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ORIGINAL ARTICLE

Associations of iron status with dietary and other factors in 6-year-old children

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Objective: To investigate the associations of iron status at 6 years of age with dietary and other factors.
Design: In a cross-sectional study, children's dietary intakes (3-day weighed food record) were recorded, body size was measured and blood samples were taken near their sixth birthday.
Subjects: A sample of 188 children, from two previous studies (cohorts 1 and 2), was contacted, and 139 (74%) agreed to participate.
Results: Multiple regression analyses with dietary and other factors showed that meat and fish consumption, multivitamin/mineral supplement intake (both positively) and cow's milk product consumption (negatively) were associated with log serum ferritin (SF) (adjusted $R^2 = 0.125$; $P = 0.028$; $n = 129$), and juices and residence (rural > urban) with haemoglobin (Hb) (adjusted $R^2 = 0.085$; $P = 0.034$; $n = 127$). Of 21 multivitamin/mineral consumers, none had depleted iron stores compared to 21 iron-depleted of 108 non-consumers ($P = 0.024$). Children living in rural areas (<10 000 inhabitants) ($n = 33$) had higher mean corpuscular volume (MCV) (83.3 ± 2.3 fl) than those living in urban areas (>10 000 inhabitants) (82.1 ± 3.2 fl; $n = 103$) ($P = 0.048$). Multiple regression analyses with dietary and other factors and growth showed in cohort 1 that residence (rural > urban), weight gain 0–1years (negatively), and meat and fish intake (positively) were associated with Hb (adjusted $R^2 = 0.323$; $P = 0.030$; $n = 51$), meat and fish (positively) with both log SF (adjusted $R^2 = 0.069$; $P = 0.035$; $n = 52$) and MCV (adjusted $R^2 = 0.064$; $P = 0.035$; $n = 52$), and in cohort 2 cow's milk product consumption (negatively) was associated with log SF (adjusted $R^2 = 0.119$; $P = 0.017$; $n = 41$) and residence (rural > urban) with MCV (adjusted $R^2 = 0.102$; $P = 0.025$; $n = 41$).
Conclusions: Consumption of meat and fish and possibly also juices, as well as multivitamin/mineral intake might affect iron status in 6-year-old children positively, whereas cow's milk product consumption might affect iron status negatively. Slower growth in the first year of life and rural residence are positively related to iron status of 6-year-olds.
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Introduction

Iron deficiency is generally recognized as the most common single nutrient deficiency worldwide (WHO, 2001; Nordic Expert Group for Nutritional Recommendations, 2004). In industrialized countries, certain high-risk groups have been identified, such as infants and young children, and women in childbearing years (Ramakrishnan and Yip, 2002). Among the most influential factors affecting iron status are dietary factors. In infants and young children, the food factor most commonly associated (negatively) with iron status is unmodified cow's milk (Michaelsen *et al*, 1995; Thane *et al*., 2000; Thorsdottir *et al*., 2003; Gunnarsson *et al*., 2004). Several other foods and nutrients have also been implicated

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in affecting iron status, such as meat and fish (Engelmann *et al*., 1998a; Thane *et al*., 2000), iron-fortified cereals (Walter *et al*., 1993) and vitamin C (Hallberg *et al*., 1987; Gibson, 1999), all positively. Findings are mixed regarding association between iron intake and iron status (Duggan *et al*., 1991; Lind *et al*., 2004).

The present study sample comes from two earlier studies, on Icelandic infants (Thorsdottir *et al*., 2003) and on 2-year-old children (Gunnarsson *et al*., 2004), where iron status was relatively poor in comparison with neighbouring countries, but was quite good at the age of 6 years (Gunnarsson *et al*., 2005). Counter to the inverse relationship observed between iron status at 1 or 2 years and growth from birth (Thorsdottir *et al*., 2003; Gunnarsson *et al*., 2004), a positive relationship was observed between iron status at 6 years, and in fact also iron status at 1 or 2 years, and growth from 1 or 2 years to 6 years (Gunnarsson *et al*., 2005). In assessment of the relationship of iron status at 1 and 6 years and development at 6 years, iron deficiency and depleted iron stores in 1-year-olds, as well as lower haemoglobin (Hb) values at 6 years, were related to lower scores in motor components of development at 6 years (Gunnarsson *et al*., 2006). In light of the effects of iron status on developmental status and health, it is important to identify possible determinants of iron status at 6 years. The aim of the present study was to examine the association between iron status and dietary intake as well as other factors in children at the age of 6 years.

Methods

Study population

Parents of 6-year-old children from two cohorts of a longitudinal study on infant nutrition in Iceland (Thorsdottir *et al*., 2003) (cohort 1), and a cross-sectional study of Icelandic 2-year-olds (Gunnarsson *et al*., 2004) (cohort 2) were invited to participate on behalf of their children. The main criterion for inclusion in the study was that children had their blood iron status indices measured and analysed in the two earlier studies. From the two cohorts, of 188 possible participants, 139 (74%) participated. All individual information was confidential, and informed consent was obtained. The sample has been described in detail elsewhere (Gunnarsson *et al*., 2005).

The study was approved by the Local Ethical Committee at Landspitali – University Hospital in Iceland and by the Icelandic Data Protection Commission.

Dietary assessment

Three-day weighed food records were used to assess energy and nutrient intake. The records were obtained once from each participant around the age of 6 years (72.3 ± 1.6 months), with help from the children's parents and carers. Parents were provided with accurate electronic scales (PHILIPS HR 2385, Koninklijke Philips Electronics N.V., Wien, Austria). They were advised to keep the record continuously for 3 days, record each food item separately and give precise information about the type of food, cooking procedure and time of served food, and to weigh and register all leftovers.

Blood samples

The children were given an appointment for blood sampling around the same time as the collection of food records in collaboration with the children's parents. The mean age of the children at the time of blood sampling was 71.9 ± 1.4 months, and the age range was 69–77 months. A detailed description regarding the blood sampling has been published (Gunnarsson *et al*., 2005).

In the blood samples, iron status was analysed. Hb and mean corpuscular volume (MCV) were analysed on the Coulter Counter STKS, and serum ferritin (SF) was analysed with electrochemiluminescence immunoassay on Elecsys Systems 1010/2010/ Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany) at Landspitali – University Hospital in Reykjavik. Depleted iron stores were diagnosed at SF below 15 μg/l. The cutoff value of 15 μg/l for SF is according to World Health Organization (WHO) criteria for children above 5 years of age (WHO, 2001). As SF is a phase reactive protein, parents were urged not to bring their children for blood sampling if they were ill or had been ill for the past couple of days; also an experienced paediatrician evaluated their health, and C-reactive protein was measured when considered appropriate by the paediatrician.

Calculation and data analysis

Nutrient calculations were performed on ICEFOOD (program of the Icelandic Nutrition Council) using The Icelandic Nutrient Database revised, as well as the Icelandic Nutrition Council Recipe Database 2002.

Statistical analyses were performed by SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Data were presented as means, s.d. and percentiles. Student's *t*-test, Mann–Whitney *U*-test and Fisher's exact test were used to identify differences between two groups, and one-way analysis of variance and Kruskal–Wallis test were used for three groups or more. The nonparametric tests were used when data were not normally distributed and could not be transformed. Spearman's ρ correlations and stepwise (forward) multiple regression analyses were computed to evaluate linear associations. Because of skewed distribution, SF was logarithmically transformed. The level of significance was taken as $P<0.05$.

Results

Iron status vs food and nutrients

Multiple regression analyses of iron status indices and the intake of chosen food items and food groups are shown in

Table 1. From the regression model, a partial correlation of borderline significance was observed between log SF and cow's milk products (apart from cow's milk, butter and cheese) ($r = -0.169$; $P = 0.058$).

When the association between log SF and the meat and fish group was analysed further, the strongest association, albeit marginal, was with variety meat (organ meat) ($r_{sp} = 0.171$; $P = 0.053$). The children that consumed any variety meat over the course of 3 days recorded had marginally higher SF values (26.9±12.3 vs 23.4±11.1; $P = 0.073$) and were less likely to have depleted iron stores (SF values <15 μg/l) than those not consuming any variety meat, and two of 44 children (5%) consuming variety meat had depleted iron stores compared to 19 of 85 (22%) not consuming variety meat ($P = 0.011$). Of other individual food items, Spearman's ρ correlations revealed that beans from the vegetable group were marginally correlated with SF ($r_{sp} = -0.168$; $P = 0.057$) and MCV ($r_{sp} = -0.159$; $P = 0.074$). From the fruit group, berries correlated marginally with SF ($r_{sp} = 0.152$; $P = 0.085$).

Multiple regression analyses on iron status indices and nutrient intake showed that copper was positively associated with log SF ($B \pm$ s.e. $= 0.207 \pm 0.077$; adjusted $R^2 = 0.08$; $P = 0.008$; $n = 129$), whereas vitamin C was positively associated with Hb ($B \pm$ s.e. $= 0.027 \pm 0.012$; adjusted $R^2 = 0.03$; $P = 0.031$; $n = 127$). Calcium was not associated with iron status, but a weak partial correlation was found between log SF and calcium ($r = -0.148$; $P = 0.098$), although not significant. Total iron intake was not associated with iron status, and mean iron intake was 10.2±4.1 mg/day (10th, 50th and 90th percentiles were 5.6, 9.5 and 15.5 mg/day, respectively), which is above the recommended 9 mg/day from the Nordic Nutrition Recommendations (Nordic Expert Group for Nutritional Recommendations, 2004).

No significant gender differences were seen in the intake of foods and nutrients, apart from consumption of fruits, which was higher in girls (93±78 g/day) than in boys (64±52 g/day) ($P = 0.044$).

Table 1 Stepwise linear multiple regression analyses of food factors influencing change in iron status indices

Dependent variable	*Independent variable*[a]	B ± *s.e.*	P	*Adjusted* R^2	n
Hb	Juices	1.238 ± 0.483	0.012	0.04	127
Log SF	Meat and fish	0.001 ± 0.000	0.024	0.07	129
MCV	None	—	—	—	128

Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; SF, serum ferritin.
[a]The independent variables used in the multiple regression analysis were as follows: cow's milk, cow's milk products (apart from cow's milk and butter and cheese), butter, cheese, meat and fish, fruits, vegetables, bread, biscuits and crackers, breakfast cereals, juices (with and without added sugar). The intake quantities used for cow's milk and juices were 100 g/day, but g/day for other food items. Only the variables reaching statistical significance are given in the table. Adjusted for gender.

Iron status vs food supplements

The strongest associations of the intake of supplements with iron status was a marginal association seen between intake of multivitamin/mineral tablets or mixture (containing iron) with SF, where those who consumed multivitamins/minerals ($n = 21$) had SF values of 30.0±16.1 μg/l compared to 23.5±10.2 μg/l in non-consumers ($n = 108$; $P = 0.070$). No child of 21 consuming multivitamins/minerals had depleted iron stores, compared to 21 of 108 children not consuming multivitamins/minerals ($P = 0.024$). Multivitamin mixtures without iron (usually containing only vitamins B and C) were not significantly associated with iron status indices. The intake of supplemental vitamin C or iron was not associated with iron status. Only six children did consume iron supplements, and none of them had depleted iron stores compared to 21 of 122 of the others (17%) (NS), or 18 of those 89 (20%) (NS) that did not receive iron or multivitamins/minerals.

Iron status vs residence

When evaluating iron status according to residence, a higher mean MCV was observed in rural areas (<10 000 inhabitants) (83.3±2.3 fl) ($n = 33$) than in urban areas (>10 000 inhabitants) (82.1±3.2 fl) ($n = 103$) ($P = 0.048$). A marginally significant tendency towards higher Hb values was also observed in rural areas compared to urban areas (130.6±7.4 vs 127.7±6.6 g/l; $P = 0.059$). No difference was observed in the intake of foods, nutrients and supplements between the two groups.

Iron status vs food and other factors

A general regression model with the food factors along with other factors analysed here is shown in Table 2.

A regression model including growth, which has been described in another paper (Gunnarsson *et al.*, 2005), was split into two separate cohorts owing to different absolute growth values between the two cohorts. The findings are shown in Table 3.

In the regression model, of other factors from cohort 1 partial nonsignificant correlations were observed between weight gain from 1 to 6 years and Hb ($r = 0.242$; $P = 0.102$), and length gain from 1 to 6 years and MCV ($r = 0.246$; $P = 0.088$). From cohort 2 partial correlation of borderline significance was found between multivitamin/mineral intake and Hb ($r = 0.278$, $P = 0.091$), as well as weight gain from 2 to 6 years and log SF ($r = 0.297$; $P = 0.067$).

Discussion

In the present study, the intake of several foods and nutrients was associated with iron status in 6-year-old children. Meat and fish, juices, copper and vitamin C were all positively associated with iron status, and cow's milk products, negatively. Multivitamin/mineral use was also positively associated with iron status, as well as rural residence compared to urban.

Table 2 Stepwise linear multiple regression analyses of food factors and other factors influencing change in iron status indices

Dependent variable	*Independent variable*[a]	B ± *s.e.*	P	*Adjusted* R^2	n
Hb	Juices	1.238 ± 0.483	0.004		
	Residence	3.679 ± 0.474	0.010	0.085	127
Log SF	Meat and fish	0.001 ± 0.000	0.034		
	Multivitamin/mineral	0.105 ± 0.045	0.023		
	Cow's milk products	−0.001 ± 0.000	0.043	0.116	129
MCV	None	—	—	—	128

Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; SF, serum ferritin.
[a]The independent variables used in the multiple regression analysis were as follows: cow's milk, cow's milk products (apart from cow's milk and butter and cheese), butter, cheese, meat and fish, fruits, vegetables, bread, biscuits and crackers, breakfast cereals, juices (with and without added sugar), multivitamin intake (yes/no), residence (rural > urban), occupation (school/kindergarten). The intake quantities used for cow's milk and juices were 100 g/day, but g/day for other food items. Only the variables reaching statistical significance are given in the table. Adjusted for gender.

Table 3 Stepwise linear multiple regression analyses of food factors and other factors, including growth, influencing change in iron status indices, divided into cohorts 1 and 2

Dependent variable	*Independent variable*[a]	*B ± s.e.*	P	*Adjusted* R^2	n
Cohort 1 (studied at first yr and 6 years)					
Hb	Residence	8.174 ± 2.054	< 0.001		
	Weight gain 0–12 months	−3.263 ± 0.931	0.001		
	Meat and fish	0.078 ± 0.035	0.030	0.323	51
Log SF	Meat and fish	0.002 ± 0.001	0.035	0.069	52
MCV	Meat and fish	0.035 ± 0.017	0.042	0.064	51
Cohort 2 (studied at 2 and 6 years)					
Hb	None	—	—	—	40
Log SF	Cow's milk products	−0.001 ± 0.000	0.017	0.119	41
MCV	Residence	2.975 ± 1.279	0.025	0.102	41

Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; SF, serum ferritin.
[a]The independent variables used in the multiple regression analysis were as follows: cow's milk products (apart from cow's milk and butter and cheese), meat and fish, juices (with and without added sugar), multivitamin intake (yes/no), residence (rural > urban), weight gain and length gain from 0 to 1 years and weight gain and length/height gain from 1 to 6 years (cohort 1), weight gain and length/height gain from 0 to 2 years and weight gain and height gain from 2 to 6 years (cohort 2). The intake quantities used for cow's milk and juices were 100 g/day, but g/day for other food items, weight was measured in kilograms and length/height in centimetres. Only the variables reaching statistical significance are given in the table. Adjusted for gender.

The positive associations observed in the present study between the intake of meat and fish and iron status indices are in accordance with what was observed in the prior study of infant nutrition where both fish products and meat products were found to be related to iron status at the age of 1 year (Thorsdottir *et al*., 2003). Comparable results have been observed elsewhere both in young children (Engelmann *et al*., 1998a; Thane *et al*., 2000) and in children at similar age and older (Thane *et al*., 2003). These foods are relatively rich in iron and contain considerable amounts of the better absorbed haeme-iron along with a 'meat-factor', probably cysteine-containing peptides, enhancing non-haeme iron absorption (Engelmann *et al*., 1998b). Here, when split into meat products and fish products, the association was weaker and did not come through in multiple regression analyses. When split further into groups of different types of meat and fish, variety meat showed the strongest association with SF. Variety meat is recognized to be an especially good source of iron, and the present findings appear to confirm that. However, as frequently reported (Duggan *et al*., 1991; Thane *et al*., 2000; Gunnarsson *et al*., 2004) iron intake is not related with iron status in the present study, but the proportion of haeme-iron is relatively high in variety meat as in other meat products; also it often contains quite large amounts of retinol, which was recently reported to enhance iron absorption, although results are mixed (Garcia-Casal *et al*., 1998; Walczyk *et al*., 2003). In the present study, retinol intake did not correlate with the iron status indices. Copper intake was, however, positively associated with SF, which is similar to what was observed in the infant study (Thorsdottir *et al*., 2003). Although these minerals are known for being competitors for absorption (Domellof and Hernell, 2002), copper has also been reported as being important for iron metabolism and absorption (Sharp, 2004), as well as being supplied partly from the meat and fish group, which was also found to be related to SF as earlier discussed.

Juices were found to be positively associated with Hb at 6 years. They were also associated with Hb and MCV at 12 months, but there the association was negative (Thorsdottir *et al.*, 2003). The reason for the positive association seen here might be the vitamin C content of the juices, as vitamin C was also associated positively with Hb. Vitamin C is generally acknowledged as being a promoter of iron absorption (Hallberg *et al.*, 1987; Fairweather-Tait *et al.*, 1995), and has been positively related to iron status before in this population at younger age (Thorsdottir *et al.*, 2003). At 6 years, juices and vitamin C are highly correlated with each other ($r_{sp} = 0.797$; $P < 0.001$), which indicates that the variables are highly inter-related, but at 12 months the correlation between juices and vitamin C was lower ($r_{sp} = 0.343$; $P = 0.001$) (unpublished observation), indicating consumption of juices with lower amounts of vitamin C at 12 months than 6 years. A probable explanation for this difference is higher consumption of citrus juices containing more vitamin C at 6 years than 12 months.

The intake of multivitamin/mineral supplements (containing iron) was found to be positively associated with SF, and correlated also marginally with Hb in cohort 2 (study of 2-year-olds), whereas vitamin mixtures without iron (usually containing only vitamins B and C) were not significantly associated with iron status indices. Relatively few children were consuming vitamin C supplements and they were not significantly different from those not consuming supplemental vitamin C. Also, only six children took iron supplements and some of those did so in conjunction with multivitamin/minerals; hence it was difficult to establish whether iron supplements had any impact but there appeared to be a small tendency to a positive effect. The positive association observed here between the intake of multivitamin/minerals and SF might indicate a positive effect of other micronutrients than vitamin C and iron, possibly copper by some part or some other vitamins or minerals or several of the vitamins and minerals combined in the dosages used in the multivitamin/mineral formulas. Although not frequently reported, several studies have found a relationship between multivitamin/mineral supplement intake and iron status in children in industrialized countries. Lawson *et al.* (1998) found better iron status in 2-year-old Indian children living in England who consumed vitamin supplements and Australian preschool children receiving vitamin supplements tended to be less likely to be iron-depleted (Karr *et al.*, 1996). Also, from the US National Health and Nutrition Examination Survey II data, vitamin–mineral users in the age groups 3–4 years and 5–10 years had higher mean Hb and SF levels, respectively (Looker *et al.*, 1987). This might suggest that certain mixtures of micronutrients in certain proportions play some role with regard to iron status.

Interestingly, no association was found between iron status and the intake of cow's milk or calcium, apart from the negative association observed between log SF and cow's milk products in the model with food and other factors and the general model from cohort 2. This is contrary to what was observed in the study of both infants (Thorsdottir *et al.*, 2003) and 2-year-olds (Gunnarsson *et al.*, 2004) where cow's milk was very strongly negatively associated with iron status indices, and similar findings have been reported widely from studies on children (Mills, 1990; Michaelsen *et al*, 1995; Gill *et al.*, 1997; Freeman *et al.*, 1998). More than 500 g cow's milk per day were consumed by 22% of 6-year-old children compared to 18% of 1-year-olds in the infant study and 16% in the study of 2-year-olds. The proportion of cow's milk and cow's milk products of the energy consumed is, however, much less at 6 years than at 1 and 2 years, 19% of energy at 6 years, 36% energy at 1 year, 27% energy 2 at years, which might partly explain why the association with cow's milk at 6 years is not stronger.

A better iron status in rural compared to urban areas is somewhat surprising. It is quite difficult to establish any plausible reasons for this, because there does not appear to be much difference in the food and nutrient intake according to residence. However, differences in dietary consumption cannot be ruled out even though they were not observed with the 3-day record administered here. To the authors' knowledge this has not been reported before, and the significance of this finding is indefinite.

When growth variables were inserted into the regression models along with the dietary and other variables, weight gain from 0 to 12 months came significantly through in model 1 as a predictor for Hb at 1 year. Other growth variables did not come through in regression analyses here, but some came close such as length gain from 1 to 6 years and weight gain from 2 to 6 years, which is according to the observations made earlier (Gunnarsson *et al.*, 2005). Fewer subjects do have growth data than the dietary and other data; therefore the number of subjects is lower in the analyses including growth variables.

The study presented here has a relatively small sample size, but is longitudinal in nature, as the sample comprises two cohorts, from an infant study and a study on 2-year-old children. Owing to methodological aspects, the associations observed between iron status and dietary components might be somewhat skewed and some might suggest that a food frequency questionnaire method should have been run with the 3-day weighed record for the estimation of some foods and nutrients less frequently consumed. A food frequency questionnaire for young children can be insufficient. Although the sample comes from an affluent well-nourished population, iron status is relatively poor in infancy (Thorsdottir *et al.*, 2003) and early childhood (Gunnarsson *et al.*, 2004), although it improves considerably when the children get older (at the age of 6 years) (Gunnarsson *et al.*, 2005). For public health purposes, given the possible association between iron status in childhood and some aspects of motor and mental development (Gunnarsson *et al.*, 2006), it is important to investigate and identify possible determinants for iron deficiency and depleted iron stores in such a well-nourished population.

In conclusion, the present findings suggest that consumption of meat and fish and possibly also juices can support better iron status in children at 6 years of age, and also intake of nutrients like copper and vitamin C, which correlates strongly to juice consumption, whereas cow's milk product consumption (apart from cow's milk and cheese and butter) might affect iron status negatively. Growth during the first year of life is also negatively related to iron status at 6 years. Intake of multivitamin/mineral supplements and residence might also play a role in iron status of children, but further studies are needed.

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