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The influence of nutrient intake on the biochemical parameters of iron status in a healthy paediatric Mediterranean population

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A random sample of 302 children, aged between 6 months and 15 years, of a healthy Mediterranean population were studied. Abnormal values of biochemical parameters of iron status were frequently found. In the 0.5–2 year group, erythrocyte protoporphyrin values were abnormal in 13.5% of subjects, serum ferritin in 9.7%, transferrin saturation capacity in 75.3%, mean red cell volume in 4.1% and haemoglobin in 9.7%. Comparing nutritional intake (24 h dietary recall over 3 days) between individuals at low and high values of biochemical iron status some significant differences were found in each age group but in no case with regard to heme iron intake. In the overall age-adjusted study population, the nutritional intake had a low but significant explanatory capacity of the variance of the measured biochemical iron status parameters (between 1.1% for transferrin saturation capacity and 4.5% for serum ferritin) and a non-significant capacity in those children younger than 3 years. In conclusion, although the dietary pattern in our area favours a good iron bioavailability, in our population the nutritional intake was shown to have a limited relationship with the parameters of biochemical iron status parameters. These data suggest that, in healthy children, abnormal biochemical iron status parameters may be related to factors other than nutritional intake.

The high prevalence of abnormal values of biochemical parameters of iron status has been considered an important nutritional problem in industrialized countries.

Nutrition is one important factor which directly influences the variability of the biochemical parameters of iron status (Cook, 1976). However, other factors can lead to discrepancies between dietary and biochemical evaluations of iron deficiency (Weinberg, 1984). Non-nutritional factors, such as inflammatory states (Dallman, 1984), could affect the biochemical assessments of iron status in children. Complex interactions between different dietary components also affect the bioavailability of ingested iron (Hallberg, Björn-Rasmussen & Rossander, 1979).

Despite this, and given the high prevalence of perceived abnormal values of biochemical iron status parameters encountered in various developed countries (Brault-Dubuc, Nadeau & Dickie, 1983; Expert Scientific Working Group, 1985; Hercberg *et al.*, 1987; Ehrhardt, 1986), prevention and correction programs have been undertaken to increase iron intake using various dietary strategies (Baker & Demaeyer, 1979).

We studied nutritional iron status in a

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Spanish Mediterranean paediatric population (0.5–15 years) in which socio-economic status and health are at acceptable levels. Nutritional intake and biochemical iron status (erythrocyte protoporphyrin (EP), serum ferritin (SF), mean red cell volume (MCV), haemoglobin (Hb) and transferrin saturation capacity (TSC)) were measured simultaneously.

We undertook to quantify the capacity of nutritional intake to explain the variability observed in the measured parameters of biochemical iron status.

Materials and methods

Study population

The study group comprised 302 children (151 boys and 151 girls) aged between 6 months and 15 years and has been described previously. The subjects were selected at random from the town census of Reus (a commercial and agricultural town in the Spanish Mediterranean area with 96 144 inhabitants) (Salas *et al.*, 1990). The random sample was stratified according to age and sex. The participation rate was 67.5% of the initial study population. Half of the non-participating cases resulted from incorrect or incomplete data in the town census and the others declined to participate.

Dietary assessment

To evaluate nutritional intake, the 24 h recall method was used (Marr, 1971) on three different days which included one non-working day. To maximize accuracy with regard to quantities of food ingested, an album of photographs of different portions of foodstuffs was used (Pekkarinen, 1970). Nutrient conversion values were from the table of foodstuff compositions of the Institut Scientifique et Technique de l'alimentation produced by the Conservatoire National des Arts et Métiers (ISTA–CNAM) in Paris. These tables were preferred to other internal tables since they reflect more closely the composition of the Mediterranean region foods.

Biochemical assessment of iron status

From children over 2 years of age, venous blood samples were drawn in the morning (9–12 a.m.) under standard conditions. Those below 2 years underwent capillary extractions from a finger prick. Hb and MCV were measured in a Model S-7 Coulter Counter; EP by fluorometry using an Automatic Model 5 Hematofluorometer; serum iron (SI) by a colorimetric method using ferrozine as chromogen adapted for semi-automatic assay; serum transferrin (ST) by a nephelometric assay; SF by ELISA. Total iron-binding capacity (TIBC) was deduced from the ST concentration (Dezier, 1986). TSC was calculated as the ratio of serum iron to TIBC and expressed as a percentage.

Statistical analysis

To reduce error term impact, since blood parameters are measured with more precision than dietary intake, subjects were classified according to the parameters of biochemical iron status. Low (equal or less than the median) and high (greater than the median) level subgroups were established. In order to assess the relationship between the measured biochemical parameters and nutrient intake, analysis of variance, bivariate and multiple regression (Drapper & Smith, 1981) methods were used. The analyses were performed using the SPSS/PC+ program package (Norusis, 1988).

To adjust for age in the multiple regression analysis between biochemical values and nutrient intake, residuals of previous bivariate regression between each parameter of iron status and age were used.

Results

Percentage of children with abnormal values of the measured parameters of biochemical iron status are presented in Table 1.

Tables 2–6 summarise the energy and nutrient intake in the low and the high levels of each parameter of biochemical iron status. In the 0.5–2-year-olds, the only significant differences found were in nutrient intake between low and high levels of MCV and EP. The differences in heme iron intake sometimes approached significance. In the 3–8 year olds, only the levels of MCV showed significant relationship with mean daily dietary intake of proteins of vegetable

origin. In the 9–15 year olds, similar relationships were found for EP and TSC and several nutrients, including non-heme iron.

Tables 2–6 contain the bivariate regressions between each measured biochemical parameter and age together with the stepwise multiple regressions between the age-adjusted parameters of biochemical iron status versus nutrient intake (last column).

Discussion

Assessments were made of iron nutritional status in a stable, homogeneous population with a good socio-economic status (per capita income ca. U.S.$6,500). These factors are of importance in relation to studies in developing countries (Hercberg *et al.*, 1987) as well as to studies conducted in large urban areas of industrialised countries (Dhur & Hercberg, 1989).

Table 1. Percentage of children with abnormal values of iron status parameters by age group

Age (years)	*n*	*Abnormal values of biochemical iron status parameters*				
		EP (%)	*SF (%)*	*TSC (%)*	*MCV (%)*	*Hb (%)*
0.5–2	73	13.5	9.7	75.3	4.1	9.7
3–8	88	3.4	11.4	22.7	2.3	6.8
9–15	141	4.9	2.1	19.7	2.1	1.4

Biochemical parameters measured: erythrocyte protoporphyrin (EP), serum ferritin (SF), mean red cell volume (MCV), haemoglobin (Hb) and transferrin saturation capacity (TSC). Abnormal values: EP > 3 µg/g of Hb; SF ≤ 12 µg/l; TSC .320 16%; MCV < 70 fl under 2 years, < 73 fl between 2 and 5 years, < 75 fl between 6 and 14 years and < 80 fl for those over 14 years; Hb < 12 g/dl om children from 6 months to 14 years and in females over 14 years, < 13 g/dl in males over 14 years. *n* = number of children.

Table 2. Daily energy and nutrient intake subgrouped on serum ferritin levels (low: ≤ median; high: > median) within the age groups together with stepwise multiple regression between age-adjusted serum ferritin and nutrient intake

Serum ferritin level (n)	*0–2-year-olds*		*3–8-year-olds*		*9–15-year-olds*		$R^2 = 0.045$, $n = 286$, $P = 0.001$
	Low (34)	*High (36)*	*Low (42)*	*High (42)*	*Low (68)*	*High (64)*	
Energy[a] (kcal)	1473 (338)	1384 (352)	1857 (376)	1820 (341)	2433 (513)	2380 (572)	β = −0.30
P value	NS		NS		NS		
Vegetable fibre (g)	10.8 (5.2)	10.0 (4.1)	13.1 (5.5)	14.9 (6.3)	19.3 (5.5)	20.3 (8.5)	β = 0.17
P value	NS		NS		NS		
Ascorbic acid (mg)	85.2 (49.0)	94.5 (40.9)	57.2 (41.2)	63.1 (45.6)	64.0 (36.4)	73.0 (41.1)	
P value	NS		NS		NS		
Heme iron (mg)	0.74 (0.41)	0.76 (0.42)	1.14 (0.53)	1.20 (0.53)	1.61 (0.62)	1.74 (0.81)	β = 0.25
P value	NS		NS		NS		
Non-heme iron (mg)	8.5 (3.9)	9.3 (4.2)	7.2 (1.5)	7.1 (1.6)	9.9 (2.7)	10.3 (3.5)	
P value	NS		NS		NS		

[a] Mean (SD) given.
n = number of individuals; NS = non-significant association.
Bivariate multiple regression between age and serum ferritin: adjusted $R^2 \times 100 = 9.9$, $P < 0.0001$.
R^2 = adjusted multiple determination coefficient; β = standardized regression coefficient.

Table 3. Daily energy and nutrient intake subgrouped on haemoglobin levels (low: ≤ median; high: > median) within the age groups together with stepwise multiple regression between age-adjusted haemoglobin and nutrient intake

	0–2-year-olds		*3–8-year-olds*		*9–15-year-olds*	
Haemoglobin level (n)	*Low (36)*	*High (35)*	*Low (37)*	*High (47)*	*Low (65)*	*High (68)*
Energy[a] (kcal)	1427 (370)	1400 (323)	1859 (392)	1822 (330)	2395 (500)	2419 (581)
P value	NS		NS		NS	
Ascorbic acid (mg)	92.8 (46.8)	86.6 (42.1)	61.2 (36.7)	59.3 (48.1)	62.2 (30.2)	74.6 (45.1)
P value	NS		NS		NS	
Heme iron (mg)	0.71 (0.40)	0.79 (0.42)	1.24 (0.60)	1.11 (0.47)	1.66 (0.69)	1.69 (0.75)
P value	NS		NS		NS	
Non-heme iron (mg)	9.1 (3.7)	8.6 (4.2)	7.2 (1.6)	7.2 (1.5)	10.0 (3.3)	10.1 (2.8)
P value	NS		NS		NS	

[a] Mean (SD) given.
n = number of individuals; NS = non-significant association.
Bivariate multiple regression between age and haemoglobin: (adjusted $R^2 \times 100 = 32.2$, $P < 0.0001$).
R^2 = adjusted multiple determination coefficient; β = standardized regression coefficient.

Table 4. Daily energy and nutrient intake subgrouped on mean red cell volume levels (low: or < median; high: > median) within the age groups together with stepwise multiple regression between age-adjusted mean red cell volume and nutrient intake

	0–2-year-olds		*3–8-year-olds*		*9–15-year-olds*		$R^2 = 0.012$ $n = 277$ $P = 0.033$
Mean red cell volume (n)	*Low (32)*	*High (39)*	*Low (40)*	*High (44)*	*Low (60)*	*High (62)*	
Energy[a] (kcal)	1399 (317)	1426 (371)	1778 (369)	1893 (341)	2402 (564)	2412 (525)	
P value	NS		NS		NS		
Proteins of vegetable source (g)	14.3 (4.0)	15.7 (6.1)	19.0 (5.1)	22.1 (6.2)	29.4 (11.7)	29.1 (9.2)	
P value	NS		0.015		NS		
Carbohydrates (g)	167.0 (44.1)	173.3 (53.1)	194.5 (42.9)	217.7 (57.0)	278.3 (85.2)	286.0 (80.4)	β = 0.12
P value	NS		0.04		NS		
Ascorbic acid (mg)	83.9 (50.2	94.5 (39.0	60.6 (44.1)	59.7 (43.0)	66.9 (32.6)	69.9 (43.6)	
P value	NS		NS		NS		
Thiamin (mg)	0.92 (0.27)	1.12 (0.40)	1.12 (0.36)	1.14 (0.36)	1.60 (0.56)	1.49 (0.47)	
P value	0.019		NS		NS		
Heme iron (mg)	0.70 (0.34)	0.80 (0.046)	1.14 (0.53)	1.19 (0.53)	1.78 (0.78)	1.59 (0.66)	
P value	NS		NS		NS		
Non-heme iron (mg)	8.5 (4.1)	9.1 (3.8)	7.1 (1.4)	7.2 (1.6)	10.4 (3.5)	9.8 (2.6)	
P value	NS		NS		NS		

[a] Mean (SD) given.
n = number of individuals; NS = non-significant association.
Bivariate multiple regression between age and mean red cell volume: adjusted $R^2 \times 100 = 16.0$, $P < 0.0001$.
R^2 = adjusted multiple determination coefficient; β = standardized regression coefficient.

Table 5. Daily energy and nutrient intake subgrouped on transferrin saturation capacity levels (low: or ≤ median; high: > median) within the age groups together with stepwise multiple regression between age-adjusted transferrin saturation and nutrient intake

	0–2-year-olds		*3–8-year-olds*		*9–15-year-olds*		$R^2 = 0.011$ $n = 287$ $P = 0.038$
Transferrin sat. cap. (n)	*Low (37)*	*High (37)*	*Low (43)*	*High (41)*	*Low (69)*	*High (63)*	
Energy[a] (kcal)	1387 (305)	1458 (391)	1780 (331)	1899 (327)	2352 (498)	2468 (538)	
P value	NS		NS		NS		
Protein of animal source (g)	47.6 (17.3)	46.4 (14.8)	49.5 (14.8)	52.8 (18.9)	56.6 (14.5)	64.5 (19.7)	
P value	NS		NS		0.009		
Ascorbic acid (mg)	96.2 (46.2)	83.2 (42.5)	58.3 (42.8)	62.1 (44.2)	66.4 (34.2)	70.8 (43.6)	
P value	NS		NS		NS		
Pyridoxine (mg)	1.64 (0.71)	1.77 (0.79)	1.74 (0.80)	1.90 (0.84)	1.95 (0.60)	2.21 (0.90)	$\beta = 0.12$
P value	NS		NS		0.049		
Heme iron (mg)	0.76 (0.45)	0.75 (0.37)	1.12 (0.53)	1.22 (0.54)	1.62 (0.68)	1.74 (0.76)	
P value	NS		NS		NS		
Non-heme iron (mg)	9.03 (3.88)	8.80 (4.31)	7.08 (1.26)	7.34 (1.83)	10.08 (3.36)	10.12 (2.83)	
P value	NS		NS		NS		

[a] Mean (SD) given.
n = number of individuals; NS = non-significant association.
Bivariate multiple regression between age and transferrin saturation capacity: adjusted $R^2 \times 100 = 18.7$, $P < 0.0001$.
R^2 = adjusted multiple determination coefficient; β = standardized regression coefficient.

In previous studies (Martí-Henneberg *et al.*, 1988; Salas *et al.*, 1987*a*) levels of food and nutrient intake in a general population in our Mediterranean region were evaluated. Mean energy intakes in paediatric subgroups were near the recommended for each group. The probability of protein intake below the estimated individual requirement was also low in these ages (between 0 and 6%). However, following the criteria of Beaton (1985), a probability of inadequate iron intake was estimated in 10%–37% of children (Salas *et al.*, 1987*b*).

The present study was to evaluate this risk of iron malnutrition in a population of children and adolescents. Subjects were considered to have iron deficiency if they had abnormal values (see Table 1) in two or more of the parameters SF, EP, CST and MCV. Based on this criterion, the prevalence of iron deficiency was 23.3% between 6 and 23 months, 8.2% between 2 and 5 years, 1.7% between 6 and 9 years, 0% between 10 and 12 years and 3.3% and 3.2% in the boys and girls, respectively, over 13 years (Salas *et al.*, 1990).

The difference in levels of iron deficiency between our study and others from Central and North European countries was related to the pattern of intake of enhancers and inhibitors of iron absorption in our Mediterranean diet (Dhur & Hercberg, 1989). A high intake of ascorbic acid and low intake of calcium are probably associated with a better iron bioavailability (Galan *et al.*, 1990).

Nevertheless, from biochemical iron status parameters, in the present study many children had abnormal values of iron status (see Table 1). This prompted a comprehensive analysis to assess the contribution of nutrient intake towards explaining the variance observed in the parameters of biochemical iron status. Other investigators

Table 6. Daily energy and nutrient intake subgrouped on erythrocyte protoporphyrin levels (low: ≤ median; high: > median) within the age groups together with stepwise multiple regression between age-adjusted erythrocyte protoporphyrin and nutrient intake

Erythrocyte protophyrin (n)	*0–2-year-olds*		*3–8-year-olds*		*9–15-year-olds*		$R^2 = 0.043$ $n = 287$ $P < 0.001$
	Low (35)	*High (37)*	*Low (41)*	*High (43)*	*Low (64)*	*High (67)*	
Energy[a] (kcal)	1510 (353)	1333 (320)	1834 (382)	1843 (336)	2445 (567)	2383 (513)	
P value	0.003		NS		NS		
Proteins of vegetable source (g)	16.3 (5.5)	14.2 (5.1)	20.8 (6.6)	20.5 (5.2)	31.6 (11.6)	27.1 (8.7)	β = −0.42
P value	NS		NS		0.014		
Lipids (g)	55.7 (18.7)	46.3 (17.3)	73.9 (20.2)	76.3 (18.7)	95.5 (26.5)	93.8 (22.5)	
P value	0.032		NS		NS		
Carbohydrates (g)	180.0 (50.6)	164.1 (49.0)	210.4 (55.3)	203.1 (48.7)	287.1 (75.9)	279.4 (88.4)	β = 0.29
P value	NS		NS		NS		
Ascorbic acid (mg)	84.9 (44.5)	95.8 (44.5)	62.1 (47.1)	58.2 (39.7)	60.6 (30.4)	76.1 (44.7)	
P value	NS		NS		0.023		
Thiamin (mg)	1.08 (0.37)	1.01 (0.37)	1.12 (0.36)	1.14 (0.36)	1.63 (0.57)	1.45 (0.44)	
P value	NS		NS		0.048		
Heme iron (mg)	0.82 (0.44)	0.70 (0.37)	1.15 (0.58)	1.19 (0.48)	1.72 (0.79)	1.65 (0.65)	
P value	NS		NS		NS		
Non-heme iron (mg)	8.73 (3.74)	9.23 (4.42)	7.13 (1.66)	7.27 (1.48)	10.73 (3.60)	9.55 (2.43)	
P value	NS		NS		0.030		

[a] Mean (SD) given.
n = number of individuals; NS = non-significant association.
Bivariate multiple regression between age and erythrocyte protoporphyrin: adjusted $R^2 \times 100 = 1.6$, $P < 0.0135$.
R^2 = adjusted multiple determination coefficient; β = standardized regression coefficient.

have postulated that the variability in biochemical iron status markers could be related to complex physiological mechanisms to which iron is, in turn, associated (Weinberg, 1984).

In a previous analysis of the present study (Salas *et al.*, 1990), significant correlations between ferritin versus food and nutrient intake within several age groups were found, despite a lack of correlation with total dietary iron intake. In order to minimize misclassification and to reduce the impact of the error term the subjects were classified on the basis of each iron status measurement, since blood parameters are more precisely estimated than dietary intake.

Stepwise multiple regression demonstrated a strong relationship between nutrient intake and biochemical iron status parameters which was not evident after adjustment for age – reflecting, probably, the inevitable increased nutrient requirement for growth. Age effect was adjusted for by using residuals of prior bivariate regression between each iron status measurement and age (expressed in whole and fractions of years).

Age was significantly correlated with all iron status measurements; more strongly with Hb, MCV and TSC than with EP and SF (Tables 2–6). The capacity of nutrient intake to explain the variation in age-adjusted iron status parameters was between 1.1% and 4.5%.

The 0.5–2 year age group contained the

largest number of abnormal parameters of iron status. The possibility that the nutrient intake in this group could explain the observed variance was negated, because after controlling for age, no nutrient sufficiently explained a significant amount of the variation in iron status measurements.

In conclusion, in a paediatric population of good socio-economic status a high number of children with evidence of abnormal biochemical iron status, especially during the first years of life, was found. However, variation in nutrient intake did not adequately explain the phenomenon.

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