

Iron metabolism in Egyptian infants with protein-calorie deficiency

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1. Studies were made of iron absorption in eighty-five infants who were suffering from protein-calorie deficiency (kwashiorkor and marasmus) and in eight infants who were suffering from Fe-deficiency anaemia: twenty-one healthy infants were used as control subjects. Factors affecting absorption – concentration of serum total protein and its fractions; serum Fe; transferrin (as indicated by total Fe-binding capacity) and its percentage saturation; blood haemoglobin; total blood Fe; and the state of Fe stores in the liver – were also investigated. Four of the infants who were suffering from severe kwashiorkor were selected for a follow-up study and were given a high-protein diet and blood transfusions.

2. Alimentary Fe absorption was retarded in infants with protein-calorie deficiency. Total serum proteins and its fractions were reduced. Blood haemoglobin, total blood Fe, serum Fe and total Fe-binding capacity were also decreased but the percentage of transferrin saturation was increased. Liver proteins per g wet weight of liver were decreased, whereas liver non-haem Fe was increased. The extent of these changes varied according to the clinical condition.

3. In infants with Fe-deficiency anaemia there was no change in liver protein concentrations or total serum proteins, except for a slight increase in the β -globulin fraction of the latter. Blood haemoglobin and total Fe were reduced, whereas serum total Fe-binding capacity was raised and the percentage of transferrin saturation was lowered. A fall in the liver non-haem Fe concentration was observed.

4. After giving a high-protein diet to infants with severe kwashiorkor, the characteristic anaemia associated with protein-calorie deficiency changed and the general manifestations of Fe-deficiency anaemia emerged. After blood transfusion, these infants showed a marked clinical improvement.

Anaemia is a health problem in most of the developing countries of the tropics and subtropics (Foy & Kondi, 1957). It has been attributed to many aetiological factors, among which are a deficiency of either dietary iron or protein (Woodruff, 1955; Foy & Kondi, 1957; Sandozai, Haquani, Rajeshvari & Kaur, 1963). Protein-calorie deficiency is prevalent among infants and young children in Egypt (Morcos, 1966); in its most advanced stage it is usually associated with anaemia (El Nabawy, Shukry, Mohyeldin & El Hawary, 1961).

Although several studies have been concerned with anaemia in protein-calorie malnourished infants (El Gholmy, Aboul-Dahab, El Essawi, Abdel Rahman & Malek, 1962; Sandstead, Gabr, Azzam, Shukry, Weiler, El-din, Mokhtar, Prasad, El Hifney & Darby, 1965), there appears to be a need for information on Fe metabolism in the two most important types of protein-calorie deficiency, namely kwashiorkor and marasmus, and a need to illustrate how far patients can benefit from Fe therapy.

The work reported here entailed the study of Fe absorption, transport and storage in these two types of protein-calorie deficiency as well as in Fe-deficiency anaemia.

METHODS

Subjects

The subjects were ninety-three infants who were suffering from moderate kwashiorkor, severe kwashiorkor, first-grade marasmus or Fe-deficiency anaemia, and who were attending Mounira Children's Hospital, Cairo University. Special care was taken to exclude any child that showed signs of any other ailment that might affect the results, especially infections, parasites, dehydration, nephropathy or liver cirrhosis. Twenty-one healthy infants were used as control subjects. For the number of subjects in each group, their age, sex and weight, see Tables 1 and 3.

Fasting blood haemoglobin, total blood Fe, and serum proteins, Fe and Fe-binding capacity

To provide information on anaemia in protein-calorie deficiency and to evaluate Fe therapy in this disorder, it was necessary to study fasting serum Fe values as well as the pattern of Fe-tolerance curves in both controls and patients. The night before doing the Fe-absorption test, the subjects were fasted. Four of the subjects with severe kwashiorkor were given protein and blood transfusions and their progress was studied during treatment up to clinical recovery.

In the morning, 5 ml blood were withdrawn from each of the fasted subjects and this amount was divided into two tubes: one containing potassium oxalate was kept for the determination of haemoglobin and total blood Fe; the other was used for the separation of serum to determine its proteins (total and fractions), Fe and Fe-binding capacity. Serum was kept at -20° till analysed.

A dose of 4 mg Fe/kg body-weight, in the form of ferrous sulphate, was then given by stomach-tube and samples of blood were taken after 2, 4 and 6 h for determination of serum Fe. Neither food nor fluid was allowed during the experimental period.

Liver protein and non-haem Fe content

Liver biopsies were done on thirty subjects who represented different ages, sexes and grades of protein-calorie deficiency: ten were clinically diagnosed as having severe kwashiorkor, ten as moderate kwashiorkor and ten as first-grade marasmus; nine were healthy controls. Biopsy samples, weighing 25–50 mg, were kept in a cellophane bag in solid CO₂ in a small Dewar's flask and analysed for protein and non-haem Fe content.

Follow-up study

Four of the infants who were suffering from severe kwashiorkor were given a high-protein diet for 10 d; they were also given blood transfusions.

Analytical techniques

Serum proteins were separated by electrophoresis and estimated by the method of King & Wootton (1956*a, b*). Haemoglobin was determined by the method of Wong (1928). Total blood Fe was determined by a modification (El-Shobaki, 1970) of the method of Sobel (1964). Serum Fe concentration and total Fe-binding capacity were determined by the technique of Ramsay (1957*a, b*). Tissue proteins were estimated by

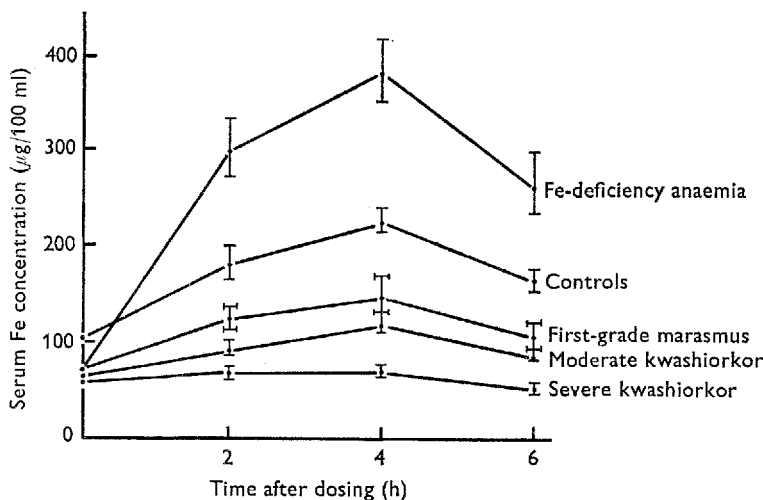


Fig. 1. Iron-tolerance curves in groups of Egyptian infants suffering from protein-calorie deficiency diseases. (Mean values with their standard errors represented by the vertical bars.)

Folin's method as described by Lowry, Rosebrough, Farr & Randall (1951) and the liver non-haem Fe was estimated by a modification (El-Shobaki, 1970) of the method of Hallgren (1953).

RESULTS

Fe absorption

The dose of 4 mg Fe/kg body-weight gave an acceptable metabolic tolerance curve for Fe absorption from the gut (Fig. 1). The period of 6 h was quite adequate to illustrate the maximum rise in serum Fe concentrations and the changes during that period were within the range to be accurately estimated by the method of Ramsay (1957*a*). Maximum rise in serum Fe concentration for both controls and patients was attained after 4 h from the administration of the test dose. The mean values for fasting serum Fe are shown in Fig. 1.

Fe-deficiency anaemia. The fasting serum Fe concentration was far below that for controls. Administration of the Fe test dose was followed by a marked increase in serum Fe concentration, to 5.94 times its initial value after 4 h. This was followed by a gradual decline, but at 6 h the concentration was still higher than the initial value.

First-grade marasmus. The pattern obtained was variable; some of the curves were below the level of that for controls and some were above. However, in all marasmic subjects, there was a considerable rise in serum Fe concentration at 4 h after the dose of Fe and the average ratio of serum Fe at this time to the original concentration was 2.3, i.e. nearly equal to that in the controls. Nevertheless, the average concentration at 4 h in this type of protein-calorie deficiency was only 0.66 of the corresponding value for controls.

Moderate kwashiorkor. The shape of the tolerance curve was more or less similar to that of the controls, though the Fe-concentration values at 2, 4 and 6 h were lower than the corresponding control values.

Severe kwashiorkor. The values for serum Fe concentration were significantly lower than those obtained for either normal or moderate cases and the curve appeared more or less a straight line.

Four hours after the Fe dose was administered, the ratios between the values for serum Fe in moderate and severe kwashiorkor and the value for the controls were 0.54 and 0.32 respectively. The ratios between serum Fe concentration at 4 h after the test dose and the initial fasting value were 1.9 and 1.38 for moderate and severe kwashiorkor respectively. The corresponding value for controls was 2.5.

Other factors

Moderate kwashiorkor. There was a moderate decrease in the concentration of total serum proteins and its fractions, especially albumin (Table 1). Total Fe-binding capacity was also reduced (Table 2) and, when considered with the concomitant decrease in β -globulin, was due to a decrease in transferrin concentration. This is substantiated by the finding of more or less normal percentage of saturation in spite of the low serum Fe concentration. Again, blood haemoglobin was markedly decreased, but the liver non-haem Fe concentration in this group was found to be about 1.5 times that of the controls (Tables 2, 3).

Severe kwashiorkor. Compared with moderate kwashiorkor, all these derangements were accentuated, resulting in a marked rise in the percentage saturation of transferrin and an obvious increase in the liver non-haem Fe concentration, reaching about twice that of the control concentration (Tables 2, 3).

First-grade marasmus. Total serum proteins were either within the normal range or slightly higher. A moderate decrease in albumin and a slight rise in α -1, α -2 and β -globulins was found in all patients. γ -Globulin values were normal or slightly higher in some patients (Table 1). Blood haemoglobin and total blood Fe values were either low or normal (Table 2). The protein concentration of liver biopsy samples relative to wet weight was lower than that of the controls but was higher than that in subjects with kwashiorkor. However, the liver non-haem Fe concentration was higher than in controls but still lower than that observed in the kwashiorkor groups (Table 3).

Fe-deficiency anaemia. Serum protein concentration was within the normal range but there was a slight increase in transferrin and a very low percentage saturation (Tables 1 and 2).

Follow-up study

The follow-up study of the four patients with kwashiorkor showed that a high-protein diet for 10 d resulted in a raised concentration of total serum proteins, though the value did not reach the normal value. The Fe-binding capacity was increased, suggesting an increase in serum transferrin. Liver non-haem Fe concentration decreased, whereas serum Fe concentration was slightly increased. Nevertheless, percentage of transferrin saturation started to decrease (Table 4).

Table 1. Total serum protein and its electrophoretically separated fractions in the groups of Egyptian infants

Group	No. of subjects		Age* (months)	Body-wt* (kg)	Total proteins (g/100 ml)	Albumin (g/100 ml)	Globulins (g/100 ml)			
	♂	♀					α-1	α-2	β	γ
Controls	4	8	10-36	9.2-12.0	6.79 ± 0.02	3.58 ± 0.09	0.43 ± 0.02	0.70 ± 0.02	0.81 ± 0.03	1.11 ± 0.02
Moderate kwashiorkor	15	8	4-36	3.5-11.4	5.39 ± 0.08	2.27 ± 0.03	0.45 ± 0.02	0.79 ± 0.02	0.68 ± 0.06	1.20 ± 0.04
Severe kwashiorkor	7	9	5-30	3.2-12.0	3.79 ± 0.12	1.39 ± 0.09	0.36 ± 0.03	0.71 ± 0.02	0.50 ± 0.03	0.83 ± 0.04
First-grade marasmus	9	7	4-36	3.0-8.5	6.96 ± 0.13	3.01 ± 0.11	0.66 ± 0.03	0.80 ± 0.03	1.27 ± 0.07	1.21 ± 0.09
Fe-deficiency anaemia	4	4	10-36	6.0-12.0	7.34 ± 0.27	3.72 ± 0.07	0.55 ± 0.07	0.86 ± 0.003	0.94 ± 0.02	1.27 ± 0.04

* Range.

(Mean values with their standard errors)

Table 2. Blood haemoglobin, total blood iron, total and unsaturated serum Fe-binding capacity of the groups of Egyptian infants

Group*	Haemoglobin (g/100 ml)	Total blood Fe (g/100 ml)	Serum Fe-binding capacity (μg/100 ml)	
			Total	Unsaturated
Controls	10.39 ± 0.22	35.1 ± 3.89	320 ± 4.62	213 ± 5.06
Moderate kwashiorkor	7.80 ± 0.02	26.6 ± 0.72	231 ± 15.30	162 ± 11.21
Severe kwashiorkor	6.42 ± 0.34	21.8 ± 1.11	133 ± 9.68	74 ± 9.68
First-grade marasmus	8.00 ± 0.38	27.0 ± 0.93	326 ± 11.59	254 ± 13.46
Fe-deficiency anaemia	7.97 ± 0.25	27.1 ± 0.07	416 ± 4.12	348 ± 15.78

* The subjects were the same as in Table 1.

(Mean values with their standard errors)

Table 3. Blood haemoglobin, total blood iron, serum total protein, total Fe-binding capacity and percentage saturation, serum Fe at 0 and 4 h after the administered Fe dose, and liver protein and non-haem Fe content of the groups of Egyptian infants

(Mean values with their standard errors)

Group	No. of subjects	Age* (months)	Body-wt* (kg)	Haemoglobin (g/100 ml)	Total blood Fe (mg/100 ml)	Total serum proteins (g/100 ml)	Total serum Fe-binding capacity (µg/100 ml)	Serum		Serum Fe (µg/100 ml)	Liver protein (g/100 g)	Liver non-haem Fe (mg/100 g)
								saturation	o h			
Controls	6	13-36	10-16	11.15 ± 0.27	37.87 ± 0.88	7.05 ± 0.08	325 ± 5.07	31.9 ± 0.88	103 ± 2.18	223 ± 10.2	21.17 ± 0.03	17.0 ± 0.71
Moderate kwashiorkor	5	10-36	6-12	7.31 ± 0.44	24.84 ± 1.61	5.31 ± 0.07	227 ± 11.0	29.6 ± 1.85	65 ± 2.68	91 ± 7.22	13.70 ± 0.77	21.3 ± 1.05
Severe kwashiorkor	5	11-30	8-12	7.20 ± 0.34	24.53 ± 1.22	3.56 ± 0.12	152 ± 8.44	42.2 ± 2.98	63 ± 3.54	70 ± 4.06	7.54 ± 0.77	29.1 ± 2.15
First-grade marasmus	5	6-24	4-8	9.14 ± 0.34	31.08 ± 1.18	6.12 ± 0.16	300 ± 11.4	22.1 ± 1.73	66 ± 3.82	151 ± 6.12	15.10 ± 0.94	19.6 ± 0.71

* Range.

Table 4. Effect of treatment on blood and serum constituents and liver non-haem iron in four Egyptian infants with severe kwashiorkor

Subject no.	Time of observation (before, during or after treatment)	Total protein (g/100 ml)	Haemoglobin (g/100 ml)	Total serum Fe-binding capacity (µg/100 ml)	Serum Fe (µg/100 ml)		Liver non-haem Fe (mg/100 g)
					o h	4 h	
1	Before	2.90	5.00	55	40	80	29.6
	During	5.20	7.30	216	70	150	12.5
	After	6.90	11.50	310	110	280	15.7
2	Before	3.10	6.90	127	60	90	25.2
	During	4.90	8.80	200	100	200	11.0
	After	6.60	10.90	290	120	285	18.2
3	Before	4.30	6.60	90	60	90	23.2
	During	5.10	8.30	243	90	190	19.1
	After	7.20	12.00	315	115	265	18.2
4	Before	3.40	6.40	138	30	80	26.7
	During	5.40	7.30	225	60	100	15.0
	After	6.80	9.90	310	95	160	17.9

DISCUSSION

In the control subjects the mean values for fasting serum Fe were similar to those previously reported in Egypt by El Gholmy *et al.* (1962); higher values (161 $\mu\text{g}/100$ ml) were reported by El Nabawy *et al.* (1961). These local variations, which were not unexpected, may be attributed to differences in age and socio-economic standards of the different groups that were studied.

The lower ratios between serum Fe concentration 4 h after the test dose of Fe and the initial fasting Fe concentration in moderate and severe kwashiorkor (1.9 and 1.38 respectively) compared with the control value (2.5) may indicate malabsorption of Fe in kwashiorkor, especially in severe cases.

In first-grade marasmus, the low average ratio (0.66) of serum Fe concentration, at 4 h after the test dose was administered, to the corresponding value for the controls was perhaps due to the low initial serum Fe concentration in marasmic patients.

In Fe-deficiency anaemia, the low fasting serum Fe concentration and the marked increase in its value after the test dose seem to be characteristic of this type of anaemia and indicate a state of Fe deficiency and a great need of the body for Fe.

The factors that can influence and control the concentration of serum Fe and rate of Fe absorption from the gut are: (1) concentration of serum proteins, especially that of transferrin, (2) haemoglobin content of blood, (3) amount of Fe in stores, (4) valency of the Fe administered and (5) presence of diarrhoeal disorders and infections.

Regarding the last two factors, the Fe that we used was divalent and none of the cases that was studied at the time of our investigation was suffering from any infection or diarrhoeal disorder. Furthermore, the test was performed after an overnight fast to obviate any nutrient interference.

The results of the estimation of total serum proteins and its fractions in controls are in agreement with those previously reported for Egyptian infants (El Gholmy, El Nabawy, Shukry, Ismail & El Hawary, 1960).

The values for Fe-binding capacity, both total and unsaturated, as well as total blood Fe, agreed with those reported by El Nabawy *et al.* (1961). These results, when associated with normal Fe absorption after the Fe test dose, suggest that Fe metabolism was normal in the controls.

The values obtained for liver non-haem Fe concentration in normal controls are the first to be reported for Egyptian infants. They are lower than corresponding values for Europeans (Sherlock, 1968). This difference may be attributed to various factors, among which is the food pattern.

The finding in marasmic patients that the liver non-haem Fe concentration was higher than in the controls and even lower than that found in the kwashiorkor group indicates almost normal mobilization of the liver non-haem Fe to meet the essential biological requirements and to a degree that exceeds the needs of the body in the marasmic state. This may be due to a deficiency in the molecules necessary to synthesize the protein moieties of haemoglobin. As excretion of Fe from the body is very limited (Nasser & Baird, 1968), the only way to get rid of the non-utilized part is to store it in the liver. The high non-haem Fe concentration in the liver of kwashiorkor

Table 5. *Differentiating biochemical derangement in three groups of Egyptian infants who were suffering from protein-calorie deficiency diseases*

Disease	Fe absorption	Serum transferrin (total Fe-binding capacity)	Percentage saturation	Non-haem Fe concentration of liver
Kwashiorkor	Considerably decreased	Decreased	Increased	Increased
First-grade marasmus	Slightly decreased	Normal or slightly increased	Slightly decreased	Slightly increased
Fe-deficiency anaemia	Considerably increased	Slightly increased	Considerably decreased	Considerably decreased

patients, in spite of the decreased liver protein value, adds support to this suggestion.

In Fe-deficiency anaemia the picture differs; here sufficient protein is usually supplied, whereas Fe intake is low. In our subjects the serum protein concentration was within the normal range, but with a slight increase in transferrin concentration and a very low percentage saturation. This seems to be an adaptive mechanism of the body to capture the slightest amount of Fe provided in the gut by means of ferritin, which seems to be normal as evidenced by the normal protein pattern and the quick transfer of the absorbed Fe in this group to different organs where it is badly needed.

From the results obtained, it may be concluded that the state of anaemia – taking as criteria serum Fe, Fe-binding capacity, percentage saturation of transferrin, blood haemoglobin concentration, liver non-haem Fe concentration and rate of Fe absorption from the gut – can be initiated through either protein deficiency or Fe deficiency or both together. It seems that the state of body proteins, especially ferritin, and transferrin and its percentage saturation, and the liver non-haem Fe concentration are the chief factors governing the type of anaemia met with in such cases.

Table 5 sums up the main differentiating biochemical derangements in the groups that were studied.

In the follow-up study the fact that percentage of transferrin saturation started to decrease suggested preference of transferrin synthesis to other proteins. This observation is similar to that reported by Lane (1968). The marked clinical improvement in these patients after blood transfusion was associated with progress of protein synthesis, especially of the ferritin and transferrin moieties. A consistent rise in serum transferrin in response to treatment was reported by McFarlane, Reddy, Cooke, Longe, Onabamiro & Houba (1970). Fe absorption from the gut was enhanced and liver non-haem Fe was transferred to different sites of the body.

Blood transfusion ensures quick provision of plasma proteins, thus enhancing serum Fe utilization as evidenced by the slight rise in serum Fe concentration observed in these four subjects despite blood transfusion.

It may be concluded that restoration of the normal serum protein pattern on

therapy would alleviate the anaemia without accumulation of Fe as evidenced by the slight rise in serum Fe and its increased utilization. Moreover, unless serum proteins are corrected, anaemia will resist Fe therapy alone.

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