Haemoglobin and ferritin concentrations in children aged 12 and 18 months

Andrea Sherriff, Alan Emond, Neil Hawkins, Jean Golding, the ALSPAC Children in Focus Study Team

Abstract

***Aims*—To define the normal ranges and investigate associated factors for haemoglobin and ferritin in British children at 12 and 18 months of age, and to estimate correlations between both haemoglobin and ferritin concentrations at 8, 12, and 18 months of age.**

***Subjects and methods*—Subjects were part of the "children in focus" sample, randomly selected from the Avon longitudinal study of pregnancy and childhood. Capillary blood samples were taken from 940 children at 12 months and 827 children at 18 months of age.**

***Results*—Haemoglobin was distributed normally and ferritin was distributed log normally at 12 and 18 months of age. Ninety five per cent reference ranges were established from empirical centiles of haemoglobin and ferritin. Haemoglobin concentrations at 18 months were associated with sex and maternal education. Concentrations of ferritin at 12 and 18 months of age were associated with birth weight and current weight. Girls at 12 months, but not at 18 months, had 8% higher ferritin concentrations than boys. Haemoglobin and ferritin concentrations were significantly correlated over time (8–12 months: r_{Hb} = 0.26, r_{Fer} = 0.46; 12–18 months: r_{Hb} = 0.37, r_{Fer} = 0.34; 8–18 months: r_{Hb} = 0.22, r_{Fer} = 0.24).**

***Conclusion*—Iron stores are depleted by rapid growth in infancy. A definition of anaemia based on the fifth centile gives cut off points at 12 and 18 months of age of haemoglobin < 100 g/l, and for iron deficiency of ferritin < 16 µg/l and < 12 µg/l, respectively. Because children below the fifth centile at one time point differ from those six months later, it is unclear whether screening would be effective.**

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Keywords: haemoglobin; ferritin; iron deficiency; anaemia

Unit of Paediatric and Perinatal Epidemiology, Institute of Child Health, University of Bristol, 22–24 Tyndall Avenue, Bristol BS8 1TQ, UK
A Sherriff
J Golding
ALSPAC Children in Focus Study Team

Bristol Royal Hospital for Children, St Michael's Hill, Bristol BS2 8BJ, UK
A Emond

AMGEN Ltd, 240 Cambridge Science Park, Milton Road, Cambridge CB4 4WD, UK
N Hawkins

Correspondence to:
Dr Sherriff.

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Iron deficiency in infants is a common problem worldwide, and in the UK it is particularly common in urban areas.[1] If left untreated it is thought to cause impairment of psychomotor development and alterations in behaviour.[2–5]

Currently, the World Health Organisation (WHO)[6] recommends cut off points for the diagnosis of iron deficiency anaemia of less than 12 µg/l for serum ferritin and less than 110 g/l for haemoglobin. These guidelines, however, cover individuals from infancy to old age, and a variety of studies[7–9] have attempted to determine age specific norms for haemoglobin and serum ferritin concentrations. Few of them have been longitudinal or non-interventionist, hence their results might not be entirely representative of the general population.

Our paper follows on from that of Emond *et al*,[7] who reported haemoglobin and serum ferritin concentrations from a randomly selected sample of 8 month old infants within the geographically based Avon longitudinal study of pregnancy and childhood (ALSPAC).[10]

We aimed: (1) to establish reference centiles for haemoglobin and ferritin concentrations in children of 12 and 18 months of age; (2) to investigate factors associated with haemoglobin and ferritin concentrations; and (3) to estimate correlations between both haemoglobin and ferritin concentrations in children at 8, 12, and 18 months of age.

Materials and methods

STUDY SAMPLE

All births in the former Avon Health Authority area with an expected date of delivery between 1 April 1991 and 31 December 1992 were eligible for the ALSPAC study. Over 80% of the known births from the geographically defined catchment area were included, resulting in a total cohort of 14 138 surviving live births. From the population cohort, we invited a 10% randomly selected sample of parents whose babies were born within the last six months of the survey to bring their 12 and 18 month old children to a research clinic (children in focus), where a number of clinical, physiological, and psychological assessments were carried out. Of those invited, 1213 (88%) parents attended with their 12 month old children and 1151 (87%) parents attended when their children were 18 months old. The mean ages of the children attending the 12 and 18 month clinics were 54 weeks and 80 weeks, respectively. Permission was given by 940 and 827 of these parents for blood to be taken from their 12 and 18 month old children, respectively. Twins were excluded from this analysis.

BLOOD ASSAYS

A heel prick sample of capillary blood was taken from the children and collected into an EDTA capillary tube. The haemoglobin concentration was assayed using the HEMOCUE B-Hb photometer. The blood was then centrifuged and the plasma removed and frozen. Plasma ferritin was assayed using the DELFIA

Table 1 Haemoglobin centiles (g/l) in 8, 12, and 18 month old children

	All children			Boys			Girls		
Centile	8 months	12 months	18 months	8 months	12 months	18 months	8 months	12 months	18 months
n	1053	900	796	582	498	435	471	402	361
5th	97	100	102	97	99	101	99	101	102
10th	102	105	106	101	104	106	103	106	106
25th	110	112	111	109	111	111	111	112	112
50th	117	118	117	117	118	117	118	118	118
75th	124	124	122	124	124	122	125	125	123
90th	131	130	128	132	130	127	131	131	128
95th	136	134	130	136	133	129	135	135	132
Mean (SD)	116.9 (11.4)	117.5 (10.4)	116.9 (9.5)	116.7 (11.7)	117.3 (10.4)	116.3 (9.6)	117.3 (10.9)	117.9 (10.5)	117.6(9.5)

time resolved fluoroimmunoassay system. If the samples clotted they were discarded. Sufficient blood was obtained to measure the haemoglobin concentration for 900 and 796 children at 12 and 18 months of age, respectively. Sufficient plasma was obtained to assay ferritin for 734 and 718 children at 12 and 18 months of age, respectively.

The quality assurance scheme operating within the laboratory and the measures taken to test the stability of the samples were reported previously by Emond *et al*,[7] when the 8 month blood samples were analysed.

Ethical approval for the study was obtained from the ALSPAC ethics committee, and the local ethics committees of United Bristol, Southmead, and Frenchay Health Care Trusts. Following their advice, the results of those children whose haemoglobin was found to be less than 90 g/l were given to the mothers, with a letter to hand to their general practitioner suggesting further investigation.

STATISTICAL ANALYSES

All data were analysed within the SAS statistical software package (SAS Institute, Cary, North Carolina, USA). Visual inspection of the histograms of haemoglobin concentrations within the sample of 12 and 18 month old children participating in this study suggested that the data were normally distributed; however, ferritin concentrations were skewed and so were appropriately transformed (natural logarithm). Normality was confirmed using the Kolmogorov-Smirnov statistic, with a Lilliefors significance level. Pearson's correlation coefficients were calculated to determine the extent of association between continuous variables. Generalised linear models using type III sums of squares were constructed to investigate factors associated with haemoglobin and ferritin concentrations in 12 and 18 month old children.

Growth velocity was expressed as the difference in weight of the child between two time points over the time between measurements. The change in ferritin concentrations was calculated as the difference in ferritin concentrations between two time points over the time between measurements.

Results

Data gathered from infants at 8 months of age are included in this paper for comparison with the data collected from children at 12 and 18 months of age. Analyses of the 8 month data have been reported in an earlier paper.[7]

HAEMOGLOBIN CONCENTRATIONS

Table 1 gives the centiles of haemoglobin ranked in ascending order for this cohort of children at 8, 12, and 18 months of age. It can be seen that median values remained fairly constant with age and between sexes, but the fifth and 10th centile cut off points rose with age and the 90th and 95th centile cut off points diminished, implying a narrowing of the distribution. Haemoglobin concentrations were most highly correlated between 12 and 18 month old children ($r = 0.371$; $p < 0.0001$; $n = 696$). The correlation was lower between 8 and 12 month old children ($r = 0.258$; $p < 0.0001$; $n = 785$) and between 8 and 18 month old children ($r = 0.217$; $p < 0.0001$; $n = 700$). Of those who attended all three clinics, 30 children had haemoglobin concentrations below the fifth centile at any one time. However, only two children had haemoglobin concentrations consistently below the fifth centile at all three clinic visits.

FERRITIN CONCENTRATIONS

Ferritin concentrations were measured when sufficient blood plasma remained after haemoglobin had been assayed. A total of 734 and 718 such samples were obtained from

Table 2 Ferritin centiles (μg/l) in 8, 12, and 18 month old children

	All children			Boys			Girls		
Centile	8 months	12 months	18 months	8 months	12 months	18 months	8 months	12 months	18 months
n	743	734	718	413	400	389	330	334	329
5th	16.8	16.2	12.3	15.9	16.0	12.1	20.1	17.3	12.8
10th	20.8	18.7	14.5	18.7	18.2	14.1	23.0	19.6	15.7
25th	26.5	23.8	19.6	25.3	23.2	18.8	28.9	25.6	20.5
50th	37.7	31.9	26.4	35.0	30.5	25.2	41.9	34.7	28.2
75th	54.1	44.2	38.6	49.3	41.0	37.6	60.8	46.1	39.9
90th	76.2	57.2	53.7	69.3	53.7	53.6	86.4	61.4	55.8
95th	99.3	69.5	66.1	90.3	59.8	59.8	105.9	75.4	67.9
Geometric mean	36.6	32.8	27.5	36.6	31.2	26.6	44.7	34.8	28.6

Table 3 Unadjusted means of haemoglobin and geometric means of ferritin concentrations in 12 and 18 month old children according to sex, ethnicity, infection status, and maternal education

	Haemoglobin (g/l)				*Ferritin (μg/l)*			
	12 months	*n*	*18 months*	*n*	*12 months*	*n*	*18 months*	*n*
Sex								
Male	11.73 (0.05)	498	11.63 (0.05)	435	31.19	400	26.58	389
Female	11.79 (0.05)	402	11.76 (0.05)	361	34.81	334	28.50	329
Ethnicity								
White	11.76 (0.04)	845	11.69 (0.04)	746	32.79	691	27.66	671
Non-white	11.72 (0.14)	22	11.79 (0.2)	18	34.81	16	26.84	16
Infection								
No	11.78 (0.04)	695	11.70 (0.04)	644	32.14	563	27.11	574
Yes	11.65 (0.07)	205	11.65 (0.08)	152	34.81	171	29.67	144
Maternal education								
CSE	11.85 (0.09)	118	11.71 (0.08)	104	34.81	98	27.66	93
Vocational	11.60 (0.11)	90	11.58 (0.13)	75	32.79	72	25.79	66
O level	11.68 (0.06)	320	11.60 (0.06)	278	31.82	267	28.79	245
A level	11.82 (0.07)	224	11.73 (0.06)	202	32.46	173	27.66	188
Degree	11.85 (0.09)	127	11.92 (0.1)	114	33.45	108	25.28	105

singletons at 12 and 18 months of age, respectively. The distribution of ferritin in both age groups is approximately log normal and the centiles are given in table 2. Both the median and the other centiles dropped with age, implying that iron stores were falling with age.

Log_e ferritin concentrations in the blood at 8, 12, and 18 months of age were correlated positively. Pearson's correlation coefficient for the association between log_e ferritin concentrations at 8 and 12 months old was $r = 0.463$ ($p < 0.0001$; n = 479); between 8 and 18 months old, $r = 0.241$ ($p < 0.0001$; n = 470); and between 12 and 18 months old, $r = 0.334$ ($p < 0.0001$; n = 518). Ferritin measures were available on 351 children at all three time points. Seventeen children had ferritin concentrations below the fifth centile at any one time point. However, only one child had ferritin concentrations consistently below the fifth centile on all three occasions.

FACTORS ASSOCIATED WITH HAEMOGLOBIN AND FERRITIN CONCENTRATIONS

Factors found to be associated with haemoglobin and log_e ferritin concentrations in the infants in this cohort at 8 months of age were investigated using multiple regression analyses. These comprised sex of the study child (boy or girl), birth weight (kg), weight on attendance at clinic (kg), and study mother's highest educational qualification (CSE, vocational, O level, A level or degree). In addition, as potential confounders, we included ethnicity of the child (white or non-white), and whether the child had a recent infection before, or during, their visit to the clinic (yes or no) because these factors are known to affect ferritin concentrations. Table 3 shows unadjusted (geometric) mean haemoglobin and ferritin concentrations for the above factors and table 4 gives adjusted regression coefficients from the multiple regression analyses.

Table 4 Multiple regression analyses of haemoglobin and log_e ferritin concentrations in 12 and 18 month old children

	Adjusted regression coefficient	*95% CI*	*p Value*
Haemoglobin at 18 months			
Intercept	11.99		
Sex (boy)	−0.141	−0.276 to −0.006	0.04
Maternal education			
CSE	−0.209	−0.462 to 0.045	0.028
Vocational	−0.350	−0.629 to −0.07	
O level	−0.324	−0.532 to −0.12	
A level	−0.191	−0.410 to 0.028	
Degree	1.0 (Reference)		
Log_e ferritin at 12 months			
Intercept	3.3		
Birth weight (kg)	0.22	0.15 to 0.29	< 0.001
12 month weight (kg)	−0.07	−0.1 to −0.03	< 0.001
Sex (boy)	−0.08	0.01 to 0.15	0.018
Log_e ferrtin at 18 months			
Intercept	3.3		
Birth weight (kg)	0.14	0.061 to 0.218	0.001
18 month weight (kg)	−0.04	−0.07 to −0.008	0.014
Infection (no)	−0.09	−0.183 to 0.006	0.066

HAEMOGLOBIN CONCENTRATIONS

At 12 months of age, none of the variables in table 3 contributed significantly to the variation in haemoglobin concentrations within the cohort. By 18 months of age, however, sex of the study child, and the study mother's highest educational qualification were significantly associated with haemoglobin concentrations. Boys had significantly lower haemoglobin concentrations than girls ($p = 0.04$; adjusted mean difference (95% confidence interval (CI)), 0.141 (0.004 to 0.319)). The effect of maternal education on haemoglobin concentrations of their children at 18 months of age appeared to follow a U shaped curve with the highest concentrations in children whose mothers attained a degree and the lowest concentrations in those children whose mothers gained a vocational qualification ($p = 0.028$).

FERRITIN CONCENTRATIONS

Log_e ferritin concentrations in infants at 12 months of age were associated with the sex of the study child, birth weight, and weight on attendance at the clinic. Girls had 8% higher ferritin concentrations than boys ($p = 0.018$). Birth weight was associated positively ($p < 0.001$), and weight on attendance at the clinic ($p < 0.001$) was associated inversely with log_e ferritin concentrations. Neither recent infection, ethnicity, or mother's highest educational qualifications were associated with log_e ferritin concentrations at 12 months of age.

At 18 months of age, the birth weight of the study child and the weight on attendance at the

Table 5 Percentages of 12 and 18 month old children within our sample identified as anaemic using five different criteria

	Haemoglobin only		*Ferritin only*		*Haemoglobin and ferritin*	
Criteria for IDA	*12 months*	*18 months*	*12 months*	*18 months*	*12 months*	*18 months*
Our study Hb < 100 g/l Fer < 16/12 μg/l	5	5	5	5	0.5	0.6
WHO[6] Hb < 110 g/l Fer < 12 μg/l	18	17.3	1.1	4.4	0.4	1.7
Inst Med[11] Hb < 110 g/l Fer < 10 μg/l	18	17.3	0.4	2.5	0.1	1.1
Milman[9] Hb < 105 g/l Fer < 10 μg/l	8	6.3	0.4	2.5	0.1	0.7
Freeman[10] Hb < 110 g/l Fer < 7 μg/l	18	17.3	0.3	0.3	0.1	0

IDA, iron deficiency anaemia; Inst Med, Institute of Medicine, USA.

clinic were significantly associated with $\log_e$ ferritin concentrations. Birth weight was associated positively, and weight on attendance at the 18 month clinic was associated inversely, with $\log_e$ ferritin concentrations. Children with reported recent infection at 18 months had 9% higher ferritin concentrations than those who had not reported a recent infection, but this difference was not significant ($p = 0.066$). There was no difference between boys and girls at this age, nor was there any effect of maternal education or ethnicity on ferritin concentrations.

Within the statistical model, when all else remains constant, an increase of 1 kg in birth weight (but no increase in clinic weight) would be associated with an increase in ferritin concentrations of 22% and 14% in 12 and 18 month old children, respectively. An increase of 1 kg in 12 and 18 month weight in children with the same birth weight would be associated with a decrease of 7% and 4% in ferritin concentrations at 12 and 18 months of age, respectively.

GROWTH VELOCITY AND DEPLETION OF FERRITIN CONCENTRATIONS

Using weights obtained at 8, 12, and 18 months, the growth velocities (g/week) were calculated for all children. The rates of ferritin depletion (μg/l/week) were calculated from 8, 12, and 18 month blood samples. In the period between 8 and 12 months of age, growth velocity correlated with the rate of change in ferritin concentrations ($r = -0.154$; $p = 0.004$). The more rapid the growth between 8 and 12 months of age, the more depleted were the iron stores. However, between 12 and 18 months of age, no significant relation was found, although a similar trend was noted ($r = -0.074$; $p = 0.168$).

Discussion

These data were derived from the "children in focus" study, a randomly selected 10% subsample of the geographically defined total population cohort enrolled in ALSPAC.[10] Using the 1991 national census data, the children in focus sample has been compared with UK national figures, and found to be very similar. The main difference affecting this particular research is that children from ethnic minority backgrounds are underrepresented in the children in focus sample compared with the UK. This is an important difference because other studies[11 12] have suggested that iron deficiency anaemia is more common in ethnic minority groups than in the white population of the UK. However, the small number of non-white children in our sample were not significantly different from the white children.

Because concentrations of haemoglobin within the samples of 12 and 18 month old children were approximately normally distributed, we have defined a one sided reference range from the fifth to 100th centile comprising 95% of the distribution from this healthy population. We defined the fifth centile, at ~ 100 g/l, as the cut off point for anaemia. However, ferritin was log normally distributed and, for iron deficiency, the fifth centiles—16 μg/l at 12 months and 12 μg/l at 18 months—were used as logical cut offs.

We have shown that between 12 and 18 months of age haemoglobin concentrations in children do not change considerably, although there was a marked change in ferritin concentrations with age. Therefore, if ferritin is going to be used for defining iron deficiency anaemia, it is important that age specific norms are used.

The most widely known guidelines for the detection of iron deficiency anaemia have been produced by the WHO.[6] In the USA, the Institute of Medicine suggests cut off points of 110 g/l for haemoglobin and 10 μg/l for ferritin, but these values are independent of age and do not take into account the differences in haemoglobin and ferritin concentrations from infancy to old age.[11] Several authors have suggested alternative cut off points for infants and young children—for example, Millman[9] and Freeman.[8] Using our data, a logical cut off would be the fifth centile values for haemoglobin (100 g/l) and ferritin (16 μg/l at 12 months and 12 μg/l at 18 months). Table 5 compares the percentage of children in our study who would be identified as having iron deficiency anaemia using our proposed criteria based on the fifth centile and four other recommended criteria. The comparison shown in table 5 suggests that a cut off of 100 g/l for haemoglobin might be more appropriate at this age than the threshold of 110 g/l recommended by WHO and others.[6] For ferritin, the comparison in table 5 suggests that the cut offs recommended by other authors might be too strict for a healthy population at this age. It is even more difficult to define appropriate cut offs using a combined haemoglobin and ferritin measure.

The lack of association between haemoglobin concentrations and birth weight or current weight was not surprising and was similar to what had been found at 8 months of age in the same cohort.[7] The sex difference in haemoglobin concentrations identified at 18 months of age, although significant, might be of little importance clinically owing to the small size of the effect. At 12 months of age, we could not show an association between haemoglobin concentrations and educational level of the mother, but there was a weak relation at 18 months when haemoglobin concentrations were found to be higher in those children whose mothers were educated to university level compared with those children whose mother's highest educational achievement was a vocational qualification. There was not a linear effect with other levels of maternal education, however, and this statistical difference might have arisen by chance.

The relation between other factors and ferritin concentrations were more complicated. The significant sex difference in mean log ferritin concentrations seen at 8 months of age was also found at 12 months but not at 18 months of age. In addition, similar to the findings at 8 months of age, the log ferritin concentrations in the children at 12 and 18 months of age were associated positively with the birth weight of the child, yet associated negatively with the weight of the child at 12 and 18 months of age, respectively. The most likely explanation for this finding is that ferritin stores are depleted by rapid growth in infancy, and that those infants most at risk of iron deficiency are infants with a low birth weight and low iron stores who show rapid catch up growth in the 1st year of life.

There is evidence from this and our previous paper that rapid growth in infancy leads to a depletion of iron stores, and it has been shown elsewhere that treatment with iron increases weight gain.[13] To explore this finding further, we examined the relation between growth rate in early infancy and ferritin depletion rates. Using unadjusted growth velocity from 8 to 12 months and from 12 to 18 months, we found a significant relation between rate of growth and rate of ferritin depletion only between 8 and 12 months. Hence, relatively large infants by 1 year of age, particularly those who have crossed up the centiles, are more likely to have depleted iron stores and be at risk of developing iron deficiency anaemia.

CONCLUSION

Our study showed that haemoglobin concentrations in the blood do not vary greatly between the ages of 8 and 18 months, and neither are they strongly dependent on sex, birth weight, or weight of the child when blood was taken. Caution is needed when using ferritin as an indicator of overall iron status in infants because ferritin concentrations change rapidly in the first 18 months of life. We have demonstrated that age, sex, and the growth process in early infancy are important determinants of ferritin concentrations in the blood, resulting in different children being iron deficient at different ages. Follow up of these children is needed to identify important outcomes, such as cognitive function, to determine whether it is appropriate to screen for iron deficiency, and at which age(s).

The Avon longitudinal study of pregnancy and childhood (ALSPAC) is part of the WHO initiated multicentre survey, the European longitudinal study of pregnancy and childhood (ELSPAC). The children in focus substudy is, however, unique to ALSPAC. Specific funds for this anaemia study have been contributed by the South and West Regional Health Authority, the Department of Health, Cow and Gate, and the Meat and Livestock Commission. We gratefully acknowledge help in planning and conducting this anaemia study from Dr J James, Prof A Oakhill, Mr D Oakes, Mr N Farron, and Ms L McGrath. We are extremely grateful to all our funders, to the midwives, and other health professionals who made the survey possible, but most of all to the parents and children who have taken part. Further information about the ALSPAC study can be obtained from Professor Golding.

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