Measurement of the total daily dietary iron absorption by the extrinsic tag model[1, 2]

Miguel Layrisse,[3] Carlos Martínez-Torres,[4] and Magdalena González[5]

ABSTRACT Measurement of the daily non-heme iron absorption from diets consumed in several areas of Venezuela was determined by the extrinsic tag method. Diets were selected from three regions (Central region, Andes and Coast) with different environment and food habits. Breakfast and lunch extrinsically tagged with ^{59}Fe and ^{55}Fe, respectively, were administered on the first 2 days of each study. Fifteen days later, blood was drawn to determine hematological characteristics and radioactivity and subjects were fed again with supper tagged with ^{59}Fe. Finally a standard dose of iron ascorbate tagged with ^{55}Fe was administered on the 16th day and blood was taken 2 weeks later to determine the increase of blood radioactivity. Neither milk nor cheese and butter improved the poor absorption of vegetal iron administered at breakfast. Absorption of 3 to 4 mg of vegetal iron was increased about twice by the effect of 50 mg of meat, about thrice by 100 g of fish and about five times by 150 g of papaya containing 66 mg of ascorbic acid. The absorption from heme iron in meat accounts for 62 and 42% of the total iron absorbed from Central Andes diets, respectively, whereas fish iron absorption accounts for about 10% of the total iron absorbed. The data presented indicate that the amount of dietary iron does not reflect the net amount of iron absorbed by the individuals and that ingredients of a meal such as beef, fish and fruits are paramount to obtain a reasonable utilization of the non-heme iron. *Am. J. Clin. Nutr.* 27: 152–162, 1974.

Several studies carried out in the last three years (1–3) have demonstrated that the extrinsic tag method for iron absorption allows measurement of the absorption from heme and vegetable food iron present either in a single food or in a complete meal containing meat and several vegetable foods. These studies provided further evidences of dissociation in the absorption from the heme iron pool represented by hemoglobin and myoglobin and the absorption from the non-heme iron pool represented by vegetal iron and iron salts.

As an extension of these studies, this paper will describe the measurement of the daily iron absorption from the diets consumed in several areas of Venezuela, using the extrinsic tag model. It will also show the importance of some animal foods and fruits in the diet as enhancing factors for iron absorption.

Material and methods

Eighty-two adult peasants from rural areas of Venezuela volunteered for this study; 37 were males and 45 were females. These subjects were in apparent good health with the exception of some individuals who have moderate and marked iron deficiency due mostly to hookworm infection. For each person, in addition to iron absorption determinations, other tests were performed for blood hemoglobin concentration (4), packed red cell volume, serum iron (5), and unsaturated iron binding capacity (6, 7).

Preparation of the extrinsic tag diets

Three types of diets were used for this study. They were selected from previous nutrition surveys carried out by the Instituto Nacional de Nutrición in areas with different environments and food habits. The diet from the central area represents an average of the foods consumed by people in the low socioeconomic class living in rural and semirural areas, but close to the cities of Aragua and Carabobo States. Due to the variety of food articles that these individuals regularly introduce in their diet within the year, it was difficult to obtain an average of the foods consumed four or five times a week, and the diet selected does not reflect entirely the various foods consumed in that area (Table 1). Maize is the staple vegetable food which is accompanied by cereals, oats at breakfast and

---

[1] From the Instituto Venezolano de Investigaciones Científicas, Apartado 1827 Caracas, Venezuela.
[2] Supported in part by World Health Organization and William Waterman Fund.
[3] Chief, Hematology Laboratory, Instituto Venezolano de Investigaciones Científicas, and Professor of Medicine, Universidad Central de Venezuela. Caracas, Venezuela. [4] Associate Investigator. [5] Chief, statistics Department.

The American Journal of Clinical Nutrition 27: FEBRUARY 1974, pp. 152–162. Printed in U.S.A.

TABLE 1
Average of a daily dietary iron intake consumed in various areas of Venezuela

Breakfast, uncooked food weight, g		Iron, mg	Lunch, uncooked food weight, g		Iron, mg	Supper, uncooked food weight, g		Iron, mg
Central areas								
Maize	40	1.13	Maize	40	1.13	Maize	40	1.13
Butter	10	0.06	Black beans	10	0.70	Black beans	10	0.70
Cheese	15	0.15	Paste	7	0.15	Paste	7	0.15
Oats	20	0.98	Plantain	80	0.71	Eggs	56	1.40
Milk	200	0.60	Tomato	50	0.40	Meat	50	1.25
			Potato	40	0.40			
			Carrot	40	0.40			
			Meat	50	1.25			
Total		2.92			5.14			4.63
Andes								
Maize	40	1.13	Maize	40	1.13	Maize	40	1.13
Milk	100	0.30	–	–	–	Milk	100	0.30
Butter	10	0.06	Plantain	90	0.80	Plantain	90	0.80
White cheese	15	0.15	Tomato	100	0.60	Tomato	100	0.60
			Rice	10	0.27	Rice	10	0.25
			Black beans	10	0.70	Black beans	10	0.70
			Meat	50	1.25	Coffee	8	0.22
						Sugar	8	0.00
Total		1.64			4.75			3.88
Coast								
Maize	40	1.20	Maize	40	1.20	Maize	40	1.20
Milk	100	0.30	Rice	10	0.25	Milk	100	0.30
Butter	10	0.06	Plantain	90	0.80	Plantain	90	0.80
Fish	60	0.30	Fish	50	0.25	Fish	50	0.25
Coffee	8	0.22	Avocado	50	0.40	Pumpkin	100	0.40
Sugar	8	0.00	Tomato	50	0.30	Papaya	150	0.60
			Onion	10	0.10	Coffee	8	0.22
			Watermelon	150	0.30	Sugar	8	0.00
Total		2.08			3.60			3.77

wheat at lunch and supper. They have a small piece of meat at lunch and supper.

The diet from the Venezuelan Andes represents an average of the foods consumed four to five times a week by people of the low or middle low economic levels living in Merida and Trujillo States. The basic vegetable food intake is maize, which is eaten at each meal; milk is usually drunk at breakfast and supper, and a small piece of meat is eaten once a day.

A survey carried out among the poor people on Margarita Island and in the coastal region of Anzoategui State served as a base to select an average diet for the inhabitants of the Venezuelan Coast. The diet these people consume four or five times a week consists of two main foods, maize and fish, eaten at each meal and accompanied by fruits at lunch and supper.

A small amount of iron (0.1 mg) in the form of ferric chloride was used as an extrinsic tag. This salt, tagged with ^{59}Fe, was mixed with a maize dough during the preparation of breakfast. The same amount of salt tagged with ^{55}Fe and ^{59}Fe was used for lunch and supper, respectively. For lunch and supper of the Andes diet and lunch of the Coast diet, the tagged ferric chloride solution was divided into two aliquots, one was mixed with maize dough and the other added to the water used to boil the rice. The foods were served separately in an aluminum dish at each meal and the subjects were free to eat them as they pleased.

The amount of calories, protein, and fat was estimated from the food composition tables (8, 9), the amount of iron determined by chemical analysis, and the ascorbic acid content was chemically determined from the meals at the time they were served.

Absorption studies

Breakfast tagged with ^{59}Fe was administered in the morning of the first day, after an overnight fast. No food or drink was allowed during the 3 hr following the feeding test. Lunch tagged with ^{55}Fe was administered the morning of the next day. Fifteen days later, blood was drawn to determine hematological characteristics and radioactivity, and the subjects were fed with the supper tagged with ^{59}Fe. Finally, a standard dose of ferrous ascorbate tagged with ^{55}Fe

(3 mg Fe) was administered on the 16th day and absorption was again determined from the increase of blood radioactivity found 2 weeks after the administration of the last test dose.

Duplicate 10-ml blood samples were prepared for radioactive counting by wet-ashing, iron precipitation, and electroplating, following the method of Dern and Hart (10, 11). Radioactivity was counted in a Tri-Carb Scintillation Counter (Packard Model 3310). Triplicate standards of tagged foods and iron salts were also digested and counted simultaneously with the blood sample. Blood volume was estimated according to the Tulane table (12). Non-heme iron from a meal was then calculated by dividing the total blood radioactivity by the total radioactivity administered with the foods. No correction was made to estimate the total iron utilization.

Radioactive iron salt. Trace doses of iron in the form of ferric chloride with a specific activity of 10 to 15 μCi/μg Fe were mixed with carrier ferrous sulfate. Two moles of ascorbic acid/mole of iron were added prior to oral administration.

Statistical analysis. As in previous studies (1, 2, 13–15), the mean absorption and standard deviation were calculated from the logarithm of the percentage of absorption and the result was retransformed as an antilogarithm to recover the original units. The comparison of iron absorption from two meals was performed in pairs by the Student *t* test (13).

Results

Table 2 shows the nutrient content of the Venezuelan diets studied for iron absorption. For the especial purpose of this study, only foods eaten four or more times a week were chosen to integrate these diets: this is the reason why the total food intake in the Central Areas and Andes diets is lower than the average intake obtained from the surveys (16, 17). The Andes diet is apparently less rich in nutrients than the others and ascorbic acid is present in high amounts in the Coast diet, especially at supper when a large piece of papaya is eaten.

Table 3 shows the hematological characteristics and iron absorption tests of the three Venezuelan populations: Central Areas, Andes, and Coast. Two studies were performed on the Coast diet and in the study on the Andes diet, each meal was administered twice on alternate days, following the technique of Brise and Halberg (18). The results on the concentration of serum iron, percent transferrin saturation, and the absorption of a standard dose of iron ascorbate indicate that the groups are not comparable in terms of individual iron stores. Apparently, group D has more iron-deficient subjects.

TABLE 2
Nutrient content from the Venezuelan diets studied

	Breakfast	Lunch	Supper	Total
Central areas				
Total food energy, kcal	386	403	330	1,119
Protein, g	16.6	17.5	21.8	55.9
Fat, g	17.8	9.7	15.2	42.7
Ascorbic acid, mg	1.0	17.1	0.0	18.1
Andes				
Total food energy, kcal	237	391	408	1,036
Protein, g	10.4	16.5	11.8	38.7
Fat, g	12.3	9.7	6.3	28.3
Ascorbic acid, mg	0.0	19.8	15.8	35.6
Coast				
Total food energy, kcal	376	454	521	1,351
Protein, g	20.5	17.6	21.0	59.1
Fat, g	12.5	10.3	8.2	31.0
Ascorbic acid, mg	0.0	29.9	75.4	105.3

The non-heme iron absorption from the Central Areas diet is low in each meal but its absorption at lunch and supper is significantly higher than that observed at breakfast. This increase was probably due to the small portion of meat eaten at these meals.

The non-heme iron absorption from the Andes diet also shows low absorption in each meal, but for the same reason mentioned above, absorption at lunch was significantly higher than at breakfast.

The non-heme iron absorption from the coast diet was high in each meal and in both studies; the mean absorption in the first study was 5% at breakfast and increased up to 7 and 13% at lunch and supper, whereas in the second study, the mean absorption was 7% at breakfast and increased to 11 and 17% in the last two meals. This diet differs from the other diets studied here in two main components; 100 g of fish is eaten at each meal and fresh fruit was eaten at lunch and supper. We direct your attention to the large portion of papaya (150 g) that the subjects eat at supper and its high ascorbic acid content (44 mg/100 g).

In order to elucidate the difference of the enhancing effect of papaya and fish on the

TABLE 3
Non-heme iron absorption from diets consumed in various areas of Venezuela

							Iron absorption, %			
Subject	Age, years	Sex	Hb, g/ 100 ml	Packed red cell, %	Serum iron, μg/ 100 ml	Transferrin saturation, %	A Breakfast ^{59}Fe	B Lunch ^{55}Fe	C Supper ^{55}Fe	D Iron ascorbate ^{59}Fe
Central areas										
1) SI	34	F	14.7	42.5	78	25	0.24	1.04	2.23	35.19
2) LV	48	F	14.9	44.0	115	44	0.30	1.49	4.18	9.11
3) CA	55	F	15.3	43.0	100	35	0.40	0.93	0.56	14.17
4) RA	35	F	13.2	38.0	78	24	0.42	2.96	2.04	15.82
5) EV	56	M	15.4	42.5	151	43	0.57	3.41	2.66	11.88
6) JB	32	F	12.6	37.5	122	39	1.00	4.55	1.50	24.90
7) JD	28	M	18.0	49.0	130	36	1.56	2.73	3.56	8.73
8) JA	50	M	15.8	46.0	106	35	1.57	3.94	2.06	18.39
9) PJV	19	M	15.0	43.5	141	49	1.59	3.09	4.00	19.84
10) GS	29	F	12.7	38.0	49	15	3.06	8.71	1.57	16.02
11) EL	17	M	17.0	49.0	68	19	8.48	9.76	3.36	46.13
12) MJV	35	F	13.5	39.0	58	18	9.57	17.15	10.75	49.56
Mean	37		14.8	42.7	100	32	1.17	3.48	2.54	19.18
Limits of 1 SE							(0.82–1.67)	(2.70–4.50)	(2.06–3.12)	(16.23–22.66)
Andes										
1) NA	58	M	15.3	42.0	132	45	0.44	1.09	0.69	8.71
2) NG	56	M	16.0	44.0	107	38	0.59	1.17	0.39	3.33
3) BA	45	M	14.8	42.0	45	13	0.63	0.56	3.04	45.76
4) AC	60	M	14.9	40.0	98	26	0.66	2.08	4.71	30.47
5) MG	54	F	14.2	41.0	119	35	0.72	1.11	1.33	9.94
6) RF	54	M	15.5	43.0	115	44	0.76	0.98	1.11	5.82
7) IG	50	F	13.8	40.0	90	28	0.95	3.28	0.89	9.61
8) RG	33	F	14.1	42.0	109	37	0.96	1.96	3.05	21.47
9) VR	37	M	15.7	43.0	98	30	1.19	0.90	0.50	3.41
10) CP	47	F	14.0	39.0	103	34	1.89	1.76	4.25	17.19
11) EC	60	F	14.3	41.0	97	32	1.90	3.63	1.19	17.69
12) YL	25	F	13.0	38.0	100	27	3.10	8.90	3.98	35.42
13) AR	29	F	14.9	42.0	106	29	3.45	4.69	1.58	8.34
14) AG	17	F	13.0	39.0	40	10	10.31	9.34	13.42	29.42
15) CV	30	F	13.8	41.0	59	15	15.43	15.99	13.85	40.48
Mean	44		14.4	41.1	95	30	1.49	2.35	2.04	14.12
Limits of 1 SE							(1.13–1.96)	(1.82–3.03)	(1.54–2.71)	(11.30–17.64)
Coast (1st study)										
1) AD	45	M	14.5	41.0	109	38	0.95	5.43	2.82	22.74
2) RI	18	M	15.0	43.0	154	45	2.13	6.47	7.74	19.66
3) AG	43	F	15.3	45.0	139	44	2.74	3.22	10.20	15.25
4) HG	14	F	15.0	42.0	145	40	3.55	6.69	16.14	25.70
5) RL	21	F	13.2	37.0	100	28	4.32	5.64	20.08	32.10
6) RB	20	F	13.5	39.0	158	44	4.40	10.07	19.94	39.61
7) NS	31	M	16.6	46.0	136	36	4.73	2.31	5.83	7.59
8) RI	33	M	15.9	46.0	160	45	5.60	3.53	6.09	8.87
9) JL	17	M	6.7	25.0	30	8	6.68	16.42	29.99	55.78
10) SU	30	M	16.2	49.0	77	18	8.67	7.46	18.80	24.25
11) MR	19	F	14.8	41.0	72	21	9.36	14.07	23.13	60.26
12) CV	38	F	14.0	41.0	77	23	10.12	9.82	19.44	61.67
13) AB	62	M	15.1	44.0	127	35	10.22	13.94	29.39	32.82

TABLE 3–*Continued*

Subject	Age, years	Sex	Hb, g/100 ml	Packed red cell, %	Serum iron, μg/100 ml	Transferrin saturation, %	Iron absorption, % A Breakfast ^{59}Fe	B Lunch ^{55}Fe	C Supper ^{55}Fe	D Iron ascorbate ^{59}Fe
14) BD	21	F	13.1	38.0	84	24	10.39	7.80	7.01	54.92
15) MCR	35	F	15.0	44.0	129	31	10.46	9.65	15.87	27.71
Mean	30		14.3	41	113	32	5.23	7.13	12.84	27.44
Limits of 1 SE							(4.37–6.27)	(6.16–8.25)	(10.74–15.35)	(23.21–32.44)
Coast (2nd study)										
1) CB	52	M	9.4	30.5	18	4	0.76	3.40	5.23	50.53
2) AH	60	F	14.9	43.0	63	19	0.99	2.87	6.44	9.33
3) AR	37	F	13.3	38.0	62	22	3.00	4.11	15.47	32.26
4) EM	50	F	13.2	39.0	61	18	3.02	6.42	7.31	10.32
5) JT	50	F	15.2	40.0	153	40	4.46	6.87	13.55	22.42
6) VV	43	M	16.8	47.0	108	37	11.06	8.62	7.73	18.69
7) GG	15	F	13.6	39.5	96	29	11.19	16.13	28.21	27.77
8) RA	40	M	15.9	46.0	57	17	11.51	8.18	12.59	24.62
9) MA	19	F	15.1	42.0	100	24	12.99	18.06	40.11	29.48
10) AP	20	F	12.4	36.0	64	15	14.98	21.09	51.09	73.98
11) RMG	17	F	13.0	38.0	36	8	22.42	30.06	19.39	68.07
12) RA	16	M	12.6	36.5	35	8	23.07	51.31	53.83	76.31
13) EB	14	M	9.9	31.0	22	5	26.48	44.03	44.59	59.30
Mean	33		13.5	39.0	67	19	7.09	11.35	17.41	31.51
Limits of 1 SE							(5.12–9.83)	(8.71–14.79)	(13.83–21.92)	(25.93–38.29)

absorption from non-heme iron, further experiments were carried out in which the absorption from maize iron given alone was compared with the absorption from maize administered with papaya and maize administered with papaya and fish muscle (Table 4). The mean iron absorption from maize administered with papaya was similar to the absorption from maize administered with papaya and fish and either mean absorption was six times higher than the iron absorption from maize given alone. These results clearly indicate the striking enhancing effect of papaya on the absorption from non-heme iron.

Another experiment was carried out in which the effect of papaya on the non-heme iron absorption was compared with the effect of ascorbic acid contained in this fruit. In Table 5, the effect of this fruit on iron absorption is shown to be due to its ascorbic acid content. In this study, maize administered with either papaya or with the amount of ascorbic acid contained in the same portion of papaya increases about six times the absorption of maize iron.

The difference in absorption from maize iron in Tables 4 and 5 is probably due to the number of iron-deficient subjects in each group, which is reflected in their respective iron ascorbate absorption.

Calibration of non-heme iron absorption data

Figure 1 shows the correlation coefficient data between the absorption from iron ascorbate in each study and iron absorption from each meal. There is a significant correlation in each instance; in some of them it is higher than 0.75. The correlation coefficient was not particularly high in the Andes meal despite the fact that the method of multiple doses was used.

Based on the significant correlation observed

TABLE 4
Effect of papaya a

Subject	Age, years
1) JM	50
2) VMT	52
3) AR	28
4) JAT	63
5) JG	46
6) BFM	47
7) MQ	36
8) VT	64
9) FD	19
10) RR	29
11) ARB	18
12) JCB	40
13) EG	20
14) TV	55
Mean	41
Limits of 1 SE	

[a] The following

TABLE 5
Effect of papaya an

Subject	Age, years
1) RA	33
2) EG	60
3) GG	54
4) RG	32
5) AE	62
6) MP	35
7) FM	34
8) PP	24
9) HL	18
10) SV	65
11) AR	16
12) SV	18
13) BV	42
Mean	38
Limits of 1 SE	

[a] The following a
acid.

TABLE 4
Effect of papaya and fish on the absorption from non-heme iron

Subject	Age, years	Sex	Hb, g/100 ml	Packed red cell, %	Serum iron, μg/100 ml	Transferrin saturation, %	Iron absorption, %[a] A Maize alone ^{55}Fe	B Maize + papaya ^{55}Fe	C Maize + papaya + fish ^{59}Fe	D Iron ascorbate ^{59}Fe
1) JM	50	M	16.2	46.0	105	33	0.82	7.62	6.89	5.36
2) VMT	52	M	15.8	43.0	105	29	1.15	37.71	26.58	85.34
3) AR	28	F	13.6	41.0	156	41	1.57	17.80	20.28	18.24
4) JAT	63	M	14.7	44.0	71	20	2.56	33.15	25.19	18.35
5) JG	46	M	14.0	44.0	60	18	3.39	14.51	30.12	46.60
6) BFM	47	F	15.2	41.0	111	32	3.85	17.65	15.87	38.89
7) MQ	36	M	12.7	39.0	62	14	5.11	38.15	41.57	62.81
8) VT	64	M	15.9	44.0	127	41	5.95	12.12	12.73	26.28
9) FD	19	F	11.5	37.0	91	23	7.91	21.41	22.62	36.96
10) RR	29	F	8.8	30.0	21	5	9.45	43.49	34.05	67.40
11) ARB	18	M	15.3	43.5	84	27	9.60	42.84	26.29	82.36
12) JCB	40	M	15.6	47.0	76	24	10.67	16.53	16.84	27.38
13) EG	20	F	13.1	40.0	112	30	11.28	60.67	50.46	74.76
14) TV	55	F	10.7	33.0	22	6	12.48	38.89	28.57	45.70
Mean	41		13.8	40.9	86	25	4.55	24.69	23.01	36.93
Limits of 1 SE							(3.58–5.78)	(21.03–28.97)	(20.09–26.34)	(30.15–45.25)

[a] The following amount of food was administered: 100 g cooked maize, 150 g fresh papaya, and 100 g fish.

TABLE 5
Effect of papaya and ascorbic acid on the absorption of non-heme iron

Subject	Age, years	Sex	Hb, g/100 ml	Packed red cell, %	Serum iron μg/100 ml	Transferrin saturation, %	Iron absorption, %[a] A Maize alone ^{59}Fe	B Maize + ascorbic acid ^{55}Fe	C Maize + papaya ^{59}Fe	D Iron ascorbate ^{55}Fe
1) RA	33	M	14.8	45.5	133	44	0.14	0.92	1.59	9.73
2) EG	60	F	12.3	37.0	79	25	0.52	2.46	3.74	7.70
3) GG	54	F	13.2	38.5	104	32	0.79	6.21	9.43	8.32
4) RG	32	M	16.8	47.0	193	38	0.86	4.07	8.88	21.28
5) AE	62	M	13.9	40.0	167	42	0.92	12.95	13.42	52.63
6) MP	35	M	15.3	45.0	46	15	1.00	2.03	8.35	16.46
7) FM	34	F	13.0	38.0	149	46	1.10	15.33	4.00	11.04
8) PP	24	F	14.4	43.0	157	60	1.85	8.87	2.95	10.91
9) HL	18	M	16.6	49.0	108	30	2.20	7.24	26.64	33.78
10) SV	65	M	14.7	44.0	102	29	2.98	13.24	8.59	14.73
11) AR	16	F	11.6	36.0	51	13	3.23	36.80	21.24	57.33
12) SV	18	F	13.5	40.0	80	20	3.39	22.76	16.33	44.27
13) BV	42	F	12.0	37.0	51	14	9.45	28.29	35.70	42.80
Mean	38		14.0	41.5	109	31	1.38	7.90	8.79	19.86
Limits of 1 SE							(1.03–1.84)	(5.83–10.70)	(6.82–11.32)	(16.17–24.39)

[a] The following amount of food was administered: 100 g cooked maize, 150 g papaya, and 70 mg ascorbic acid.

TABLE 7
Average of the daily dietary iron absorption from Venezuelan diets

Meals	Type of iron component	Iron intake, mg	Iron absorption, %	Total iron absorption: From observed absorption	Total iron absorption: From calibrated absorption[a]
Central areas					
Breakfast	Non-heme	2.92	1.17	0.03	0.04
Lunch	Non-heme	3.89	3.48	0.14	0.15
	Heme[b]	1.25	17	0.21	0.24
Supper	Non-heme	3.38	2.54	0.09	0.10
	Heme[b]	1.25	17	0.21	0.24
Total		12.69		0.68	0.77
Andes					
Breakfast	Non-heme	1.64	1.49	0.02	0.04
Lunch	Non-heme	3.50	3.25	0.11	0.13
	Heme[b]	1.25	12	0.15	0.24
Supper	Non-heme	3.88	2.04	0.07	0.12
Total		10.27		0.35	0.53
Coast (1st study)					
Breakfast	Non-heme	1.78	5.23	0.09	0.07
Lunch	Non-heme	3.35	7.13	0.24	0.19
Supper	Non-heme	3.52	12.04	0.42	0.36
Fish ingested in three meals	Ferritin[c] mainly	0.80	12	0.10	0.08
Total		9.45		0.85	0.70
Coast (2nd study)					
Breakfast	Non-heme	1.78	7.09	0.12	0.09
Lunch	Non-heme	3.35	11.35	0.38	0.25
Supper	Non-heme[c]	3.52	17.41	0.61	0.43
Fish ingested in three meals	Ferritin mainly	0.80	14	0.11	0.08
Total		9.45		1.22	0.86

[a] The calibration of the non-heme iron absorption was made by multiplying the mean observed absorption data by the ratio of A/B, where A is the composite mean absorption of the reference dose of all individuals and B the mean absorption of the reference dose for a given diet. [b] The iron absorption from meat was calculated by multiplying the absorption of the reference dose found in a given study by 0.87, which is the mean absorption ratio of veal iron to the reference dose. [c] The iron absorption from fish was calculated by multiplying the absorption of the reference dose found in a given study by 0.44, which is the mean absorption ratio of fish iron to the reference dose.

Previous studies (1–3, 20) have shown that extrinsic tag absorption reflects the absorption from the native vegetal iron and both absorptions are modified in the same proportion when the food is administered with substances such as meat or ascorbic acid which enhance the absorption, or with desferrioxamine which reduces it. The results presented here are in full agreement with earlier studies and provide further information on those animal foods not yet tested for their effect on a meal. It is worthwhile, therefore, to add some comments on the effects of these foods on the absorption of non-heme iron.

All our subjects drank milk at breakfast and those in the Andes also included it at supper. Milk did not improve the absorption of vegetal iron administered in these meals, as the absorption data observed do not differ from those observed when the same vegetable foods were served alone (2, 14). The same observation is also valid for milk products (cheese and butter). Milk by itself contains chelating inhibitor agents for iron absorption; thus, the absorption of ferric or ferrous iron salt is approximately five times less when it is administered with milk than when given alone (21, 22).

Eggs contain some inhibitors for iron absorption that affect the native iron (23, 24) and iron salts administered with them (3, 24). Eggs were served with meat and vegetable foods at supper in the Central Areas diet. It may be possible that the mean non-heme absorption from this meal, 2.5%, which was midway between the mean absorption from breakfast (1.2%) and lunch (3.5%), reflects the inhibiting effect of this food.

Meat has shown an enhancing effect on the absorption from vegetal iron in humans (13, 25) and in experimental animals (26). In spite of the small amount of meat (50 g) that the subjects had at lunch and supper in the Central Areas diet and at lunch from the Andes diet, the increase in iron absorption from non-heme iron was nearly twice that observed at breakfast when no meat was given. This difference would be more relevant if 100 g of meat had been served, as shown in previous reports (13).

Fish muscle was also demonstrated to have an enhancing effect on the absorption from vegetal iron (25). This effect was clearly demonstrated from the absorption obtained at breakfast and lunch in the Coast diet.

Early studies have shown the enhancing effect of ascorbic acid on the iron absorption from vegetable foods (27–30) and animal foods (24, 31). Recent studies (2, 32) also demonstrate its enhancing effect on both extrinsic and intrinsic tag vegetal iron. Sayers et al. (32) have shown that a dose of ascorbic acid above 50 mg enhances significantly the absorption from 2 mg of maize iron (32). Ascorbic acid content of cooked foods is minimal in the Central areas and the Andes diet. The plantain and tomato in these diets are so overcooked that the ascorbic acid content is considerably reduced. On the other hand, the vitamin is present in relatively high proportions in the fruits eaten at lunch and supper in the Coast diet; 29 mg of ascorbic acid are provided by watermelon, avocado, and plantain eaten at lunch, and 75 mg of this vitamin come mainly from 150 g of papaya eaten at supper. This amount of ascorbic acid was definitely the enhancing factor that produced such a high increase of absorption from non-heme iron at supper.

Besides the difference in absorption in foods, there are two other variables that play an important role in the variation of iron absorption. They can be grouped into two categories, namely, *a*) intrasubject variation, and *b*) intersubject variation.

The former is due to daily physiological variation presumably due to changes in the motility and secretion of the gut. The method of multiple dose (18) from each food or meal administered on several days reduces somewhat the effect of this variation. In this study, we have tried to minimize this variation by using the mean absorption of each meal instead of individual absorptions (33).

The intersubject variation in absorption is due to differences in the iron stores between individuals. There are several methods with various degrees of sensitivity to detect the degree of iron tissue store (34); however, in the studies on iron absorption, it is customary to test the individuals for serum iron concentration, percent transferrin saturation, and absorption of a reference dose of iron salt. Previous studies (13, 14, 33) have shown that although serum iron concentration and the percent of transferrin saturation are good indices to detect tissue iron stores, there was a poor correlation between these variables and the absorption from food iron. Nevertheless, the absorption of 3 mg iron as iron ascorbate correlates well in most instances with the iron absorption from either vegetable or animal foods.

The data presented here provide further evidence for the use of a reference dose test whenever it is necessary to compare the iron absorption of two or more groups of subjects consuming different foods. The calibration of non-heme and heme iron absorption from the four groups of subjects tested with three different diets have permitted us to see more clearly the difference in absorption between these diets and to determine the ingredients that have contributed to an increase in non-heme iron absorption. It is evident that the iron absorption from vegetable foods was approximately doubled by the effect of 50 g meat, nearly thrice by that of 100 g fish, and almost five times by the effect of 75 mg ascorbic acid.

The two studies performed with the Coast diet provide some indications on the reproducibility of these enhancing effects. The differences between the mean of these studies in each meal was approximately 20%.

Another observation that can be drawn from this study is the difference in the iron absorption between normal and iron-deficient subjects. Only the two groups of subjects consuming the Coast diet have an adequate number of iron-deficient subjects to make such a comparison. In normal individuals with a transferrin saturation above 24%, the total iron absorption was 0.80 mg/day. In those with a transferrin saturation between 16 and 24%, which includes subjects with normal and slight reduction of iron stores, the absorption was 0.98 mg, and in severely iron-deficient individuals with a 15% transferrin saturation, the absorption was 2.13 mg, that is, more than twice the absorption found in normal individuals.

All the data presented indicate that the amount of dietary iron does not reflect the net amount of iron absorbed and utilized by the individuals and that the components of a meal such as beef, fish muscle, and fruits are paramount in order to obtain a reasonable utilization of the vegetal iron. Beef muscle is of special importance because of its relatively high iron content, high absorbability, and enhancing effect on the absorption of vegetal iron.

Summary

The measurement of the daily non-heme iron absorption from diets consumed in several areas of Venezuela was determined by the extrinsic tag method. The diets were selected from three areas (Central areas, Andes, and the Coast) with different environments and food habits. Extrinsic ^{59}Fe or ^{55}Fe maize or maize and rice were administered with the other items in each meal. Breakfast and lunch tagged with ^{59}Fe and ^{55}Fe were administered on the first 2 days of each study, and lunch and a standard dose of iron ascorbate on the 15th and 16th days.

The results presented show the effect of various animal foods and fruits on the absorption from vegetal iron. Neither milk, cheese, nor butter improved the poor absorption from vegetable iron. Beef and fish muscle enhanced its absorption but the most striking enhancing effect was obtained from the papaya that was eaten at supper in the Coast diet. Further experiments demonstrated that the effect of papaya is due entirely to its ascorbic acid content.

The daily iron absorption from these diets shows that heme accounts for 62 and 42% of the total iron absorbed from the Central Areas and Andes diets, respectively. Fish iron absorption accounts for 12 and 9% of the total iron absorbed from the Coast diet, but this food was vital to the increased absorption from vegetal iron.

The absorption from the standard dose of iron ascorbate performed in each study showed a significant correlation with the absorption from each meal. It was possible, therefore, to calibrate the non-heme iron absorption between groups of subjects without the interference of intersubject variation in absorption. Absorption from vegetal iron was approximately doubled by the effect of 50 g meat, almost thrice by the effect of 100 g fish, and nearly five times by the effect of 150 g papaya containing 66 mg ascorbic acid. These effects were reproducible as far as fish and papaya are concerned, with a margin of approximately 20% difference between the mean of two groups consuming the same diet.

The data presented indicate that the amount of dietary iron does not reflect the net amount of iron absorbed by the individuals and that the ingredients of a meal such as beef, fish, and fruits are paramount to obtain a reasonable utilization of the non-heme iron.

The authors would like to thank Mr. Guillermo López-Calzón, Miss Irene Leets, and Mrs. Marta Renzi for their technical assistance.

References

1. LAYRISSE, M., AND C. MARTÍNEZ-TORRES. Model for measuring the dietary absorption from heme iron. Test with a complete meal. Am. J. Clin. Nutr. 25: 401, 1972.
2. COOK, J., M. LAYRISSE, C. MARTÍNEZ-TORRES, R. WALKER, E. MONSEN AND C. A. FINCH. Food iron absorption measured by an extrinsic tag. J. Clin. Invest. 51: 805, 1972.
3. BJÖRN-RASMUSSEN, E., L. HALLBERG AND R. B. WALKER. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic iron salt added to food: studies on maize, wheat and eggs. Am. J. Clin. Nutr. 25: 317, 1972.
4. CROSBY, W. H., J. L. MUNN AND R. W. FURTH. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forced Med. J. 5: 693, 1954.
5. International Committee for Standardization in Hematology: proposed recommendations for measurement of serum iron in human blood. J. Clin. Pathol. 56: 543, 1971.
6. RAMSAY, W. N. M. The determination of the total iron binding capacity of serum. Clin. Chim. Acta 2: 221, 1957. Modified by Iron Panel (Intern. Comm. Standard. Haematol.) Unpublished.

7. COOK, J. D. An evaluation of absorption method for measurement of plasma iron-binding capacity. J. Lab. Clin. Med. 76: 497, 1970.
8. IBARRA, C. Tabla de composición de alimentos para uso práctico. Instituto Nacional de Nutrición (Venezuela). Cuaderno No. 17, 1954.
9. INCAP-ICNND. Tabla de composición de alimentos para uso de América Latina. Instituto de Nutrición de Centro América y Panamá. Ciudad de Guatemala, Guatemala, C.A., 1961.
10. DERN, J. R., AND W. L. HART. Studies with double labelled iron. I. Simultaneous liquid scintillation counting isotopes of Fe^{55} and Fe^{59} as ferrous perchlorate. J. Lab. Clin. Med. 57: 322, 1961.
11. DERN, J. R., AND W. L. HART. Studies with double labelled iron. II. Separation of iron from blood samples and preparation of ferrous perchlorate for liquid scintillation counting. J. Lab. Clin. Med. 57: 460, 1961.
12. NADLER, S. B., J. U. HIDALGO AND T. BLOCH. The Tulane table of blood volume in normal men. Surgery 51: 224, 1962.
13. MARTINEZ-TORRES, C., AND M. LAYRISSE. Iron absorption from veal muscle. Am. J. Clin. Nutr. 24: 531, 1971.
14. LAYRISSE, M., J. D. COOK, C. MARTÍNEZ-TORRES, M. ROCHE, I. N. KUHN AND C. A. FINCH. Food iron absorption: a comparison of vegetable and animal foods. Blood 33: 430, 1969.
15. MARTINEZ-TORRES, C., AND M. LAYRISSE. Nutritional factors in iron deficiency. Food iron absorption. In: Clinical Hematology. Philadelphia: Saunders, vol. 2, p. 339.
16. Interdepartmental Committee on Nutrition for National Defense. Nutrition Survey, Venezuela. Washington, D.C.: U.S. Govt. Printing Office, 1963.
17. VELEZ, F. Estudios acerca de la nutrición en Venezuela de 1950-1971. Revista Venezolana de Asistencia Social. In press.
18. BRISE, H., AND L. HALLBERG. Iron absorption studies. II. Acta Med. Scand. 171 (Suppl. 376): 7, 1962.
19. MARTÍNEZ-TORRES, C., AND M. LAYRISSE. Iron absorption from ferritin. In preparation.
20. LAYRISSE, M., C. MARTÍNEZ-TORRES, J. D. COOK, R. WALKER AND C. A. FINCH. Iron fortification of food. Its measurement by the extrinsic tag method. Blood 41: 333, 1973.
21. LAYRISSE, M., C. MARTÍNEZ-TORRES, M. RUPHAEL-DIVO, W. JAFFÉ AND J. E. TORRES-SUÁREZ. Iron absorption from skim milk enriched with iron glycerophosphate. Arch. Latinoam. Nutr. 23: 145, 1973.
22. STEKEL, A. Communication presented at Pan American Health Organization Meeting on Food fortification with iron. Sao Paulo (Brazil), 1972.
23. MOORE, C. V. Iron nutrition–iron metabolism. Ciba Intern. Symp. Springer-Verlag, 1964, p. 244.
24. CALLENDER, S. T., S. R. MARNEY AND G. T. WARNER. Eggs and iron absorption. Brit. J. Haematol. 19: 657, 1970.
25. LAYRISSE, M., C. MARTÍNEZ AND M. ROCHE. The effect of interaction of various foods on iron absorption. Am. J. Clin. Nutr. 21: 1175, 1968.
26. AMINE, E. K., AND D. M. HEGSTED. Effect of diet on iron absorption in iron-deficient rats. J. Nutr. 101: 927, 1971.
27. STEINKAMP, R., R. DUBACH AND C. V. MOORE. Studies in iron transportation and metabolism. Arch. Internal Med. 95: 181, 1955.
28. CALLENDER, S. T., AND G. T. WARNER. Iron absorption from bread. Am. J. Clin. Nutr. 21: 1170, 1968.
29. ELWOOD, P. C., D. NEWTON, J. D. EAKINS AND D. A. BROWN. Absorption of iron from bread. Am. J. Clin. Nutr. 21: 1162, 1968.
30. KUHN, I. N., E. R. MONSEN, J. D. COOK AND C. A. FINCH. Iron absorption in man. Am. J. Lab. Clin. Med. 71: 715, 1968.
31. MOORE, C. V., AND R. DUBACH. Observation on the absorption of iron from food tagged with radioiron. Trans. Assoc. Am. Physicians 64: 245, 1951.
32. SAYERS, M. H., S. R. LYNCH, P. JACOBS, R. W. CHARLTON, T. H. BOTHWELL, R. B. WALKER AND F. MAYET. The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. Brit. J. Haematol. 24: 209, 1973.
33. MARTÍNEZ-TORRES, C., AND M. LAYRISSE. Effect of amino acids on iron absorption from a staple vegetable food. Blood 35: 669, 1970.
34. BAITON, D. F., AND C. A. FINCH. The diagnosis of iron deficiency anemia. Am. J. Med. 37: 62, 1964.

Compa
amino

C. L. Long, B.

It is generally
nitrogen in the
made on the b
requirements of
must consider t
certain metaboli
nonessential am
synthesis. Moreo
on gluconeogene
tion resulting f
insulin derived f
should also be co
Until recently
nutrition were
zymatic or min
amino acid soluti
the nutritional c
many years. Beca
ing from the infu
in the peripheral
glucose solutions
concentrations co
infusion. Thus, in
able nitrogen and
sary to accept the
as well. These large
for clinical reasons