Iron absorption by humans from fish

CARLOS MARTÍNEZ-TORRES, IRENE LEETS
AND MIGUEL LAYRISSE
Instituto Venezolano de Investigaciones Científicas,
Apartado 1827, Caracas, Venezuela

SUMMARY

Iron absorption from fish (snapper) was tested in 75 subjects. The geometrical mean absorption of 0.5 mg of fish iron in both normal and iron deficient subjects was 8% but the mean absorption increased up to 18% in subjets with marked iron deficiency. The mean absorption ratio of fish to a reference dose of iron ascorbate was 0.46, about half the ratio found in veal muscle and veal liver. Its absorption was increased by desferrioxamine and maize. Fish iron contribution to the diet is meager because of its low content and relatively low absorbability; its main benefit lies in its capacity to increase significantly the absorption of vegetable iron.

Fish is a foodstuff especially consumed by populations of low socio-economic class living on the coast. Its iron content is very low, it ranges from 0.4 to 1 mg per 100 g of fish (1, 2). Preliminary tudies (3, 4, 5, 6, 7) have shown that iron absorption from fish is not as high as that from meat (4) and liver (7) but it enhances the absorption of vegetal iron (8).

This paper provides information on iron absorption from fish muscle on large number of individuals, as well as the effect of interaction of this food with chelating agents and vegetals on iron absorption. The results showed that the absorption of fish iron is lower than meat and liver iron. Its absorption is markedly decreased when it is administered with vegetals or desferrioxamine but is increased when it is administered with ascorbic acid.

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Materials and methods

Seventy five adult peasants from agricultural areas of Venezuela volunteered for this study; 44 were males and 31 females. They were in apparent good health with exception of those with moderated or marked iron deficiency anemia. In addition to iron absorption determinations, each subject was tested for blood hemoglobin concentration (9), packed red cell volume, serum iron (10) and unsaturated iron binding capacity, using radioactive iron and magnesium carbonate (11).

Absorption studies

Fish muscle biosynthetically labeled with radioactive iron was administered in the morning, after an overnight fast, no additional food or drink was allowed for three hours following the test dose. The next day a dose of ferrous ascorbate or other food, labeled with different radioactive iron was administered. Blood was drawn 15 days after the administration of food to determine hematological characteristics and radioactivity. Some subjects were fed again on day 15 and 16 with another radioactive material and blood was taken on the 30th day.

Duplicate 10-ml blood samples were prepared for radioactive counting by wet ashing and electroplating, following the technique of Dern and Hart (12, 13). Radioactivity was measured in a liquid scintillation spectometer (Packard model 3310). Triplicate standars of the material administered were counted simultaneously with the blood samples. The iron absorption from the foods was calculated from ^{59}Fe and ^{55}Fe activity in the subject's blood using an estimated blood volume based on sex, weight and height (14). No correction was made to determine total iron utilization.

Preparation of labeled foods

Snappers (*Lutjanus* sp.) weighing about 200-400 g were placed in tanks, about two weeks later, the fishes were anesthetized with Tricain methasulfonate and 50 to 100 μCi of ^{55}Fe in the form of ferrous citrate was injected intramuscularly, close to the cuadal fin. After three months the fish were killed and filleted. The part of the fish which was less than 4 cm from the site of the injection was discarded, the rest of the fish (about 60% of the total body weight) was washed with water, in the same fashion used for culinary purposes, and

then boiled and ground. The muscle was mixed and divided into portions of about 50 g each and fried before it was given to the subjects. Except for the area near the site of ^{55}Fe injection, the muscles had a fairly uniform radioactivity with a variation of less than 20%. This was also demonstrated by radioautography. Only about 1-2% of the total radioactivity injected was recovered in the muscles. The iron content of the fish muscle ranged from 0.4 to 0.6 mg of iron per 100 g.

The distribution of radioactive iron into the iron compounds of fish muscle was determined by grinding about 8 g of the muscle suspended in 20 ml of 0.05 M phosphate buffer, pH 7.4 with 1% triton added to it in a glass tissue homogenizer. The supernatant was separated by centrifugation (3.000 x g). The insoluble portion was washed twice with the buffer.

A 100 x 5 cm colum of Sephadex G-100 stabilized with the same phosphate buffer. The soluble fish meat preparation was applyed on Sephadex bed. Fractions of 6 ml each were collected and read at a wawe length of 400 mu to measure optical density.

Standard dose of radioactive iron ascorbate. Trace doses of ^{59}Fe in the form of ferric chloride with a specific activity of 10-20 μCi/ug Fe were mixed with carrier ferrous sulfate. Two moles of ascorbic acid/mole of iron were added prior the oral administration.

Statistical analysis. As in previous studies (3, 4, 5, 6) the mean absorption and standard deviation were calculated from the logarithm of the percentage of absorption and the results were retransformed as antilogarithm to recover the original units. The comparison of iron absorption from two meals was performed in pairs by the student's test (15).

RESULTS

Characteristics of the biosynthetical radioactive fish

Four assays were performed to determine the distribution of the radioactive and nonradioactive iron in to the iron component of fish muscle using a Sephadex column. About 12% of the total radioactivity was in the insoluble fraction. The column separated from the supernatant one fraction with about 86-88% of activity containing high molecular weight substances corresponding to ferritin, less than 2% was found in

the fraction corresponding to hemoglobin and no activity was found in the fraction corresponding to myoglobin.

The chemical analysis showed that about 14% of the total fish muscle iron was in the insoluble portion and about 86% in the fraction corresponding to ferritin.

TABLE 1
IRON ABSORPTION FROM FISH

Identification	Age	Sex	Hb g/ 100 ml	Packed red cell (%)	Serum iron ug/ 100 ml	Transferrin saturation (%)	IRON ABSORPTION (%)		
							A Fish (0.5 mgFe)	B. Iron ascorbate (3 mg Fe)	A/B ratio
1) DA	35	F	12.9	40	127	39	1.0	5.7	0.17
2) MI	42	M	14.7	42	185	54	1.4	18.0	0.08
3) MC	60	M	15.3	43	83	24	1.5		
4) JF	21	M	15.7	43	137	36	1.5		
5) AV	47	M	13.3	42	58	17	1.9	2.8	0.68
6) MT	36	F	13.9	39	131	51	2.1	2.2	0.95
7) EM	41	F	15.2	42	47	14	2.3		
8) AT	45	M	15.9	45	69	21	2.4	21.7	0.11
9) EI	49	M	15.2	40	121	42	2.5	5.2	0.48
10) JRG	31	M	16.4	47	170	56	2.6	14.1	0.18
11) DU	57	M	15.2	41	130	32	2.7		
12) CP	44	F	13.0	38	86	26	3.1		
13) RR	41	M	16.0	47	198	45	3.4	5.3	0.64
14) ST	42	M	14.7	45	188	75	3.6	1.4	2.57
15) JER	39	M	16.0	46	216	58	3.7	5.5	0.67
16) BG	35	M	14.9	47	204	53	4.3	20.5	0.21
17) CM	19	F	13.1	41	92	31	4.3	27.8	0.15
18) PS	40	M	16.0	48	85	26	4.6	13.0	0.35
19) OC	30	M	16.2	44	135	31	4.8		
20) RV	36	F	14.8	41	143	34	4.8		
21) EC	60	F	14.2	43	66	14	4.9	20.2	0.24
22) AP	23	F	14.7	43	129	28	4.9	43.2	0.11
23) RP	32	M	17.2	52	122	28	4.9	28.7	0.17
24) AP	17	M	16.4	46	112	30	5.2	3.9	1.33
25) JEH	31	M	16.2	48	103	30	5.2	8.8	0.59
26) FDA	29	M	16.8	48	122	50	5.3	4.1	1.29
27) SJE	38	F	14.6	41	191	51	5.3	35.1	0.15
28) RD	24	F	13.8	39	159	44	5.5	32.5	0.17
29) PCG	55	M	13.3	40	70	22	6.3	60.6	0.10
30) MP	27	M	14.9	44	164	45	6.4	17.8	0.36
31) BA	35	M	15.4	40	82	21	6.5		
32) LM	29	M	17.5	47	145	39	6.7		
33) MP	23	F	13.3	38	73	19	6.9	17.9	0.39
34) MCH	16	F	14.9	44	77	20	7.0	59.3	0.12
35) AA	34	M	14.3	43	81	24	7.2	23.4	0.31
36) PA	53	M	15.8	48	149	36	7.3	21.1	0.35
37) GG	46	M	13.2	40	114	28	7.4	10.9	0.68
38) LC	60	M	10.9	33	69	20	7.4	27.7	0.27
39) RA	21	F	13.6	36	50	14	7.5		
40) JMR	19	M	13.8	43	154	58	7.7	19.0	0.41
41) JM	44	M	17.4	51	106	25	7.7	21.8	0.35
42) LFR	42	M	14.1	43	88	37	8.9	15.8	0.56
43) JGB	30	M	15.2	42	193	49	10.5	8.3	1.27
44) LF	47	M	15.6	46	86	20	10.5	73.8	0.14
45) EA	18	M	11.8	40	25	6	11.5	50.5	0.23

TABLE 1 (continued)

Identification	Age	Sex	Hb g/100 ml	Packed red cell (%)	Serum iron ug/100 ml	Transferrin saturation (%)	IRON ABSORPTION (%)		
							A Fish (0.5 mgFe)	B Iron ascorbate (3 mgFe)	A/B ratio
46) PG	33	F	15.1	40	71	19	11.7	42.4	0.28
47) AG	29	F	13.6	41	107	33	12.6	28.7	0.44
48) JD	43	M	16.2	47	207	58	12.7	12.5	1.02
49) CD	29	F	11.8	36	35	9	13.4	18.9	0.71
50) ADD	30	F	12.1	37	29	6	13.4	47.8	0.28
51) JSH	27	M	17.8	48	105	31	13.6	3.4	4.00
52) JAZ	42	F	12.9	40	94	32	14.8	74.6	0.20
53) RC	34	F	12.0	35	63	20	15.4	7.1	2.17
54) CP	38	F	12.2	37	116	33	15.5	4.2	3.69
55) NP	13	M	13.9	43	46	11	16.0	27.0	0.59
56) FC	40	F	11.7	34	70	20	16.7	32.6	0.57
57) RA	54	F	12.6	37	135	40	16.9	23.5	0.72
58) CA	20	F	14.2	40	102	32	17.3	52.0	0.33
59) MV	37	F	11.6	35	69	20	17.8	15.2	1.17
60) AM	28	F	11.8	37	73	18	19.3	43.1	0.45
61) PA	15	M	13.2	40	50	12	20.1	51.7	0.39
62) AF	67	F	3.4	16	32	7	20.6	65.9	0.31
63) MA	37	F	14.6	41	111	27	20.7		
64) CC	17	F	15.0	43	97	24	22.1		
65) AG	24	F	13.0	39	33	7	24.6	58.8	0.42
66) RJG	29	F	13.0	41	52	14	25.1	63.2	0.40
67) GM	16	F	14.1	37	127	34	26.8	31.1	1.16
68) MR	42	M	14.2	40	141	38	26.9	8.3	3.24
69) NP	14	F	11.3	36	53	14	27.2	21.6	1.26
70) MB	60	M	12.5	37	130	36	27.7	35.5	0.78
71) LF	25	M	15.8	49	144	31	32.2	82.0	0.39
72) BA	34	M	11.8	39	72	29	37.2	41.6	0.89
73) AM	23	M	14.6	45	80	24	37.6	42.0	0.90
74) LEA	20	M	12.9	42	86	24	41.7	94.0	0.44
75) CLT	18	F	11.2	39	44	10	42.4	86.0	0.49
Mean			14.3	42	105	30	8.23	19.80	0.46
Limits of 1 S.E.							(7.41-9.14)	(17.54-22.57)	(0.41-0.51)

Characteristics of iron absorption from fish

Table 1 shows the hematological characteristics and the results of iron absorption from fish and iron ascorbate. In this study 56 new cases and 19 cases published elsewhere were included (3, 8). The criteria recommended by WHO (Hemoglobin level below 13 g/100 ml for men and 12 g/100 ml for women) was used to define anemia (16). Nine subjects had anemia and 5 of these had serum iron concentration below 50 μg/100 ml, 12 had a transferrin saturation below 20% and 8 of these had saturation equal to or below 15%.

The geometrical mean absorption of fish iron was 8%. absorption from both fish and iron ascorbate was determined in 63 subjects, the mean absorption was 9.1% and 19.9% respectively and the mean ratio of fish to iron ascorbate absorp-

tion was 0.46. This ratio is about half of the absorption ratio found in veal muscle (4) and liver (7).

There was a highly significant correlation ($r = 0.59$) between the absorption of fish and iron ascorbate but poor correlation was obtained when the absorption of fish iron was compared with the percentage of transferrin saturation ($r = -0.34$) and when compared with the serum iron concentration ($r = -0.32$).

According to these results the per cent absorption of a reference dose of iron ascorbate was used to differenciate between absorption from fish muscle in normal and iron deficient subjects. Thus in subjects with iron ascorbate absorption below 20% which includes most subjects with normal iron stores, the mean fish iron absorption was 6%, in those with iron ascorbate absorption between 20% and 39% representing subjects with

TABLE 2
EFFECT OF ASCORBIC ACID ON THE IRON ABSORPTION FROM FISH MUSCLE

Case number	PER CENT OF IRON ABSORPTION		
	A Fish (0.5 mg Fe) given alone ^{55}Fe	B Fish (0.5 mg Fe) + ascorbic acid (500 mg) ^{55}Fe	B/A ratio
2	1.4	4.9	3.50
10	2.6	5.3	2.04
13	3.4	5.0	1.47
14	3.6	5.9	1.64
15	3.7	6.2	1.68
16	4.3	17.9	4.16
18	4.6	16.4	3.57
25	5.2	5.0	0.96
26	5.3	10.2	1.92
48	12.7	8.4	0.66
58	17.3	28.4	1.64
Mean	4.6	8.5	1.85
Limits of 1 S.E.	(3.7-5.7)	(7.1-10.2)	(1.56-2.18)
Probability			<0.001

normal and moderate iron deficiency, the mean was 9% and finally those with iron ascorbate absorption above 39% the mean absorption was 18%.

Effect of chelating agents Tables 2 and 3 show the effect of ascorbic acid and desferrioxamine on the iron absorption from fish muscle. Eleven subjects were given first 0.5 mg of fish iron (100 g of food) alone and 15 days later blood was taken for radioactivity determination and the subjects were fed again with with the same amount of fish labeled with the same or different radioiron and the chelating agent. Five hundred mg of ascorbic acid induced a significant enhancement in the iron absorption from 4.6% when the food was given alone to 8.5% when it was administered with the vitamin. On the contrary, five hundred mg of desferrioxamine reduced significantly the fish iron absorption, from 5.0% to 1.3% respectively.

TABLE 3
EFFECT OF DESFERRIOXAMINE ON THE IRON ABSORPTION FROM FISH MUSCLE

Case number	PER CENT OF IRON ABSORPTION		
	A Fish (.5 mg Fe) given alone ^{55}Fe	B Fish (.5 mg Fe) + Desferrioxamine (500 mg) ^{59}Fe	B/A ratio
10	2.6	0.3	0.12
13	3.4	1.9	0.56
14	3.6	1.8	0.50
15	3.7	0.9	0.24
16	4.3	1.3	0.30
25	5.2	2.0	0.38
26	5.3	0.7	0.13
27	5.3	1.8	0.34
30	6.4	2.1	0.33
34	7.0	1.5	0.21
50	13.4	1.8	0.13
Mean	5.0	1.3	0.26
Limits of 1 S.E.	(4.3-5.7)	(1.1-1.6)	(0.22-0.31)
Probability			< .001

Effect of vegetable foods

The effect of vegetable foods on the iron assorption from fish was tested by feeding 12 subjects with fish given alone and 15 days later, after taking blood for radioactivity determination, they were given the same amount of fish and 2 mg of maize iron (100 g of food). The mean absorption was reduced from 4.8% when the food was administered alone to 2.2% when it was administered with maize (Table 4).

TABLE 4
EFFECT OF MAIZE ON THE IRON ABSORPTION FROM FISH MUSCLE

Case number	PER CENT OF IRON ABSORPTION		
	A Fish (0.5 mg Fe) given alone ^{55}Fe	B Fish (0.5 mg Fe) + maize (2 mg Fe) ^{55}Fe	B/A ratio
3	1.5	1.8	1.20
4	1.5	1.8	1.20
7	2.3	2.9	1.26
11	2.7	1.0	0.37
12	3.1	1.1	0.35
19	4.8	4.1	0.85
20	4.8	3.5	0.73
31	6.5	1.0	0.15
32	6.7	3.7	0.55
39	7.5	3.5	0.47
63	20.7	2.3	0.11
64	22.1	2.7	0.12
Mean	4.8	2.2	0.46
Limits of 1 S.E.	(3.7-6.2)	(1.9-2.5)	(0.35-0.59)
Probability			< 0.001

TABLE 5
EFFECT OF INTERACTION OF FISH AND FERRIC CHLORIDE ON IRON ABSORPTION

Case number	A Fish ^{55}Fe	B Ferric ^{59}	B/A ratio
	0.5 mg of fish iron + 0.1 mg Fe as ferric chloride		
6	2.1	1.5	0.71
8	2.4	4.4	1.83
9	2.5	3.9	1.56
28	5.5	12.4	2.25
33	6.9	15.2	2.20
43	10.5	17.3	1.65
46	11.7	24.8	2.12
51	13.6	37.0	2.72
56	16.7	35.2	2.11
67	26.8	62.1	2.32
Mean	7.17 (5.41-9.50)	13.26 (9.16-19.21)	1.85 (1.64-2.08)

Effect of interaction of fish and ferric chloride on iron absorption

A small amount of ^{59}Fe ferric chloride (0.1 mg Fe) was mixed with ground fish labeled with ^{55}Fe in order to test if its absorption is indicative of that of intrinsic fish iron in the same fashion as this iron salt reflects the absorption of vegetable foods iron (17, 18, 19). Table 5 shows that the iron absorption from the salt is distinctly higher in each subject tested: the mean absorption ratio of ferric chloride to fish iron was 1.85.

DISCUSSION

Chemical and radioactive determinations from the fish selected for this study showed that myoglobin was absent and hemoglobin accounted only for a small proportion of the total iron content. The absorption of fish iron confirms that its iron content is mainly from ferritin and hemosiderin. Its absorption is relatively low (8%) and the mean absorption ratio of fish iron ti iron ascorbate is 0.46 which is about half the ratio found in veal muscle (4) and veal liver (7), in which heam iron content was in high proportion. Its absorp-

tion is increased by ascorbic acid and decreased by desferrioxamine and maize in the same fashion as observed in the absorption of feritin (20).

The nutritive value of fish in terms of iron absorption and utilization in a diet should be analyzed according to the interaction of this food with other foods ingested in the same meal. Fish iron content is very low but its absorption is increased by foods containing a large amount of ascorbic acid and decreased by the presence of vegetable foods. All these facts lead to the conclusion that the contribuiton of fish iron to the diet is meager. On the other hand fish increases the absorption of vegetal iron, a dose of 0.5 mg of fish iron increased about twice the absorption of 2-3 mg of vegetal iron, represented by either black beans (8) maize or other vegetals (21). It is possible that products derived from protein degradation during digestion from fish (8) as well as from meat (4) and liver (7) are responsible for such an effect. Absorption studies mixing vegetables with amino acids present in fish demonstrated that cysteine is the only amino acid which enhances iron absorption from plant sources (8).

It is probable that fish contribute a great deal to prevent iron deficiency anemia in low socio-economic populations living on the coast of Venezuela, in which dietary iron intake is relatively low and fish is practically the only source of animal food (21, 22).

RESUMEN

Absorción del hierro del pescado por humanos

La absorción del hierro presente en pescados fue medida en 75 voluntarios. El promedio geométrico de la absorción de 0.5 mg de hierro, tanto en personas normales como en aquellas que presentaban una moderada deficiencia, fue de 8%, pero el promedio de absorción aumentó a 18% en los sujetos con marcada deficiencia de hierro. La relación promedio de absorción de hierro del pescado a una dosis de referencia de ascorbato de hierro fue de 0.46, aproximadamente la mitad de la encontrada en músculo y en hígado de ternera. Su absorción fue incrementada por ácido ascórbico y disminuída por desferroxiamina o maíz. La contribución del hierro presente en el pescado es escasa, debido al bajo contenido en dicho elemento y al hecho de ser relativamente poco absorbible; la ventaja del pescado como alimento radica en su capacidad para aumentar significativamente la absorción del hierro de los vegetales.

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