monsen and Balintey (1982) to estimate the quantity of dietary absorbable iron is another interesting tool because it provides a means of estimating the adequacy of diets with respect to iron. The main purpose of the present study was to determine the potential iron bioavailability in the Imbo region of Burundi by evaluating the amount of soluble and absorbable iron in usual diets.

* Author to whom correspondence should be addressed [phone, (418) 656-2131 ext 7150; fax, (418) 656-3353; e-mail, John.Zee@aln.ulaval.ca].

Table 1. Composition of Experimental Diets (g)

	diets[a]							
food items	A	B	C	D	E	F	G	H
cassava flour		443						335
rice	326		277	241	289	326	311	
beans		179		230	212			
peas						140		
cowpeas							242	
dry fish		41	47		40			54
cassava leaves	43							
amaranth leaves		23						
tomatoes	33	29	31	443	25	24	33	22
onions	15	16	16	19	18	24	20	20
palm oil	16	16	15	16	19	15	15	18
salt	6.5	5.5	4.7	5.3	4.6	4.2	5.8	6.2

[a] Identified in a dietary assessment conducted previously in the Imbo region (Nkunzimana et al., 1995). These diets are generally eaten in one or two sittings.

MATERIALS AND METHODS

Preparation of Test Meals. In the present research, eight usual meals from the Imbo region of Burundi were studied. These diets have been previously identified during a food consumption survey that was conducted in the same region in 1992 (Nkunzimana et al., 1995). Each meal is habitually served and eaten in one or two sittings. Quantities reported in Table 1 correspond to the daily food consumption of housewives. All food items were purchased and imported from Burundi to Canada where traditional methods were used for cooking. All the meals contained tomatoes, onions, palm oil, and salt in different proportions. They also included rice and cassava flour as staples, legumes (beans, peas, cowpeas), dry fish, and plant leaves (cassava and amaranth). After cooking, each diet was homogenized and lyophilized. Thereafter, they were vacuum-packed and frozen at −20 °C until used.

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Estimation of Iron Bioavailability. The potential iron bioavailability was estimated using an *in vitro* method adapted from Politz and Clydesdale (1988) and Slatkavitz and Clydesdale (1988). The method involved a two-stage pepsic and pancreatic digestion. The resulting amount of soluble iron was used as an indicator of potential iron bioavailability.

Glassware. All glassware was washed in a laboratory dishwasher, rinsed in distilled water, soaked overnight in concentrated HCl, and rinsed with distilled deionized water (DDW) to remove contaminant iron. Distilled, deionized, iron-free water was used throughout the experiments.

Reagents. All reagents were of analytical grade and prepared with DDW. Pepsin enzyme (ICN Biochemicals, Cleveland, OH) was suspended into 25 mL of 0.1 N HCl. Purified pancreatin (ICN Biochemicals, Cleveland, OH) and bile extract (Sigma Chemical Co., St. Louis, MO) were dissolved in 250 mL of Tyrode buffer which contained the following ingredients: 2 g of NaCl, 0.05 g of KCl, 0.065 g of $MgSO_4 \cdot 7H_2O$, 0.014 g of $NaH_2PO_4 \cdot 2H_2O$, 0.25 g of glucose, 0.25 g of $NaHCO_3$, 0.066 g of $CaCl_2$, and 0.05 g of NaN_3. The pH of the Tyrode buffer was adjusted to 5 with 6 N HCl. Respective quantities of pepsin, pancreatin, and bile to be used in the experiments were determined according to the protein content of meals which was previously determined with Kjeldhal analysis (N × 6.25). A pepsin-to-protein ratio of 1:50, pancreatin-to-protein ratio of 1:30, and bile-to-protein ratio of 1:19.2 were utilized.

Procedure. A 20 g sample of each diet was brought to 100 g (w/w) with DDW in 250 mL flasks and incubated with pepsin in a 37 °C incubator (Lab-Line Orbit Environ shaker, Lab-Line Instruments Inc., Melrose Park, IL) for 2 h at pH 2 to simulate gastric conditions. Every 35 min, the pH was reajusted with 1 N NaOH. Following the pepsic digestion, the pH was increased to 5 with 3 N NaOH and a pancreatin−bile extract mixture was added. Then, the incubation was continued for an additional 2 h.

Iron Analysis. All samples were analyzed in triplicate for total and soluble iron. At the end of the incubation, 15 g of the digest was taken and left for 48 h in an oven at 100 °C and for one night at 500 °C in a muffle furnace (Thermolyne Type 18200, Sybron, IA). After cooling, ashes were recovered with nitric acid and samples filtered through Whatman #42 paper. The filtrate was then brought to 25 mL with 0.1 N HCl. Total iron content was measured by atomic absorption spectroscopy (AAS). An IL 751 Model spectrophotometer (Instrumentation Laboratory Spectrophotometer, Wilmington, MA) was used. The remaining portion of the digest was centrifuged in 250 mL tubes at 10000*g* for 20 min at 4 °C and filtered through Whatman No. 41 paper. Analysis of soluble iron was also performed by AAS on the supernatant. Iron concentrations were read directly at 248.3 nm against 0, 0.5, 2, 5, 10, and 15 ppm iron standards, which were prepared using a certified atomic absorption reference solution (Fisher Scientific, Nepean, Ontario). The precision within runs was 1%, and it was 2% between runs.

Estimation of Absorbable Iron. Absorbable iron was estimated with the model of Monsen et al. (1978) assuming 0 mg iron stores. Calculations were made on a meal basis of the amount of heme and non-heme iron as influenced by iron stores and the meal's content of enhancing factors (Table 2). Also, the model assumes that approximately 40% of the iron in fish, pork, beef, lamb, and chicken is in the form of heme iron.

Statistical Analysis. *In vitro* data were evaluated statistically by analysis of variance. Individual means were compared by Duncan's multiple range test (Steel and Torrie, 1980). Correspondence between estimated absorbable iron and *in vitro* soluble iron was calculated with Pearson's test of correlation.

RESULTS AND DISCUSSION

The energy and macronutrient contents of the diets are presented in Table 3. The energy content ranged from 1308 to 2444 kcal. This wide range in energy intake may be explained by the availability of food in households, but the family size and the daily meal frequency are also important determinants of food consumption levels (Nkunzimana et al., 1995). As indicated in Table 3, only one of the eight diets (diet B) was in the range of the recommended energy intake (FAO/WHO, 1986; Agbessi Dos-Santos and Damon, 1987; Savage and Burguess, 1992). In a context where diets are monotonous and not diversified, meals which fail to cover the energy requirements are most likely too poor to provide an adequate iron intake (DeMaeyer, 1989b; Nkunzimana et al., 1996).

Table 2. Model for Estimating Absorbable Iron

	iron stores (mg)			
	0	250	500	1000
heme iron	35%	28%[a]	23%[a]	15%
non-heme iron				
low-availability meal	5	4	3	3
1. meat, poultry, or fish <30 g lean, raw wt or				
2. ascorbic acid <25 mg				
medium-availability meal	10	7	5	3
1. meat, poultry, or fish = 30−90 g lean, raw wt or				
2. ascorbic acid = 25−75 mg				
high-availability meal	20	12	8	4
1. meat, poultry, or fish >90 g lean, raw wt or				
2. ascorbic acid >75 mg or				
3. meat, poultry, or fish = 30−90 g lean + ascorbic acid = 25−75 mg				

[a] These factors are approximate values based on a semilogarithmic relationship between iron stores (the linear function) and heme iron absorption (the logarithmic function), from Monsen et al. (1978).

The heme and non-heme iron, vitamin C, and absorbable iron contents of the experimental diets appear in Table 4. Values are calculated from the dietary information and given for cooked ingredients. Diets A, D, F, and G did not contain heme iron. Non-heme iron represented 81.3−100% of the total iron content in all units. Differences in dietary intake of the Imbo population have been observed earlier and discussed in former papers (Nkunzimana et al., 1995a,b). Diet A provided a sufficient amount of vitamin C; others provided less than 25 mg/day. Important losses of ascorbic acid have been reported to occur in African households during local processing and traditional cooking, resulting in very low values in cooked foods and, hence, in very low iron absorption (Oteng-Gyang and Mbachu, 1987; Lyimo et al., 1991; Okeibuno, 1991; Galan et al., 1990, 1991). According to Hallberg et al. (1979) and Monsen (1988), meat, poultry, and fish in the diet not only provide heme iron but also enhance the absorption of non-heme iron from vegetable foods. Hence, much of the iron deficiency in many developing countries might be ascribed to the virtual lack of these products (Hercberg et al., 1987; Hercberg and Galan, 1992). In the present study, the quantity of absorbable iron ranged from 0.8 to 4.2 mg, and five diets out of eight (diets A, C, D, F, G) were under allowances in absorbed iron for pregnant (4.4−6.3 mg) and nonpregnant (2.38 mg) women (FAO/WHO, 1988). As expected, the potential iron bioavailability was higher in meals containing fish, i.e., cassava−beans−fish meals (diet B), rice−beans−fish meals (diet E), and cassava−fish meals (diet H). The levels of absorbable iron in these diets varied from 2.4 to 4.2 mg and met the requirements of menstruating and lactating women (FAO/WHO, 1988). Unfortunately, our inves-

Table 3. Energy and Macronutrient Intakes[a] in Usual Diets from the Imbo Region of Burundi

	diets								
	A	B	C	D	E	F	G	H	RDI[b]
energy (kcal/day)	1362	2444	1308	1791	2070	1788	2084	1525	2140−2640
proteins (% kcal)	9	13	16	16	19	13	15	11	7−12
total fat (% kcal)	14	9	17	12	13	11	10	14	15−25
carbohydrates (% kcal)	77	78	67	70	68	76	75	75	65−75

[a] Calculated from the dietary information by using food composition tables (SNES, 1987). [b] Recommended dietary intake (FAO/WHO, 1986; Agbessi Dos-Santos and Damon, 1987; Savage and Burguess, 1992).

Table 4. Heme Iron, Non-heme Iron, Vitamin C, and Absorbable Iron Contents[a] of Usual Diets from the Imbo Region of Burundi

	diets							
	A	B	C	D	E	F	G	H
total iron (mg)	8.3	37.4	10.7	24.3	27.6	32.1	25.2	18.0
heme iron (% total iron)	0	5.4	18.7	0	5.8	0	0	12.3
non-heme iron (% total iron)	100	94.6	81.3	100	94.2	100	100	87.7
vitamin c[b] (mg)	68.2	24.2	7.5	7.4	8.5	7.4	8.3	15.7
absorbable iron (mg)	0.8	4.2	1.6	1.6	3.2	1.6	1.3	2.4
absorbable iron (% total iron)	10.0	11.0	14.2	5.0	11.4	5.0	5.0	12.9

[a] Calculated for the eight experimental units by using food composition tables (SNES, 1987). Values represent the summation of the nutrient intakes from each of the ingredients. [b] Values are given for cooked foods.

Table 5. Total and Soluble Iron Contents (mg/100 g) of Usual Diets from the Imbo Region of Burundi[a]

	diets							
	A	B	C	D	E	F	G	H
total iron	$2.28^{b} \pm 0.03$	$3.45^{d} \pm 0.12$	$2.34^{b} \pm 0.02$	$1.12^{a} \pm 0.01$	$2.08^{b} \pm 0.05$	$1.52^{a} \pm 0.04$	$1.43^{a} \pm 0.02$	$2.91^{c} \pm 0.07$
soluble iron	$0.17^{a} \pm 0.01$	$0.67^{e} \pm 0.03$	$0.27^{b} \pm 0.02$	$0.19^{a} \pm 0.01$	$0.41^{c} \pm 0.02$	$0.30^{b} \pm 0.01$	$0.20^{a} \pm 0.01$	$0.57^{d} \pm 0.03$
% soluble iron	$7.81^{a} \pm 2.22$	$19.72^{d} \pm 8.69$	$11.90^{b} \pm 3.37$	$17.26^{c} \pm 2.15$	$24.07^{e} \pm 2.81$	$20.24^{d} \pm 4.51$	$14.25^{b} \pm 1.47$	$19.34^{d} \pm 4.86$

[a] Means (±SD) followed by the same superscript within a row are not significantly different ($p < 0.05$).

tigation indicated that these diets are scarce in rural areas of Burundi (Nkunzimana et al., 1996).

Table 5 presents the amount of total and soluble iron following the *in vitro* digestion. The total iron content per 100 g of wet matter ranged from 1.1 to 3.4 mg, whereas the mean soluble iron content varied from 0.17 to 0.67 mg. As for absorbable iron, the highest amount of soluble iron was found in meals containing fish. Therefore, the consumption of these types of diets must be encouraged in the Imbo region as potential sources of bioavailable iron.

In the present study, the potential iron bioavailability expressed as a percentage of soluble iron ranged from 7.8% to 24%. *In vivo* studies carried out on meals of the same nature as those in our study indicated a relatively lower iron absorption (Galan et al., 1990, 1991; Guiro et al., 1991). Galan et al. (1991) observed non-heme iron absorption ranging from 0.9% to 23.1% in Zairian meals which contained rice and cassava as the staple foods. They also reported iron absorption of 0.2−11.8% in Beninese meals based on maize (Galan et al., 1990). Similar values (1.2−11.4%) were observed in Senegalese meals based on cereals (Guiro et al., 1991). These results are consistent with our data using Monsen's model which indicated that absorbable iron represented 5−14.2 % of the total amount of iron (Table 4). The higher *in vitro* values observed in our study might be partly explained by the fact that a major part of the dietary iron in African countries is comprised of contaminant iron which may not be absorbed in spite of its relative solubility (Guiro and Hercberg, 1988; Galan et al., 1990). However, insufficient information is available to make any quantitative prediction of the absorption of contaminant iron.

In the present study, we also obtained a highly significant correlation ($r^2 = 0.93$; $p = 0.0001$) between soluble iron intake as determined *in vitro* and absorbable iron estimated with the model of Monsen et al. (1978). Values ranged from 0.76 to 5.08 and from 0.80 to 4.2 mg/person/day, respectively. In estimating absorbable iron with Monsen's model, calculations do not take into account inhibitory factors of iron absorption. Brune et al.(1989) and Brune (1991) have shown *in vivo* iron absorption being significantly affected by the presence of phytates and phenolic compounds in diets. Several papers involving *in vitro* methods have also shown dietary fiber binding iron and other trace minerals (Gillooly et al., 1984; Torre and Rodriguez, 1991). However, in our study, we found a significant agreement between Monsen's model and the *in vitro* method. Thus, the influence of inhibitory factors of iron absorption should have been partly reduced by enhancing factors such as ascorbic acid and fish. It should be noted, however, that the ascorbic acid content of most of our experimental meals was lower than 25 mg (Table 4). According to Monsen et al. (1978) and Monsen and Balintey (1982), such a level in a single meal is related to a low iron bioavailability. Further research will be necessary to evaluate the content of potent enhancers and inhibitors of iron absorption and their respective effects on iron solubility in typical diets from Burundi.

Evaluating the potential iron bioavailability in usual diets from Burundi is an important prerequisite for effective dietary modifications which would improve local diets and the nutritional status of vulnerable populations in developing countries. The low energy and total and absorbable iron intakes in the Imbo region of Burundi must be one of the dietary causal factors of iron deficiency anemia. Increasing the intake of heme iron from fish and of vitamin C from vegetables must be encouraged in this population, since this may provide a potential solution to prevent iron deficiency in the long run.

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