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ORIGINAL COMMUNICATION

Iron status in 6-y-old children: associations with growth and earlier iron status

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Objective: To investigate the iron status of 6-y-old children and its association with growth and earlier iron status.
Design: In a cross-sectional study, children's body size measurements were recorded and blood samples taken near their sixth birthday.
Subjects: A sample of 188 children, randomly selected in two previous studies, was contacted, and 139(74%) agreed to participate.
Results: No children had iron deficiency anaemia, one was iron-deficient (serum ferritin (SF) <15 μg/l and mean corpuscular volume (MCV) <76) fl but 16% had depleted iron stores (SF<15 μg/l). Iron status indices were generally higher than at 1 and 2 y, but correlation was seen between iron status indices at 6 y and earlier values. Haemoglobin concentration at 6 y was negatively associated with length gain from birth to 1 y ($B \pm$s.e.$=-1.269 \pm 0.452$; $P=0.007$; adj. $R^2=0.119$) ($n=52$), and proportional weight gain from birth to 1 y was higher among children with SF<15 μg/l at 6 y (295±33%; $n=10$) than those with SF≥15 μg/l (258±31%; $n=49$) ($P=0.001$). MCV at 2 y predicted weight gain from 2 to 6 y ($B \pm$s.e.$=1.721 \pm 0.581$; $P=0.005$; adj. $R^2=0.153$) ($n=44$); also, children with SF<15 μg/l at 6 y ($n=9$) gained 7.8±1.2 kg from 2 to 6 y, while children with SF≥15 μg/l ($n=35$) gained 9.6±2.8 kg ($P=0.007$), furthermore a difference was seen in proportional weight gain from 2 to 6 y between children with depleted iron stores at 2 y and not, or 156±13 *vs* 169±18% ($P=0.038$).
Conclusions: The results suggest that low iron status at 1 and 2 y might lead to slower growth up to 6 y of age. Low iron status at 1 and 2 y and/or slower growth from 1 and 2 y up to 6 y might contribute to worse iron status at 6 y, while faster growth in early childhood is related to lower iron status.
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Introduction

Iron deficiency is generally acknowledged as one of the most common nutritional problems in the world. It is particularly common during periods of rapid growth, such as in late infancy and early childhood. Iron deficiency anaemia in early childhood is known to have serious consequences for learning ability and mental and motor development (Walter *et al*, 1989). Iron status tends to improve in older children (Thane *et al*, 2000; Domellof & Hernell, 2002) when growth slows down and diet gets more diverse, and tracking of iron status has been reported during the first year (Michaelsen *et al*, 1995). Iron status in late preschool- and primary school-aged children in more developed countries is not particularly well documented, and this pertains to tracking of blood iron status from younger ages.

Fast growth in the first year has been negatively related to iron status in several studies (Morton *et al*, 1988; Sherriff *et al*, 1999; Thorsdottir *et al*, 2003), and weight gain from birth can affect iron status negatively, even up to 2 y of age (Gunnarsson *et al*, 2004). To the contrary, growth stunting in

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infants and children has been associated with iron deficiency in less developed countries (Bougle *et al*, 2000), and some studies on toddlers and older children have shown that iron supplementation can affect growth positively (Angeles *et al*, 1993; Lawless *et al*, 1994), that might indicate the importance of iron for growth. However, the evidence for this is equivocal, since some studies have shown no positive effect of iron supplementation on growth (Rosado *et al*, 1997; Rahman *et al*, 1999). More information on the relationship of these two variables, iron status and growth, is therefore needed, with regard to causality, strength of the relationship and other factors.

The study sample comes from two studies, on Icelandic infants (Thorsdottir *et al*, 2003) and 2-y-old children (Gunnarsson *et al*, 2004). In both these studies iron status was relatively poor in comparison with neighbouring countries, 20 and 9% were defined to be iron deficient in the infant study and 2-y-old study, respectively, and 41 and 27% had depleted iron stores. The aim of the present study was to examine the iron status in children at the age of 6 y and examine tracking of blood iron status by comparing current iron status with earlier iron status, that is, at the ages of 12 months and 2 y. Furthermore, to investigate the association of both earlier (12 months and 2 y) and current (6 y) iron status with growth up to 6 y and body size at 6 y.

Methods

Study population

Parents of 6-y-old children from two random cohorts of a longitudinal study on infant nutrition in Iceland (Thorsdottir *et al*, 2003) (cohort 1), and a cross-sectional study of Icelandic 2-y olds (Gunnarsson *et al*, 2004) (cohort 2), were contacted and invited to participate on behalf of their children. The main criterion for inclusion 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. The main reasons for nonparticipation were refusal ($n = 28$) and dropouts ($n = 15$). No differences were observed between the two cohorts in maternal factors, birth weight and breastfeeding rates. The study period was from 2001 to 2002. All individual information was confidential, and informed consent was obtained.

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

Blood samples

Fasting blood samples were taken from the children's antecubital fossa. The children were appointed to blood sampling after 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.

In the blood samples, iron status was analysed. Haemoglobin (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. The cutoff points used for iron deficiency anaemia were Hb < 115 g/l, SF < 15 μg/l and MCV < 76 fl, and the two latter indices with same cutoff points were used to identify iron deficiency. Depleted iron stores were diagnosed at SF below 15 μg/l. The cutoff values for Hb of 115 g/l and SF of 15 μg/l are according to WHO criteria for the age group of 5–11 y in the case of Hb and above 5 y of age in the case of SF (WHO, 2001). The cutoff value for MCV of 76 fl is according to the criteria used in NHANES II for 5–10-y-olds (Expert Scientific Working Group, 1985). Since SF is a phase reactive protein, parents were urged not to bring their children to blood sampling if they were ill or had been ill the last couple of days; also, an experienced paediatrician evaluated their health, and CRP was measured when considered appropriate by the paediatrician. CRP was measured with sandwich immunoassay (value > 10 mg/l excluded the child from participation).

Body size measurements

Weight was measured to the nearest 0.1 kg using a Taniter BWB-620 electronic scale, and height to the nearest 0.5 cm at the age of 6 y in a clinical examination at Landspitali—University Hospital (mean age at examination 72.3 ± 1.6 months).

Summary of collected data

The two cohorts used in the 6-y-old study have been described in earlier papers (Thorsdottir *et al*, 2003; Gunnarsson *et al*, 2004). An overview of the data collected there regarding body size and iron status, as well as in the 6-y-old study is given in Figure 1.

Calculation and data analysis

Statistical analyses were carried out by SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Data were presented as means, s.d. and percentiles. *t*-test, Mann–Whitney *U* test and Fisher's exact test were used to identify differences between two groups, and Kruskal–Wallis test was used for three groups or more. The nonparametric tests were used when data was not normally distributed and could not be transformed. Stepwise (forward) linear regression analyses were performed to evaluate the associations of food and nutrient intake as well as other parameters with iron status indices. As a result of skewed distribution, SF was logarithmically transformed. The level of significance was taken as $P < 0.05$.

Results

Iron status of 6-y-old children and association with earlier iron status

Values of iron status indices in children at the age of 6 y are shown in Table 1.

No children had iron deficiency anaemia, one was iron-deficient (SF < 15 μg/l and MCV < 76 fl) but 16% had depleted iron stores (SF < 15 μg/l).

The measured iron status indices were generally higher in most children at the age of 6 y than they had been in early childhood. In paired *t*-tests significant differences were found between 6 y on the one hand and 12 months or 2 y on the other for all iron status indices (Table 2). However, a correlation was seen between iron status indices at 6 y and earlier values, as shown in Table 3. The correlation was especially strong with regard to MCV, and Hb at 6 y did correlate with earlier values. Ferritin at 6 y did not correlate with earlier ferritin values, although a borderline significance was seen. Also, six of 19 children (32%) having SF < 15 μg/l at 2 y are still below this level at the age of 6 y, while three of 29 (10%) that did have SF > 15 μg/l at 2 y are below that level at 6 y (Fisher's exact test, $P = 0.073$). Ferritin at 6 y did correlate with both Hb and MCV at 2 y. At 6 y, MCV correlated both with ferritin ($r_{sp} = 0.237$; $P = 0.005$; $n = 136$) and Hb ($r_{sp} = 0.182$; $P = 0.034$; $n = 136$), while no correlation was found between Hb and ferritin.

Gender difference was observed in mean SF values, with the boys ($n = 70$) having 22.4±9.8 μg/l, while the girls ($n = 67$) had 27.3±13.4 μg/l ($P = 0.019$). MCV values were also slightly, but not significantly, higher in girls (82.9±2.9 fl; $n = 67$) than boys (81.9±3.1 fl; $n = 69$) ($P = 0.097$).

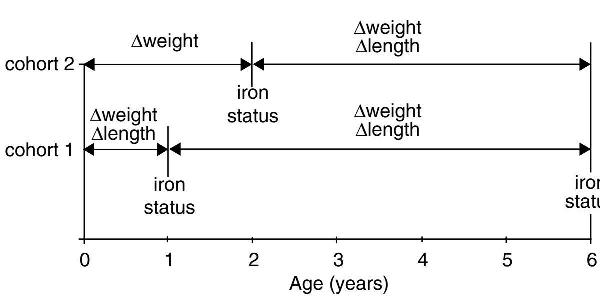

Figure 1 Schematic presentation of data from the two cohorts used in the analyses.

Iron status and body size measurements at 6 y

No association was seen between iron status indices and body size, that is, weight, height and BMI, at 6 y, and birth weight did not appear to have any effect on iron status 6 y later.

Iron status and growth — cohort 1

In a separate analysis of the data from the infant study (cohort 1), regression analyses of iron status and growth variables from birth to 12 months and 12 months to 6 y (Table 4) revealed that Hb concentration at 6 y was negatively associated with length gain from birth to 12 months, when adjusted for gender and other growth variables.

In these regression analyses no association between length gain or weight gain from 12 months to 6 y was observed; however, a partial correlation ($r = 0.25$) was observed between Hb and weight gain from 12 months to 6 y, indicating a positive relationship. Also, a correlation between MCV and length gain from 12 months to 6 y ($r_{sp} = 0.26$; $P = 0.052$), is a further indication of a positive trend between growth after the age of 1 y and iron status. Proportional weight gain from birth to 12 months was higher among the children who had depleted iron stores at 6 y ($n = 10$) than those who were not ($n = 49$), or 295±33 *vs* 258±31% ($P = 0.001$), but the difference was not significant with absolute values, or 6.9±1.3 kg *vs* 6.1±0.9 kg ($P = 0.074$).

Hb at 12 months correlated with weight gain ($r_{sp} = 0.28$; $P = 0.029$; $n = 60$) and length gain ($r_{sp} = 0.26$; $P = 0.044$; $n = 61$) from 12 months to 6 y. However, Hb appeared to be

Table 2 Mean values and paired *t*-test outcomes between iron status indices at 12 months and 6 y on the one hand (cohort 1) and 2 and 6 y on the other (cohort 2)

	Mean values at 12 months or 2 y	*Mean values at 6 y*	n	P *(for difference)*
Cohort 1				
Hb (g/l)	115.2	127.4	77	<0.001
MCV (fl)	76.5	82.3	77	<0.001
SF (μg/l)	17.4	24.3	75	<0.001
Cohort 2				
Hb (g/l)	122.9	131.9	49	<0.001
MCV (fl)	78.4	82.3	49	<0.001
SF (μg/l)	17.8	24.8	48	0.003

Table 1 Iron status indices at 6 y of age

	Mean	*s.d.*	*Median*	*10th percentile*	*90th percentile*	*no (%) <cutoff*[a]	n
Hb (g/l)	128.8	8.6	128.0	120.0	138.3	2 (1.4)	136
SF (μg/l)	24.8	11.9	22.0	12.8	39.2	22 (16.1)	137
MCV (fl)	82.4	3.1	82.2	78.4	86.3	3 (2.2)	136

[a]Cutoff values were as follows: Hb < 115 g/l; MCV < 76 fl; SF < 15 μg/l.

Table 3 Correlation between iron status indices at 6 y and earlier values

	Iron status at 6 y		
	SF	*Hb*	*MCV*
SF at 2 y	$r_{sp}=0.258$; $P=0.076$ ($n=48$)	$r_{sp}=-0.256$; $P=0.082$ ($n=47$)	$r_{sp}=0.254$; $P=0.082$ ($n=48$)
Hb at 2 y	$r_{sp}=0.302$; $P=0.035$ ($n=49$)	$r_{sp}=0.319$; $P=0.027$	$r_{sp}=0.084$; $P=0.566$ ($n=49$)
MCV at 2 y	$r_{sp}=0.376$; $P=0.008$ ($n=49$)	$r_{sp}=0.087$; $P=0.557$ (n$=48$)	$r_{sp}=0.700$; $P<0.001$ ($n=49$)
SF at 12 mo	$r_{sp}=0.202$; $P=0.082$ ($n=75$)	$r_{sp}=-0.173$; $P=0.142$ ($n=74$)	$r_{sp}=0.027$; $P=0.818$ ($n=74$)
Hb at 12 mo	$r_{sp}=0.209$; $P=0.066$ ($n=78$)	$r_{sp}=0.328$; $P=0.004$ ($n=77$)	$r_{sp}=0.367$; $P=0.001$ ($n=77$)
MCV at 12 mo	$r_{sp}=0.125$; $P=0.276$ ($n=78$)	$r_{sp}=0.257$; $P=0.024$ ($n=77$)	$r_{sp}=0.650$; $P<0.001$ ($n=77$)

mo = months.

Table 4 Stepwise linear regression analyses of growth variables influencing change in iron status indices at 6 y in the data from the infant study

Dependent variable		*Independent variable*[a]	B ± *s.e.*	P	*Adj.* R^2	n
Hb	Univariate	Length gain 0–12 mo	−1.268 ± 0.404	0.003	0.134	58
		Weight gain 0–12 mo	−0.002 ± 0.001	0.011	0.094	58
		% length gain 0–12	−0.635 ± 0.180	0.001	0.168	58
		% weight gain 0–12	−0.089 ± 0.028	0.002	0.139	58
	Multivariate	Length gain 0–12 mo	−1.269 ± 0.452	0.007	0.119	52
	Multivariate	% length gain 0–12	−0.644 ± 0.197	0.002	0.160	52
Log SF		None				
MCV		None				

mo = months.
[a]The independent variables used in the multivariate regression analyses were as follows (both in absolute values and as proportions): Length gain from birth to 12 months, weight gain from 0 to 12 months, length gain from 12 months to 6 years, weight gain from 12 months to 6 y. Only the variables reaching statistical significance are given in the table. Adjusted for gender.

a predictor for absolute weight gain from 12 months to 6 y only in univariate regression analysis, adjusted for gender, but when tested with other iron status indices, nothing emerged as significant, and when weight gain from birth to 12 months was added to the model with the iron status indices, it became the only predictor observed. However, with proportional weight gain from 12 months to 6 y, Hb at 12 months remained significant (B ± s.e. = 1.175 ± 0.476; $P=0.017$; Adj. $R^2 = 0.081$) ($n=59$), even if proportional weight gain from birth to 12 months was added to the model. Hb at 12 months was also the only predictor for absolute length gain from 12 months to 6 y in a model including iron status indices and length gain from birth to 12 months as independent variables (B ± s.e. = 0.129 ± 0.61; $P=0.038$; Adj. $R^2 = 0.057$) ($n=59$).

Iron status and growth — cohort 2

In a separate analysis of data from the study on 2-year olds (cohort 2), a correlation was seen between SF at 6 y and weight gain from 2 to 6 y ($r_{sp}=0.30$; $P=0.048$) ($n=44$). When analysed further, a difference in weight gain from 2 to 6 y was observed between those who had depleted iron stores at 6-y and those who had not. The children with depleted iron stores ($n=9$) gained 7.8 ± 1.2 kg from the age of 2 y through the age of 6 y, while the children who were not iron depleted ($n=35$) gained 9.6 ± 2.8 kg during the same period ($P=0.007$). A difference was seen in proportional weight gain between those who had depleted iron stores at 2 y and those who had not, or 156 ± 13 *vs* 169 ± 18% ($P=0.038$), but the difference in absolute weight gain did not reach statistical significance.

A correlation was found between MCV at 2 y and weight gain from 2 to 6 y, both absolute ($r_{sp}=0.349$; $P=0.020$) and proportional ($r_{sp}=0.456$; $P=0.002$), and furthermore SF and almost Hb correlated with proportional weight gain from 2 to 6 y, $r_{sp}=0.350$ ($P=0.020$) and $r_{sp}=0.296$ ($P=0.051$), respectively. In regression analysis with weight gain from 2 to 6 y as a dependent variable and the iron status indices at 2 y as well as weight gain from birth to 2 y as independent variables, only MCV at 2 y came through as a predictor for weight gain from 2 to 6 y (B ± s.e. = 1.721 ± 0.581; $P=0.005$; Adj. $R^2 = 0.153$) ($n=44$).

Iron status and growth from the two cohorts combined

The two data sets were combined by using quartiles of weight gain and length gain. In Figure 1a and b, a trend is seen towards lower MCV and SF in upper quartiles of weight gain from birth to 12 months and 2 y, while from 12 months and 2 to 6 y of age, the highest quartile in both weight gain and height gain is also highest in MCV and SF (see Figures 2a and b, 3a and b and 4a and b).

Discussion

The current results indicate a low prevalence of iron deficiency in Icelandic 6-y-old children and generally a much better iron status and lower prevalence of ID than at 12 months and 2 y of age. This is similar to findings from other studies. In the US's NHANES III the prevalence of iron deficiency was much lower in the age groups 3–5 and 6–11 y than in children aged 1–2 y (Looker *et al*, 1997) and in the British National Diet and Nutrition Survey (NDNS), children aged 3.5–4.5 y had significantly higher mean Hb and SF values than children aged 1.5–2.5 y (Thane *et al*, 2000). However, in the present study significant tracking in iron status indices was observed, mainly in MCV, but also in SF and Hb. Similar tracking was observed in SF between 6 and 9 months in a study of Danish infants (Michaelsen *et al*, 1995) and in a cohort study of Irish children, earlier iron status was found to be a predictor for iron status at 36 months of age (Freeman *et al*, 1998). To the authors' knowledge, no studies have examined tracking of iron status from infancy to as late as 6 y of age.

Iron deficiency has been implicated as a cause of stunting (Bougle *et al*, 2000). Although common in less developed countries where nutrient deficiencies are widespread, stunting is practically unknown in Iceland. Nevertheless, indications were seen that worse iron status at 12 months and 2 y might predict slower growth up to 6 y, supporting the view that even modest iron deficiency can lead to somewhat slower growth in a well-nourished population although growth was usually within the normal range. On the other hand, iron status at 12 months and 2 y was affected negatively by growth from birth, as reported earlier (Thorsdottir *et al*, 2003; Gunnarsson *et al*, 2004). This might imply that growth rate affects iron status during the period of rapid growth in infancy and early childhood, which has been widely documented elsewhere (Michaelsen *et al*, 1995; Freeman *et al*, 1998; Sherriff *et al*, 1999), where iron demand tends to exceed supply, and also that iron status at the vulnerable age between 1 and 2 y influences growth in later childhood. This is supported by the suggestion of Bougle *et al* (2000) that iron deficiency can cause stunting and many findings showing that growth can be improved in iron-deficient children that receive supplementary iron (Aukett *et al*, 1986; Briend *et al*, 1990; Latham *et al*, 1990). However, some researchers have failed to show improvements of iron supplementation on growth (Rosado *et al*, 1997; Rahman *et al*, 1999).

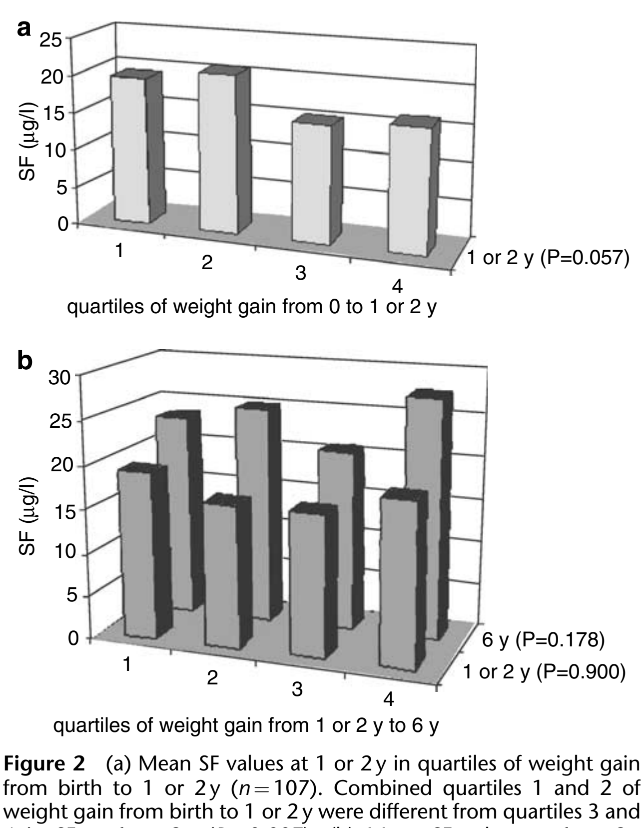

Figure 2 (a) Mean SF values at 1 or 2 y in quartiles of weight gain from birth to 1 or 2 y ($n=107$). Combined quartiles 1 and 2 of weight gain from birth to 1 or 2 y were different from quartiles 3 and 4 in SF at 1 or 2 y ($P=0.007$). (b) Mean SF values at 1 or 2 y ($n=103$) and at 6 y ($n=102$) in quartiles of weight gain from 1 or 2 to 6 y. No difference was observed for combined quartiles 1 and 2 *vs* 3 and 4 from 1 or 2 y to 6 y in SF at 1 or 2 y and at 6 y.

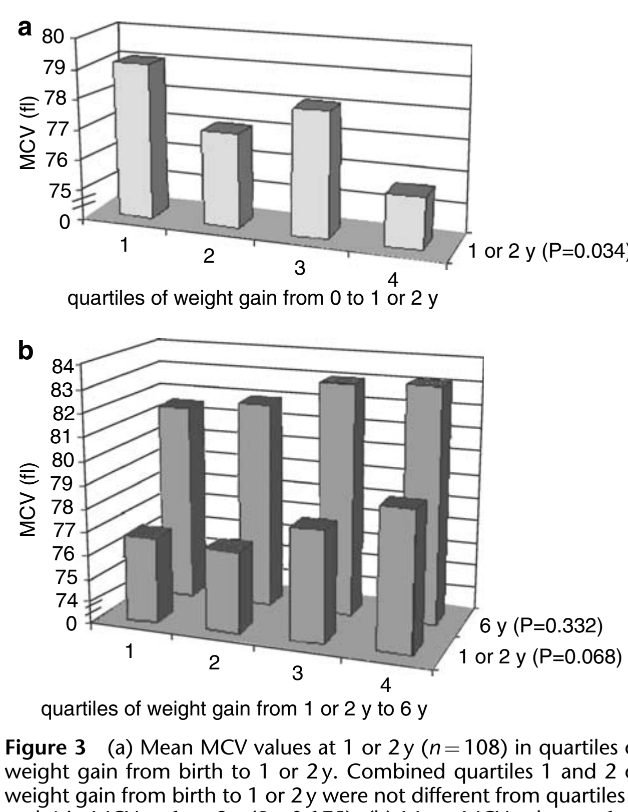

Figure 3 (a) Mean MCV values at 1 or 2 y ($n=108$) in quartiles of weight gain from birth to 1 or 2 y. Combined quartiles 1 and 2 of weight gain from birth to 1 or 2 y were not different from quartiles 3 and 4 in MCV at 1 or 2 y ($P=0.175$). (b) Mean MCV values at 1 or 2 y ($n=104$) and at 6 y ($n=101$) in quartiles of weight gain from 1 or 2 to 6 y. Difference was observed for combined quartiles 1 and 2 *vs* 3 and 4 from 1 or 2 to 6 y in MCV at 1 or 2 y ($P=0.018$) but not in MCV at 6 y ($P=0.111$).

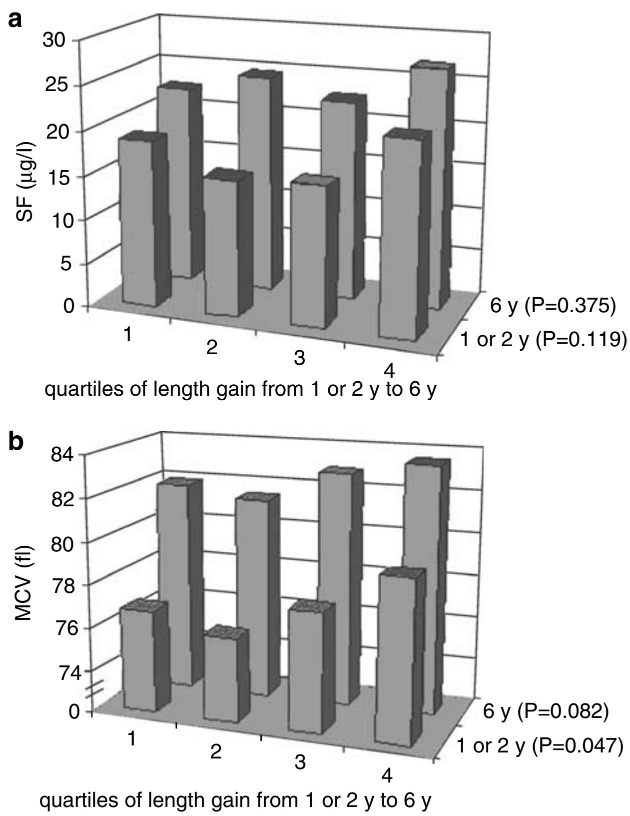

Figure 4 (a) Mean SF values at 1 or 2 y ($n = 102$) and 6 y ($n = 102$) in quartiles of length gain from 1 or 2 y to 6 y, respectively. Combined quartiles 1 and 2 were not significantly different from quartiles 3 and 4. (b) Mean MCV values at 1 or 2 y ($n = 104$) and 6 y ($n = 101$) in quartiles of length gain from 1 or 2 y to 6 y, respectively. Combined quartiles 1 and 2 were different from quartiles 3 and 4 for MCV at 1 or 2 y ($P = 0.018$) and 6 y ($P = 0.021$).

Iron status at 6 y was also positively associated, to some extent, with growth from 12 months and 2 to 6 y. However, growth from birth to 6 y did not seem to affect iron status at 6 y; neither did body size measurements at 6 y, as they had done in the infant and 2-y-old studies (Thorsdottir *et al*, 2003; Gunnarsson *et al*, 2004). However, length gain from birth to 12 months seemed to affect Hb levels at 6 y of age, possibly indicating some long-term effects of fast growth in infancy on iron status.

Figures 2–4 depict the different associations between growth and iron status in early and later childhood. Fast growth in the first 2 y is associated with lower iron status at 1 and 2 y, but the situation turns around regarding growth from 1 and 2 to 6 y and iron status at 6 y, and good iron status at 1 and 2 y also seems to influence subsequent growth positively. However, in the latter cases the question about cause and consequence is not clear; probably low iron status at the ages of 1 and 2 y affect later growth negatively to some extent, influencing iron status 4–5 y later. Owing to good nutritional status of the children, confounding of the observed association between iron status and growth by other micronutrient deficiencies is quite unlikely. Therefore, the findings give specific information about growth and iron status in childhood, which is harder to concisely detect in a population of poorer nutrient status in general.

A gender difference was observed in SF levels, the girls having higher levels. This is consistent with the findings from the infant study (Thorsdottir *et al*, 2003), but not the 2-y-old study (Gunnarsson *et al*, 2004), where no gender difference in iron status was observed. Mixed results are also seen in other studies (Wharf *et al*, 1997; Sherriff *et al*, 1999; Thane *et al*, 2000).

In the present study, a disadvantage is a relatively small sample size with limited power. An advantage of the study is that it is a longitudinal study and retains good portion of the original sample. In conclusion, the results show that iron status of 6-y-old Icelandic children is in general good and has improved from what was observed at 1 and 2 y of age. No children were defined to be iron deficiently anaemic, one child was iron-deficient while 16% had depleted iron stores. Faster growth in infancy is related to lower iron status at 6 y, as well as at 1 and 2 y, which was shown earlier. The results suggest that iron deficiency and, in general, lower iron status at 1 and 2 y might have a negative effect on growth up to 6 y of age, and perhaps also lead to worse iron status at 6 y, possibly through slower growth.

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