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## Symposium on ‘Nutrition and health in children and adolescents’ Session 2: Dietary quality and dietary recommendations in children and adolescents

### Dietary quality and adequacy of micronutrient intakes in children

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Presented are longitudinal studies, extending from infancy ( $n$  180) to 2 years of age ( $n$  130) and 6 years of age (>70% participation) of diet and Fe status in a population with high birth weight, high frequency of breast-feeding and, at the time of the study, high intake of cow's milk during the weaning period. The association between socio-demographic and dietary factors was also studied, together with Fe status in early childhood and developmental status at 6 years. Fe status was found to be poorer than in the neighbouring Nordic countries. Every fifth 1-year-old was Fe-deficient (serum ferritin <12 µg/l and mean corpuscular volume <74 fl). It was demonstrated by regression analysis that Fe status was negatively associated with cow's milk consumption at 9–12 months (significant at >460 g/d) and was weakly positively associated with fish, meat and Fe-fortified cereal consumption. Fe-deficient infants had a shorter duration of breast-feeding, and breast-feeding was related to slower growth, which can protect from worsening Fe status. Fe deficiency was less common at ages 2 and 6 years. Maternal factors associated with lower adherence to the recommended infant diet were less education, lower age and smoking. In a multiple stepwise regression analysis that included food factors, socio-demographic factors were not found to be associated with Fe status. Fe-depleted and Fe-deficient 1-year-olds had lower fine motor scores when they were 6 years old than those who were not Fe-deficient or Fe-depleted. The findings of these studies have already led to changes in the local recommendations for diet in infancy. The results suggest that Fe deficiency at 12 months of age affects development at 6 years of age. The studies indicated that mothers with less education, who smoked and who were younger needed more guidance concerning recommendations about diet in infancy.

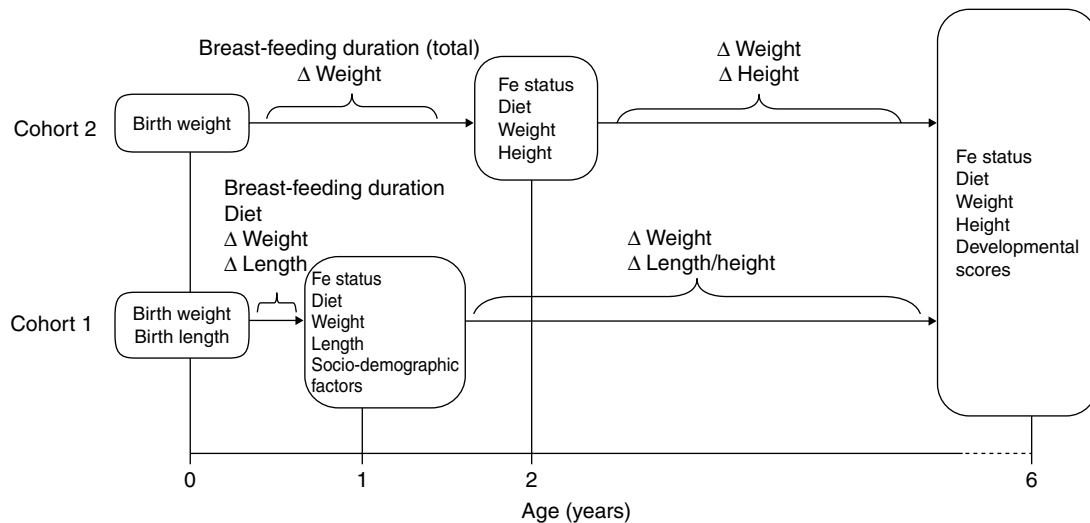
#### Iron status: Early childhood: Diet quality: Socio-demographic factors: Developmental status

Dietary quality, including sufficient micronutrients, is one of the most important factors for the health and well-being of today's children in developing and developed countries. The nutritional needs of children should be constantly reviewed and the levels for requirements and recommended intakes redefined according to the latest evidence. Late in 2004 a new edition of *Nordic Nutrition Recommendations, Integrating Nutrition and Physical Activity* was published (Alexander *et al.* 2004). These recommendations are based on the present nutrition situation in the Nordic countries and are to be used for planning diets that satisfy the nutritional needs of each age-group, i.e. covering the physiological requirement for growth and

metabolism. In general, an adequate diet is a prerequisite for overall good health and contributes to the risk reduction of diet-associated diseases. In many cases the values for infants and children have been derived from adult data using either body weight or energy requirement as a basis for the estimation. Recommendations for single nutrients are not given for the age-group 0–6 months except for vitamin D. Vitamin D supplementation is required at northern latitudes in order to ensure infants do not develop rickets. Fe deficiency is probably the most frequently observed nutrient deficiency worldwide.

The main reasons for inadequate Fe status in infants and young children are considered to be fast growth in infancy,

**Abbreviations:** MCV, mean corpuscular volume;  $r_{sp}$ , Spearman's rho correlation; SF, serum ferritin.  
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**Fig. 1.** Schematic representation of the study design showing the data collected and used in the analysis for the two cohorts of Icelandic children: infant cohort (cohort 1); 2-year-old cohort (cohort 2).

which leads to a high demand for Fe (Dallmann *et al.* 1980), and also both low Fe content and Fe bioavailability in the weaning diet (Lind *et al.* 2003). A full-term infant of normal weight usually has sufficient Fe stores for the first 6 months of life, but subsequently Fe from dietary sources play an increasing role in supplying Fe for the body (Department of Health, 1994). Fe homeostasis is maintained through absorption. Compared with many nutrients Fe is poorly absorbed, and another feature of human Fe metabolism is the absence of an excretory pathway. From studies on adults it has been shown that absorption in the intestine depends on the Fe status of the body, the amount and type of Fe in the diet and the composition of the meal (Bothwell *et al.* 1989; Lynch *et al.* 1989; Brune *et al.* 1992; Lonnerdal & Hernell, 1994). It has been suggested that during the first 6 months the infant may not be able to down regulate their absorption of Fe as efficiently as children and adults (Domellof & Hernell, 2002). Thus, it is important to have an appropriate diet that simultaneously avoids Fe deficiency and Fe overload.

The negative association between cow's milk intake and Fe status in infancy is the best-known interrelationship affecting Fe status in young childhood (Michaelsen *et al.* 1995; Gill *et al.* 1997; Freeman *et al.* 1998; Lawson *et al.* 1998; Bramhagen & Axelsson, 1999; Thane *et al.* 2000). Prolonged duration of exclusive breast-feeding, i.e. up to 9 months of age, has also been associated with poor Fe status (Pizarro *et al.* 1991). Birth weight is positively associated with Fe status in infancy (Wharf *et al.* 1997) and at  $\leq 12$  (Persson *et al.* 1998) and 18 months of age (Sherriff *et al.* 1999). However, fast growth during the first year is negatively associated with Fe status (Michaelsen *et al.* 1995) and birth weight is inversely related to post-natal growth (Thorsdottir & Birgisdottir, 1998; Atladottir & Thorsdottir, 2000). Thus, it was important to evaluate the association between Fe status and body size and growth, as well as dietary factors.

The official recommendation for the infant diet in Iceland has been similar to that of most countries;

however, Iceland has differed from most countries in its recommendation on weaning: cow's milk after the age of 6 months (Steingrimsdottir, 1995; Palsson *et al.* 1996). However, cow's milk is known to negatively influence the Fe status in infants (Tunnessen & Oski, 1987; Mills, 1990). It was therefore of great concern and theoretical interest to investigate the effect of the frequent use of cow's milk for infants between 6 and 12 months of age on the Fe status at 12 months in the Icelandic population.

In the present studies the effects of food intake and growth on Fe status were investigated at the ages of 12 months, 2 years and 6 years in Icelandic children who were born into an affluent society with a high birth weight (among the highest in the world) and a high frequency of breast-feeding (Thorsdottir & Birgisdottir, 1998) and, at the time of the infant study, a high intake of cow's milk during the weaning period. The effects of Fe deficiency in early childhood on developmental status were also studied on starting school or at 6 years of age. The findings of these studies have already led to a change in the recommendations in Iceland, and a special milk product for infants from 6 months of age and children  $\leq 2$  years has been developed. As social factors may affect food habits and the feeding of infants, the effects of several socio-demographic factors on adherence to recommendations for feeding infants were also investigated.

## Subjects and methods

### Study design

The study design is outlined in Fig. 1.

### Study population

The studies are based on two random cohorts: a longitudinal study on infant nutrition, cohort 1 ( $n$  180); a cross-sectional study of 2-year-olds, cohort 2 ( $n$  130). Of the eligible participants  $>70\%$  were involved in the Fe-status surveys at age 1, 2 and 6 years.

Parents were contacted and given information about the studies and invited to participate on behalf of their children. All individual information was confidential and informed consent was obtained. The studies were approved by the Local Ethical Committee at Landspítali-University Hospital and by the Icelandic Data Protection Commission. The two cohorts and methods have been described in Thorsdottir *et al.* (2003) and Gunnarsson *et al.* (2004).

### Growth

Growth data were collected at healthcare centres. In the infant study information on weight and length was gathered from the maternity wards involved in the study and the participating families' healthcare centres. At 2 and 6 years of age the children's height and weight were measured in a clinical examination at Landspítali-University Hospital and other centres. Information was also obtained about the children's birth weight from their birth records.

### Food records

Consumption was recorded throughout the first year (once monthly) in the infant study and information was collected about breast-feeding for the participants who were recruited at the age of 2 years. Weighed-food records for 2 d at 2, 4, 6, 9 and 12 months of age and for 3 d at 2 and 6 years of age were used in the analysis of food and nutrient intake.

### Blood samples

Blood samples were taken at a visit to the University Hospital at 1, 2 and 6 years of age for analyses of Hb, serum ferritin (SF), serum transferrin receptors, mean corpuscular volume (MCV) and erythrocyte distribution width (for details of analytical methods, see Thorsdottir *et al.* 2003; Gunnarsson *et al.* 2004, 2005).

The cut-off points used to identify Fe-deficiency anaemia at 1 and 2 years of age were Hb <105 g/l, SF <12 µg/l and MCV <74 fl, and SF and MCV, with the same cut-off points, were used to identify Fe deficiency. If children had an SF of <12 µg/l they were diagnosed as having depleted Fe stores. The cut-off value for Hb of 105 g/l has been used and considered to be appropriate for infants (Siimes *et al.* 1984; Michaelsen *et al.* 1995), as well as the cut-off values for MCV of 74 fl for infants and small children (Hercberg *et al.* 1987; Fuchs *et al.* 1993; Gill *et al.* 1997). The cut-off value for SF of 12 µg/l is in accordance with WHO criteria (International Nutritional Anemia Consultative Group/World Health Organization/UNICEF, 1998; World Health Organization, 2001). At 6 years of age the cut-off points used for Fe-deficiency anaemia were Hb <115 g/l, SF <15 µg/l and MCV <76 fl, and SF and MCV, with same cut-off points, were used to identify Fe deficiency. Depleted Fe stores were diagnosed as SF of <15 µg/l. The cut-off values for Hb of 115 g/l and for SF of 15 µg/l are in accordance with World Health Organization (2001) criteria for the age-group 5–11 years in the case of Hb and for >5 years of age in the case of SF. The cut-off value for MCV of 76 fl is in accordance with the criteria used in the National Health and Nutrition Examination Survey II for 5–10-year-olds (Expert Scientific Working Group, 1985).

### The Icelandic Developmental Inventory

At 6 years of age the Icelandic Developmental Inventory was completed by the mothers of seventy-seven participants from cohort 1, but as there were only thirty-six participants from cohort 2 no results are presented.

The Icelandic Developmental Inventory evaluates the motor and verbal development of children aged 3–6 years by collecting information from their mothers. The mothers check a list of 208 standardized questions about their child's abilities, giving scores on six subtests: (1) gross motor; (2) fine motor; (3) self help; (4) comprehension; (5) expression; (6) learning. The development and standardization of the inventory has been described previously (Thorsdottir *et al.* 2005) and has been proven to be reliable and to give valid information comparable with other tests (Gudmundsson & Gretarsson, 1993, 1994).

### Socio-demographic factors

The study of infants provided information about the parents' age, education, occupation and smoking status, the family income and the number of the child's siblings. Three levels of education were used: secondary school (<11 years); gymnasium (13–14 years); university level (>15 years). The parent's educational level was measured as the highest level completed. Mothers were divided into three categories in relation to their smoking status: mothers who did not smoke; mothers who smoked, but not during pregnancy; mothers who smoked, including throughout the period of pregnancy and lactation. Family income was measured as the monthly income of the family and divided into five groups. The number of other children was divided into two groups: mothers who had previously not had a child or had one child; mothers who had two or more children before giving birth to the child in the present study.

### Calculations and statistical analyses

Statistical analyses were performed using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Data are presented as means, standard deviations and percentiles. Student's *t* test, Mann-Whitney U test and Fisher's exact test were used to identify differences between two groups, and Kruskal Wallis test or ANOVA test was used for three or more groups. Spearman's rho correlations ( $r_{sp}$ ) as well as stepwise linear multiple regression analyses were performed to evaluate linear associations between food and nutrient intakes, as well as other parameters, and Fe status indices. As the distribution was skewed, SF values were logarithmically transformed. The level of significance was taken as  $P < 0.05$ .

## Results

### Iron status, diet and growth

Table 1 shows Fe status indices at age 12 months, 2 years and 6 years and the number of children below (or above) the respective cut-off values. Table 2 shows the frequency of depleted Fe stores, Fe deficiency and anaemia for the participating children at different ages, indicating that the prevalence of Fe deficiency at 12 months was high and

**Table 1.** Iron status indices at the ages of 12 months, 2 years and 6 years for cohorts of Icelandic children\* (Values are means and standard deviations)

	Mean	SD	Fe index < or > cut-off value†		n
			No.	%	
<b>At 12 months</b>					
Hb (g/l)	115.1	7.7	10	8.8	114
SF (µg/l)	17.3	11.3	46	41.4	111
MCV (fl)	76.4	4.2	33	28.9	114
TfR (mg/l)	7.3	2.1	26	25.5	102
<b>At 2 years</b>					
Hb (g/l)	121.8	8.5	2	2.9	70
SF (µg/l)	17.6	9.8	19	27.5	69
MCV (fl)	78.5	4.8	6	8.5	71
RDW	14.0	1.3	2	2.9	70
<b>At 6 years</b>					
Hb (g/l)	128.8	8.6	2	1.4	136
SF (µg/l)	24.8	11.9	22	16.1	137
MCV (fl)	82.4	3.1	3	2.2	136

SF, serum ferritin; MCV, mean corpuscular volume; TfR, serum transferrin receptors; RDW, erythrocyte distribution width.

\*For details of subjects and procedures, see pp. 367–368.

†Cut-off values: for 1- and 2-year-old children: Hb <105 g/l, MCV <74 fl, SF <12 µg/l, TfR >8.5 mg/l, RDW >18%; for 6-year-old children: Hb <115 g/l, MCV <76 fl, SF <15 µg/l.

that Fe deficiency was less common at the age of 2 and at 6 years.

It has been reported previously (Thorsdottir *et al.* 2003) that the Fe-deficient infants had a shorter duration of breast-feeding (5.5 (SD 2.3) months) than the non-Fe-deficient children (7.9 (SD 3.2) months;  $P=0.001$ ). Furthermore, regression analyses, adjusted for gender and duration of cow's milk feeding, have shown associations between log SF and the duration of both exclusive (B 0.04 (SE 0.02); adjusted  $R^2$  0.05;  $P=0.011$ ;  $n$  105) and total breast-feeding (B 0.03 (SE 0.01); adjusted  $R^2$  0.14;  $P=0.027$ ;  $n$  105), and between serum transferrin receptors:log SF and duration of total breast-feeding (B -0.44 (SE 0.15); adjusted  $R^2$  0.07;  $P=0.005$ ;  $n$  94), indicating a better Fe status with longer duration of breast-feeding.

The strongest food factor associated with Fe status at 12 months and 2 years of age was found to be cow's milk (Thorsdottir *et al.* 2003; Gunnarsson *et al.* 2004). Fe status indices at 1 year of age were negatively associated with cow's milk consumption at 9–12 months, and were significant at a consumption of >460 g/d ( $P<0.05$ ; see also Thorsdottir *et al.* 2003). All Fe status indices, except Hb, differed significantly between the children receiving <500 ml cow's milk daily and those receiving >500 ml cow's milk daily (Table 3). The effect of cow's milk was not seen at the age of 6 years.

Multiple regression analyses of Fe status indices and the intake of selected food items revealed that the consumption of cow's milk was almost universally negatively associated with Fe status indices at both 1 and 2 years of age, but not at 6 years of age (Table 4) (Gunnarsson *et al.* 2006a). At 6 years of age a partial correlation of borderline significance was observed between log SF and cow's milk

**Table 2.** Frequency (%) of depleted iron stores, iron deficiency and iron-deficiency anaemia for 1-, 2- and 6-year-old Icelandic children participating in the study\*

Age (years)	Fe depleted	Fe deficient	Fe-deficiency anaemia
1	41	20	2.7
2	20	9	1.1
6	16	0.7	0

\*For details of subjects and procedures, see pp. 367–368.

**Table 3.** Mean iron status indices at 1 and 2 years of age in Icelandic children consuming either >500 g cow's milk/d or <500 g cow's milk/d at 9–12 months and 2 years respectively\*

	Cow's milk <500 g/d		Cow's milk >500 g/d		Statistical significance of difference between groups: $P$
	Mean	$n$	Mean	$n$	
<b>At 1 year</b>					
Hb (g/l)	114.8	80	115.8	17	NS
SF (µg/l)	20.1	78	9.7	16	0.001
MCV (fl)	77.3	80	73.1	17	0.001
<b>At 2 years</b>					
Hb (g/l)	122.2	58	117.3	11	NS
SF (µg/l)	18.7	58	11.2	10	0.024
MCV (fl)	79.6	59	72.5	11	0.010

SF, serum ferritin; MCV, mean corpuscular volume.

\*For details of subjects and procedures, see pp. 367–368.

products (apart from cow's milk, butter and cheese;  $r$  -0.169;  $P=0.058$ ). At 1 and 6 years of age the consumption of meat and fish emerged as a positive predictor for log SF, while juice consumption was associated with Hb at both ages, negatively at 1 year and positively at 6 years. At 6 years of age juice consumption and vitamin C intake were highly correlated ( $r_{sp}$  0.797;  $P<0.001$ ), while at 12 months of age the correlation was lower ( $r_{sp}$  0.343;  $P=0.001$ ), indicating the consumption of juices with a lower amount of vitamin C at 12 months than at 6 years. At both 1 and 2 years of age biscuits and crackers were positively associated with Fe status indices. At 6 years of age no associations were observed between MCV and the food items selected for analysis. In general, the association between the total intake of single nutrients and Fe status was similar to that for the food items containing those nutrients, e.g. a negative association between Ca and Fe status indices at 1 and 2 years of age. Breast-feeding was also related to slower growth, during the first year in particular, which seemed to protect from worsening Fe status. The effects of growth and body size on Fe status have been thoroughly described (Thorsdottir *et al.* 2003; Gunnarsson *et al.* 2004, 2005). In general, faster growth from birth to 12 months and 2 years of age seems to indicate a worse Fe status at that age, but Fe deficiency at 12 months and 2 years of age is associated with slower growth up to 6 years of age.

The effect of supplement intake was tested for 6-year-olds. The associations between multivitamin-mineral supplement intake (containing Fe) and Fe status was at best

**Table 4.** Multiple regression analyses of food factors influencing iron status indices of cohorts of Icelandic children at 1 year (consumed at 12 months and the average for 9 and 12 months), 2 years and 6 years\*

Dependent variable	Independent variable†	B	SE	P	Adjusted R <sup>2</sup>	n
At 12 months	At 9–12 months					
Log SF	Cow's milk	-0.078	0.014	0.000		
	Fe-fortified breakfast cereals	0.010	0.004	0.009		
	Fish products	0.007	0.003	0.009		
	Butter and cheese	-0.009	0.004	0.042	0.34	94
TfR	Cow's milk	0.326	0.113	0.005	0.08	85
MCV	Cow's milk	-0.812	0.199	0.000		
	Butter and cheese	-0.189	0.061	0.002	0.21	97
TfR : log SF	Cow's milk	1.135	0.201	0.000	0.31	83
Hb	Porridges	0.102	0.042	0.017		
	Butter and cheese	-0.285	0.124	0.024	0.10	97
At 12 months	At 12 months					
Log SF	Cow's milk	-0.035	0.014	0.012		
	Meat products	0.004	0.001	0.009		
	Biscuits and crackers	0.009	0.004	0.034	0.18	99
TfR	Cow's milk	0.397	0.102	0.000		
	Bread	0.027	0.011	0.013	0.16	90
MCV	Cow's milk	-0.468	0.189	0.015		
	Juices	-0.891	0.359	0.015	0.09	102
TfR : log SF	Cow's milk	0.988	0.190	0.000		
	Juices	0.863	0.334	0.011	0.26	88
Hb	Juices	-1.996	0.673	0.004		
	Butter and cheese	-0.233	0.086	0.008	0.13	102
At 2 years	At 2 years					
Hb	Cow's milk	-1.369	0.536	0.013		
	Cow's milk products	-0.025	0.012	0.045	0.11	69
Log SF	Cow's milk	-0.042	0.017	0.015	0.07	68
MCV	Cow's milk	-1.145	0.306	<0.001		
	Biscuits and crackers	0.133	0.055	0.018	0.20	70
RDW	Cow's milk	0.241	0.089	0.008	0.09	69
At 6 years	At 6 years					
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

SF, serum ferritin; MCV, mean corpuscular volume; TfR, serum transferrin receptors; RDW, erythrocyte distribution width.

\*For details of subjects and procedures, see pp. 367–368.

†The independent variables used in the multiple regression analysis were as follows: breast milk (only in the infant study); cow's milk; other cow's milk products apart from butter and cheese; porridges (only in the infant study); meat products; fish products (meat and fish products combined in studies of 2- and 6-year-olds); fruits; vegetables (fruits and vegetables were combined in the infant study); bread, biscuits and crackers; cakes (only in the infant study); Fe-fortified breakfast cereals, butter and cheese (separated in the study of 6-year-olds); juices (with and without added sugar). The intake quantities used for cow's milk and juices were 100 g/d, but g/d for other food items. Only the variables reaching statistical significance are given in the table. Variables are adjusted for gender.

marginal (SF ( $\mu\text{g/l}$ ): mean non-consumers ( $n$  108) 23.3 (SD 10.2); supplement consumers ( $n$  21) 30.0 (SD 16.1);  $P = 0.070$ ). None of the twenty-one children with depleted Fe stores consumed multivitamin–mineral supplements compared with twenty-one of the 108 non-Fe-depleted children ( $P = 0.024$ ). The intake of supplemental vitamin C and Fe was not associated with Fe status.

#### Early iron deficiency and development

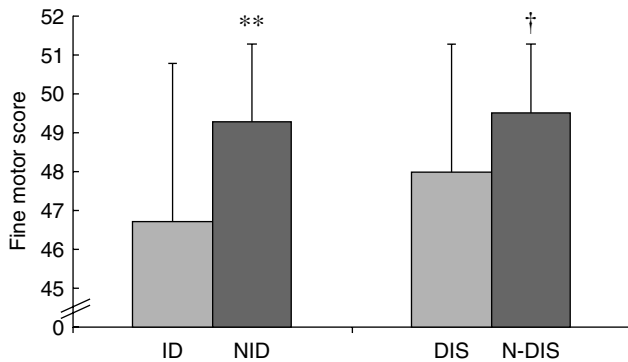
Children who were Fe-deficient at 1 year of age ( $n$  10) had lower fine motor scores at 6 years of age (46.7 (SD 4.1)) than non-Fe-deficient children (49.3 (SD 2.0);  $n$  56;  $P = 0.011$ ). Children with depleted Fe stores at 1 year of age ( $n$  26) also had lower fine motor scores (48.0 (SD 3.3)) than their non-Fe-depleted peers (49.5 (SD 1.8);  $n$  40;  $P = 0.045$ ; see Fig. 2) (Gunnarsson *et al.* 2006b).

Fine motor score was marginally negatively correlated with the level of serum transferrin receptors ( $r_{sp} -0.21$ ;  $P = 0.071$ ;  $n$  63), but significantly correlated with serum transferrin receptors:log SF ( $r_{sp} -0.28$ ;  $P = 0.032$ ;  $n$  61).

Stepwise linear multiple regression analyses, including Fe status at 1 or 2 years and 6 years, showed that Hb at 6 years was positively associated with the gross motor score (adjusted  $R^2$  0.05;  $P = 0.019$ ;  $n$  96), while Hb at 1 and 2 years was positively associated with the expression score along with gender (boys > girls; adjusted  $R^2$  0.11;  $P = 0.037$ ;  $n$  96).

#### Diet and socio-demographic status

Multiple regression analysis that included socio-demographic factors showed that longer exclusive breast-feeding is associated with more education ( $P = 0.022$ ) and non-smoking ( $P = 0.013$ ), and that longer total breast-feeding



**Fig. 2.** Fine motor scores at 6 years of age in a cohort of Icelandic children who at the age of 1 year were iron-deficient (ID) or non-iron-deficient (NID), or who had depleted iron stores (DIS) or non-depleted iron stores (N-DIS). For details of the study population, the evaluation of motor development and the study design, see p. 367, p. 368 and Fig. 1 respectively. Values are means and standard deviations represented by vertical bars. Mean value was significantly different from that for the ID group: \*\*  $P = 0.011$ . Mean value was significantly different from that for the DIS group: †  $P = 0.045$ .

(exclusive+partial) is associated with non-smoking ( $P = 0.004$ ), which explains 22 and 19% of the variance respectively. Mothers with the least education (<11 years;  $n = 25$ ) breast-fed exclusively for an average of 2.2 (SD 1.6) months, while mothers with a medium level of education (13–14 years;  $n = 27$ ) breast-fed for 3.4 (SD 1.7) months and mothers with a high level of education (>15 years;  $n = 44$ ) for 3.4 (SD 1.6) months (the least-educated group was significantly different from the other two groups;  $P = 0.023$  and  $P = 0.014$  respectively). The duration of total breast-feeding was shorter among mothers who smoked (3.6 (SD 3.6) months;  $n = 8$ ) than among mothers who did not smoke (7.6 (SD 3.1) months;  $n = 80$ ;  $P = 0.003$ ), and also among mothers who smoked but not during pregnancy (9.0 (SD 3.0) months;  $n = 8$ ;  $P = 0.003$ ).

When examining the intake of certain dietary factors at 9 and 12 months of age, linear multiple regression analysis showed that smoking and the age of the mother were significantly associated with added sugar intake when controlling for all the other socio-demographic factors. The intake of added sugar among children of mothers with the least education (15 (SD 9) g;  $n = 20$ ) was higher than that among children of mothers in the two higher education classes combined (11 (SD 6) g;  $n = 67$ ;  $P = 0.018$ ). Also, a difference was seen in the intake of added sugar according to the mothers' smoking habits ( $P < 0.001$ ); children of mothers who smoked ( $n = 5$ ) consumed more added sugar (23 (SD 8) g) than children of mothers who smoked, but not during pregnancy (6 (SD 6) g;  $n = 7$ ;  $P < 0.001$ ) and non-smoking mothers (12 (SD 6) g;  $n = 75$ ;  $P = 0.001$ ).

Multiple regression analyses showed that when adjusted for all the other socio-demographic factors the education of the mother was associated with fruit intake and mothers' smoking habits were associated with vegetable intake. Socio-demographic factors explained 11% of the variance for fruit intake and 13% of the variance for vegetable intake. The intake of fruit was lower among the children of mothers with the least education (65 (SD 50) g;  $n = 20$ )

than among children of mothers with a high level of education (104 (SD 57) g;  $n = 43$ ;  $P = 0.021$ ). The fruit consumption of children whose mothers had a medium level of education (91 (SD 49) g;  $n = 24$ ) was not different from that for the other groups. The vegetable intake of children of mothers who smoked but not during pregnancy ( $n = 7$ ) was lower (8 (SD 7) g) than that of children whose mothers did not smoke (46 (SD 32) g;  $n = 75$ ;  $P = 0.008$ ). Children of mothers who smoked (35 (SD 42) g;  $n = 5$ ) were not significantly different from the other two groups in their intake of vegetables.

## Discussion

In a generally well-nourished population with high birth weight and a high frequency of breast-feeding, but in which cow's milk has been commonly used between 6 and 12 months of age, 20% of 12-month-old children and 9% of 2-year-old children were found to be Fe-deficient. The Fe status improved from 12 months of age to 6 years of age. The percentage of children with depleted Fe stores at 1, 2 and 6 years of age were 41, 28 and 16 respectively. Three children at the age of 1 year (2.7%), one child at 2 years and none at 6 years had Fe-deficiency anaemia.

In comparison with other studies, Fe status seems to be lower in Icelandic 1-year-olds than in children from neighbouring countries. In a Swedish study 26% of 12-month-old infants were defined as having depleted Fe stores (SF <12 µg/l; Persson *et al.* 1998). In Danish 9-month-old infants the average Hb and MCV values reported are similar to those found in the present study, but none of the Danish infants was observed to have Fe-deficiency anaemia, and mean SF levels (37 µg/l; Michaelsen *et al.* 1995) are higher than those for the Icelandic 1-year-olds (17 µg/l). The Icelandic 2-year-olds had a slightly lower Fe status than that observed in other studies of children in that age-group from other Nordic countries (Fagerli *et al.* 1996; Bramhagen & Axelsson, 1999), but a similar Fe status to that found in the UK National Diet and Nutrition Survey of children aged 1.5–2.5 years (Thane *et al.* 2000). On the other hand, for 2-year-old Irish children (Freeman *et al.* 1998) the values reported for Hb and erythrocyte distribution width are similar to those in the present study, although their mean SF values are lower, and about 50% were found to have an SF of <10 µg/l. Although Fe status has been shown to be much better in the 6-year-olds than in the younger children (Gunnarsson *et al.* 2005), Fe status indices show tracking, mainly in MCV but also in SF and Hb, demonstrating for the first time tracking of Fe status from 1 to 6 years of age. Tracking has been observed in SF between 6 and 9 months in a study of Danish infants (Michaelsen *et al.* 1995), and in a cohort of Irish children earlier Fe status was found to be a predictor for Fe status at 36 months of age (Freeman *et al.* 1998).

### Dietary factors

Total breast-feeding duration was positively associated with Fe status. One possible explanation for this finding is that children who are breast-fed longer grow slower

(Atladdottir & Thorsdottir, 2000), and slower growth induces better Fe status at the age of 12 months (Michaelsen *et al.* 1995; Thorsdottir *et al.* 2003). Additionally, breast-fed infants receive less cow's milk. The positive effects of breast-feeding on Fe status in the present study do not reflect the results of several other studies (Pizarro *et al.* 1991; Oti-Boateng *et al.* 1998). However, the negative effect on Fe status in the study of Pizarro *et al.* (1991) was observed when breast milk was the only milk provided for  $\geq 9$  months; in the present study the median duration of total breast-feeding was 8 months and that of exclusive breast-feeding was shorter.

Cow's milk consumption was the dietary factor most strongly associated (negatively) with Fe status indices in both the infant study and the study of 2-year-olds. A negative association between Fe status and cow's milk consumption has also been reported in many other studies of infants and young children (Michaelsen *et al.* 1995; Gill *et al.* 1997; Freeman *et al.* 1998; Lawson *et al.* 1998; Bramhagen & Axelsson, 1999; Thane *et al.* 2000). This strong association has led to the widespread recommendation that cow's milk should be avoided as the infant's main drink until the age of 12 months; many of the Fe-containing follow-on formulas are recommended by the manufacturer for use until the age of 2 years. The results have also led to a change in the Icelandic recommendation, and the effect of this change is now being investigated in about 300 infants. That study will be completed in 2007.

In both the infant study and the study of 2-year-olds the effect of cow's milk on Fe status was observed in those consuming  $>500$  g/d. Ca has been shown to inhibit Fe absorption in single-meal studies (Cook *et al.* 1991; Hallberg *et al.* 1991), but whether the effect of Ca on Fe absorption persists in a complete diet has been questioned (Reddy & Cook, 1997). In the present study Fe status indices were not found to be as strongly associated with Ca intake as with cow's milk, which might indicate that some other factors in cow's milk play a contributing role in affecting Fe status. High consumption of cow's milk might displace foods from other food groups in the diet, which has been indicated with cow's milk consumption  $>400$  g/d in the UK National Diet and Nutrition Survey (Thane *et al.* 2000). Gastrointestinal bleeding associated with early cow's milk intake, probably associated with cow's-milk proteins, has been mentioned as a possible cause of Fe deficiency in infants (Ziegler *et al.* 1990). Tunnessen & Oski (1987) have concluded that the main factor responsible for Fe inadequacy in infants receiving cow's milk from 6 months of age is the low content of Fe rather than gastrointestinal bleeding in infants caused by cow's milk proteins. Also, gastrointestinal bleeding diminishes in late infancy and is probably negligible at 12 months (Ziegler *et al.* 1999). Interestingly, no effect of cow's milk consumption on Fe status was seen at 6 years of age. Indeed, the percentage of the energy consumed in the form of cow's milk and cow's milk products was much less than that at 1 and 2 years of age (19 v. 36 and 27 at 1 and 2 years respectively), which might partly explain why the association with cow's milk at 6 years of age is not stronger.

Consumption of meat and fish was found to be positively associated with Fe status indices at 12 months and

6 years old, but not at 2 years old. Comparable results have been observed both in young (Michaelsen *et al.* 1995; Engelmann *et al.* 1998a; Thane *et al.* 2000) and older children (Thane *et al.* 2003). These foods are relatively rich in Fe and contain considerable amounts of the better-absorbed haem-Fe together with a 'meat-factor', probably cysteine-containing peptides, that enhances the absorption of non-haem-Fe (Engelmann *et al.* 1998b). However, Fe intake was not related to Fe status at 1, 2 or 6 years of age in the present study, which is similar to observations in some other studies (Duggan *et al.* 1991; Thane *et al.* 2000). Several reasons have been proposed for this lack of association, e.g. Fe absorption increases in individuals with low Fe stores and Fe bioavailability depends both on the form of Fe in foods and the presence of dietary enhancers or inhibitors of absorption in meals (Gibson, 1999). Juices were found to be positively associated with Hb at 6 years of age, but negatively associated with Hb and MCV at 12 months. The reason for the discrepancy observed 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 Fe absorption (Hallberg *et al.* 1987; Fairweather-Tait *et al.* 1995), and in the present studies was positively related to Fe status both in infancy and at 6 years of age. The explanation for this difference is the higher consumption of citrus juices containing more vitamin C at 6 years of age than at 12 months, which was indicated by a high correlation between juice and vitamin C at 6 years of age but not at 1 year.

Fe-fortified breakfast cereals were positively associated with SF, an effect that has not been shown in comparably young children elsewhere, although Fe-fortified infant cereals have been shown to improve Fe status (Walter, 1993). The negative effect of bread consumption on Fe status seen in the infant study has been reported elsewhere (Michaelsen *et al.* 1995). The association of biscuits and crackers with Fe status in 1- and 2-year-olds cannot easily be explained, since not only do the biscuits and crackers most popular with children in Iceland contain relatively small amounts of Fe, but they also contain more known inhibitors of Fe absorption than enhancers.

In 6-year-olds the intake of multivitamin-mineral supplements containing Fe was positively associated with SF, but multivitamin mixtures without Fe, usually containing only B-vitamins and vitamin C, were not significantly associated with Fe status indices. Relatively few children were consuming vitamin C supplements, and their Fe status indices were not significantly different from those of children not consuming supplemental vitamin C. Also, only six children took Fe supplements, and some of these children did so in conjunction with multivitamin-minerals, so it was difficult to establish whether Fe supplements as such had any impact, but there appeared to be a small tendency to a positive effect. Several studies have found a relationship between multivitamin-mineral supplement intake and Fe status in children in industrialized countries. Lawson *et al.* (1998) have found better Fe status in 2-year-old Indian children living in England who consume vitamin supplements, and Australian preschool children receiving vitamin supplements tend to be less likely to be Fe-depleted (Karr *et al.* 1996). Also, the US National

Health and Nutrition Examination Survey II data indicate that vitamin–mineral supplement users in the age-groups 3–4 years and 5–10 years have higher mean Hb and SF levels respectively (Looker *et al.* 1987). Multivitamin supplements including minerals, or Fe-fortified cereals, may be of importance for those children at risk for Fe deficiency, but it does not seem to be of importance for healthy children to take supplements to prevent Fe deficiency. Varying the diet to include fish or meat as well as cow's milk, and including foods containing vitamin C may be more important.

#### *Early iron deficiency and development*

The possible long-lasting negative effect of Fe deficiency, especially when severe and accompanied by anaemia, has been widely documented (Palti *et al.* 1983; Dommergues *et al.* 1989; Lozoff *et al.* 1991, 2000; Hurtado *et al.* 1999). Only one study has shown complete reversal of test score differences after treatment in infancy (Idjradinata & Pollitt, 1993), but these results do not necessarily exclude possible differences in intellectual function in later life (Gordon, 2003). It is still uncertain whether the long-lasting developmental deficit in children with Fe-deficiency anaemia in infancy and early childhood is a result of Fe deficiency or possibly some confounding factors. Most studies relating to this area have been conducted in populations that are relatively deprived socially and economically, where there are many confounding environmental factors (for review, see Grantham-McGregor & Ani, 2001). The present study was conducted in an environment in which many of these confounding factors were absent. Data from cohort 1, the infant study, did not identify differences in Fe status indices at the age of 1 year according to socio-demographic variables (Gunnarsson *et al.* 2006b). The current findings are different from those of most other studies, which in general have reported anaemia to be associated with developmental deficit. One of the few exceptions is the study by Oski *et al.* (1983), which found Fe-deficient children (defined as Hb >110 g/l; SF <12 µg/l; erythrocyte protoporphyrin >0.3 mg/l) to have lower developmental scores than non-Fe-deficient children, but the scores were corrected after 1 week of treatment. Similar findings have been reported in a short-term study by Walter *et al.* (1983).

The present results show that early Fe deficiency is mainly associated with less development in motor ability. Long-lasting motor differences have been related to delayed myelination, although the mechanisms behind this association are not understood (Lozoff *et al.* 2000). Lozoff *et al.* (1987) have also reported that a decline in motor development occurs in infants at a higher cut-off value for Hb (<105 g/l) than a decline in mental development (<100 g/l), possibly suggesting that motor development can be impaired with less-severe Fe deficiency than mental development.

#### *Infant diet and socio-demographic status*

The present findings from the infant study show that in mothers with similar healthcare support throughout pregnancy and infancy and with infants who are healthy at birth

adherence to recommendations relating to breast-feeding, as well as other infant feeding recommendations, is associated with mothers' education and smoking, with the age of mother also playing a part (Gudnadóttir *et al.* 2006). Similarly, the education of the mother also appears to influence the intake of added sugar, as has been observed elsewhere (van den Boom *et al.* 1995), and is the only significant factor associated with the fruit intake of 9–12-month-old infants. Similar associations with socio-demographic factors have been observed in other Nordic countries (Hornell *et al.* 1999; Lande *et al.* 2003).

These findings indicate the need for altered support to increase adherence to new recommendations in the case of mothers with the least education (secondary school or less), mothers who smoke throughout pregnancy and lactation, and mothers who are younger than 27 years. The same support may be needed for parents with the lowest income, as they appeared to show a similar tendency to low compliance with recommendations. It is important to offer these groups support and education to ensure better nutritional status of their children.

#### *Dietary quality and adequate micronutrient intake*

The present studies show that a child's micronutrient status is affected by diet, even the diet in the years before the measurements, as well as growth. This outcome underlines the importance of diet and growth in infancy for adequate nutrition. There are many micronutrients that need to be studied, and the recent focus on vitamin D will probably further increase the interest in *n*-3 fatty acids and marine food. A varied diet is of major importance for preschool and older children to ensure adequacy of micronutrients, in addition to breast-feeding in the first months; exclusive breast-feeding is recommended up to 6 months of age and further breast-feeding as part of a healthy diet as long as it suits the mother and the child (Alexander *et al.* 2004). In relation to the Fe status of Icelandic children, it is important to monitor the effects of the altered recommendations. Furthermore, it is of high importance for the micronutrient status of the children to investigate how a high fish intake can be re-introduced into the Icelandic diet to ensure the previous high intake of vitamin D, marine fatty acids and I. Recent studies in Europe, the ProChildren Project, have also shown that Icelandic 11-year-olds have the lowest fruit and vegetable intake among this age-group in Europe (Klepp *et al.* 2005; Yngve *et al.* 2005). These studies also suggest approaches, through increased availability, self-efficacy and other social factors, that can be used to encourage increased intake of fruits and vegetables (Sandvik *et al.* 2005).

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