Journal of Biomedical Investigation, 2008; 6(2):39-46

Original Article

Iron Indices in School children in Ceres District of the Western Cape South Africa.

Benson Ogboko, David Fisher and R. Swart

Department of Medical Biosciences, University of the Western Cape Bellville, Private Bag X17 7535, Bellville Cape Town South Africa. Human ecology and Dietetic department.

For Correspondence:

ABSTRACT

Iron status of children from communities in South Africa has been a subject of debate and the lack of single biochemical indicator makes it more challenging, hence this study was designed to assess the iron status of children in a Ceres district of the Western Cape in South Africa. For this study, 265 children were randomly recruited and blood sample collected from the participating children was used for the analysis of the following parameters; soluble transferrin receptors (sTfR), haemoglobin (Hb), mass cell volume (MCV) and ferritin (Fert).

The result showed that the mean serum iron level of 13.45 umol/l, mean serum ferritin level of 1.85mg/l, mean serum transferring receptor of 30.11 ug/l were recorded in >75% of the participating children. The Haemoglobin level of less than 12.5 g/l and mass cell volume of 83.10 ft were observed in >50%, of participating children. There were no gender differences for any of the iron measure. The haemoglobin level showed marginal iron deficiency while only serum iron was significantly correlated with the heamoglobin level. The serum transferrin receptors, ferritin and MCV did not indicate possible iron deficiency. The results of other factors such as socioeconomic status and demographic data did not show any significant interference either. The implication of the finding is discussed.

Key Words: Blood, iron, children, Hb, STR, (MCV), (Fert).

Introduction

Iron deficiency is the most common nutritional deficiency in Africa, Asia and most developing world; affecting as much as 66–80% of the world's population (1, 2) and is the leading nutritional cause of anemia (3). It is easily noticed in anemia and those particularly at risk include: children, pregnant women, women with heavy menstruation and people with mal-absorption problems.

Although over the years, iron status of children has improved according to data published by (National Health and Nutrition Examination Survey 111 or NHANES 111) and (NHANES 11) (4), despite these improvement, iron deficiency is still common in most countries.

Infants and young children are especially vulnerable to iron deficiency because of their rapid growth and increased physiologic demands for iron (3) in other to fulfill major body functions. Also, diets high in cereals and low in meat and fish products may cause iron deficiency due to poor dietary iron bioavailability. Studies also show that ascorbic acid increases iron absorption (5). Of particular significance is the fact that iron deficiency in children can adversely affect cognitive and psychomotor development during vulnerable periods such as the toddler years (6). Iron deficiency during childhood has multiple consequences like neurochemistry disorder; alteration of dopamine receptors (7) and decreased monoamine oxidize activities (8).

Dietary intake requirement, inflammation and most biochemical indicators are age-related and constitute a major factor influencing iron concentration in children (9). Iron deficiencies in children are generally estimated from studies

using healthy adults, but they differ in many ways from that of adults. In children, the most likely cause is an inadequate amount of iron in the diet, coupled with the extra requirement for iron because of growth, also children present particular problem as they are highly susceptible to diseases and require more nutrients than adults (9). Other features are non-hematological but are clinically important and are clearly defined in children than in adults. Presently, there is no single biochemical indicator available to reliably access iron inadequacy in children (10).

This study focused on determination of such parameters as serum ferritin, serum transferrin receptor, MCV, and Haemoglobin values as possible indices for assessment of iron status in the Western Cape.

Subjects

This paper presents the biochemical indicators of iron in school children in Ceres district. A total number of 265 grade one learners aged between 7-9 years in six primary schools in Ceres district of the Western Cape, South Africa, were involved in this survey. These schools serve communities that duly represent the diverse ethnic groupings as well as socio-economic groupings within the bigger South African community. Informed consent was received.

Exclusion and inclusive criteria

Children with CRP concentration greater than 10mg/l were excluded while those with CRP concentration less than 10mg/l were included. The CRP concentration was used to include participants as a sign of apparent healthy state and to exclude participants as a sign of inflammation and ill health. The 265 pupils met the criteria of ≥ abnormal iron measure.

Data collection

Sample and data collection took place during school hours at each school over a period of one week in 2003 and 2004 respectively. Samples were prepared for analyses within one week of sample collections according to the following methodology:

Blood analysis: 5mL of whole venous blood was collected in a zinc free heparinised tube[12]. This process was carried out by a trained community staff nurse. Through the process care was taken to avoid any health risk situation that will endanger the subject or the nurse through blood contamination, infections or psychological situation. All blood samples collected were adequately marked and labelled. The blood samples were then stored in coolers under ice bags at 0 to - 4 °C, for usually < 24hrs[13].

In the laboratory the whole sample was then centrifuged to separate the serum. The serum was then stored at –15°C for all analyses. Prior to each analysis the serum was then thawed once only.[14]. Iron reagent was used to measure the iron concentration by a timed-endpoint method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the FerroZine Iron Reagent. The SYNCHRON LX ^{R} i725 (15) system automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 8 parts reagent. The system monitors the change in absorbance at 560 nm. The change in absorbance is directly proportional to the concentration of iron in the sample and is used by the SYNCHRON LX (15, 16) system to calculate and express the iron concentration.

Serum ferritin measurement was based on radio-imunometric assay (Pathcare laboratory Ltd. Cape Town). Combined measurement of ferritin, soluble transferrin receptor, and C-reactive protein was performed by a sandwich enzyme-linked immunosorbent assay technique. The external standard for ferritin consisted of the IBS standard diluted to 19.2 µg/l ferritin. The coefficient of variation for within assay-variation was < 4.5% for all ELISA assays, with a between-batch coefficient of variation of 6.2–7.4%.

All infants had data for Hb (hemoglobin), MCV (mean cell volume). Values for transferrin

saturation, ferritin, TfR, and body iron were available for all infants. A total of 236 infants had complete data for the measures used to determine iron status in NHANES II or III (i.e., Hb, MCV, transferrin saturation, and ferritin) (13, 16). Missing data were due to technical problems, such as trouble obtaining sufficient blood, samples with CRPC conc. >10mg/L and ethical exceptions.
For CRP, within-batch variation was <3% and between batches, it was <5%.

Precautions: Blood specimen storage and stability
Tubes of blood were kept closed at all times in a vertical, stopper-up position. Serum was physically separated from contact with cells as soon as possible. A maximum limit of two hours from the time of collection is needed for the assay to expire if not used (17). Separated serum should not remain at 15-30°C longer than 8hrs. Serum assays should be completed within 8hrs and the separated samples should be stored at 2-8°C. If the assays are not completed within 24hrs, serum samples should be re-centrifuged and separated from precipitates before testing. Frozen samples should be thawed only once. Analysis deterioration may occur in samples that are repeatedly refrozen and thawed (17).

Ethical considerations
The Senate Research committee of the University of the Western Cape provided ethical approval for this study (SHD of 2004/6). The participation of learners was voluntary following informed consent by parents or guardians. The participants were free to terminate participation at their convenience. Confidentiality of the data collected and subsequent findings were assured by using only code numbers for each participant.

Statistical analysis
Statistical analyses were performed using SAS Version 8.12 (18). The theory of Oliver Jean Dun (1971) was used in comparing of test of quality of dependent correlation coefficient (Pearson Correlation Coefficients) between blood indexes, food, vegetables and water samples. The health of learners plays a major role in determining the response of learners to elements utilization, therefore, paired student t-tests were used to relate variability to physical factors as well as objective observational conditions.

Results:
The male: female ratio in this study is 1:1. The mean age was 7.73 ±0.60yrs. The average weight and height of the children are 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributors were 2 persons and that of income was R250–R999 per month (Table 1).

The mean value of the serum iron (umol/l) of the participating children was within the range of the standard reference but 20%, 75% and 5% of the children presented with serum iron value below, within and above the standard reference range. This showed no significant drop in serum iron P>0.05. See table 2.

Similarly, the mean soluble transferrin receptor (mg/l) and mean serum ferritin of the participating children were within the range of the standard reference. However, 24%, 75% and 1% of the children presented with values below, within and above the standard reference range in each case. This value did not show any significant drop in soluble transferrin value P>0.05). See table 2.

The mean haemoglobin (g/dl) was within the range of the standard reference but 5%, 95%, and none of the children presented with values below, within and above the standard reference range. There was no significant drop in haemoglobin P>0.05. See table 2.

The mean mass cell volume (ft) of the participating children was also within the range of the standard reference, however, 15% and 85% of the children presented with values below and within the standard reference range. There was no significant drop in MCV P>0.05. See table 2.

In comparing each biochemical indicator against serum iron using SAS (Pearson correlation

coefficient), there was a significant (P<0.01) correlation between serum iron and hemoglobin, as shown in Graph 1 with few out-layers. Removing all out-layers (Graph 2) a strong correlation could be seen, indicating marginal serum iron decrease in children investigated and the lack of consistence of serum iron decrease was also observed.

The ferritin model utilized in NHANES II (transferrin saturation, and ferritin) resulted in a considerably lower estimate of 25% iron deficiency for children aged 7 to 9yrs (11). Corresponding estimates of iron deficiency with anemia were high, respectively, using Hb < 13 g/dl, the cutoff in NHANES II and 111 (4). The approach recommended by a world health Organization (WHO) Expert Committee at ≤14yrs of age (NHANES 11) and the Sweden/Honduras study (NHANES 111), with more stringent cutoffs given for five iron status measures and Hb, yielded prevalence estimates of 5% for iron deficiency and >50% for iron deficiency anemia (graph 2). There are yet no established norms for TfR in infants, but the Sweden/Honduras study (NHANES 111) considered "abnormal" TfR > 11 mg/l. Only one infant in our study had a value above this cutoff. MCV showed a corresponding level of low iron deficiency. Graph 3 shows a detail description of iron source and potential iron deficiency and anemia complications.

Discussion:

The results have clearly showed a non prevalent of iron deficiency situation, with two or more biochemical indicators (indices) not showing abnormal levels of iron in serum as noticed in whole serum iron. This finding is in agreement with the theory that at least two biochemical indicators must be abnormal to ascertain iron deficiency[10]. Also that iron deficiency is the most common nutritional deficiency in the world, affecting as much as 66–80% of the world's population[19] and is the leading nutritional cause of anemia in the developing world[3]. Anemia been the third stage of iron deficiency may occur only when the total iron hemoglobin level is reduced below normal which is ≤13 for infants [10, 20].

It is contrary to most findings that showed high level of iron deficiency among disadvantage

Table 1: Socio-economic characteristics of participating children and family

		Age of participants (years)	Weight (kg)	Height (cm)	Family members contributing to household income	Family average wage
Phase One		Average in yrs	(Kg)	(Cm)	Income *	
1	November 2003	7.60	20.46	118.71	4.82	2.19
2	September 2004	7.84	22.48	118.62	4.12	1.84
1 & 2	2003/04	7.73 **SD** 0.60	21.93 **SD** 4.82	118.69 **SD** 7.23	4.73 **SD** 1.28	2.73 **SD** 1.24

Table 2: Blood and serum biochemical makers for iron

Parameters	Mean ± Sd	**Standard reference range for category of participants	Number of participants with values within, above and below the standard reference range			SEM
			Below (%)	Within (%)	Above (%)	
Serum iron (umol/l) (n=236)	13.45 ± 5.19	9.5 – 21.3	20	75	5	0.34
sTfR (mg/l) (n=235)	1.83 ± 0.67	0.8 – 2.3	24	75	1	0.04
Ferritin (ug/l) (n=235)	30.11 ± 15.52	20 - 100	24	75	1	1.01
HB (g/dl) (n=232)	12.53 ± 1.10	11.5 – 15 5	5	95	0	0.07
MCV (ft) (n=232)	83.10 ± 4.42	77 - 95	15	85	0	0.29

sTfR = Soluble transferrin receptor, Hb = Hemoglobin, MCV = Mass cell volume, **Ref Std (12)

children, although other factors might have been responsible but the appropriate cutoffs for iron deficiency in infant and children will remain controversial. Individual iron index levels showed a non-perfect distribution in comparison with the laboratory references, except in MCV and Hb where a strong relationship can be observed and statistical significance showed a strong correlation between samples mean and standard means, as observed in serum iron and hemoglobin, sTfR, and MCV.

One of the factors that is regularly overlooked when assessing iron status of children is the concentration of several nutrients such as zinc, vitamin A and iodine. Dietary intake requirement, inflammation and most biochemical indicators are age-related[6]. Although there were higher levels of sTfR and very low Ferritin and MCV observed in one of the samples, the results did not show exact relationship between both, but need further investigation especially when there are very high levels of sTfR which might result in lowering of both Ferritin and MCV. However, the proportion meeting criteria for iron deficiency was considered low (≤5%) and regular iron absorption and storage is not fully developed in infants therefore making it difficult to interpret measures connected to iron storage such as ferritin and sTfR and standards used for children results will tend to lack consistency.

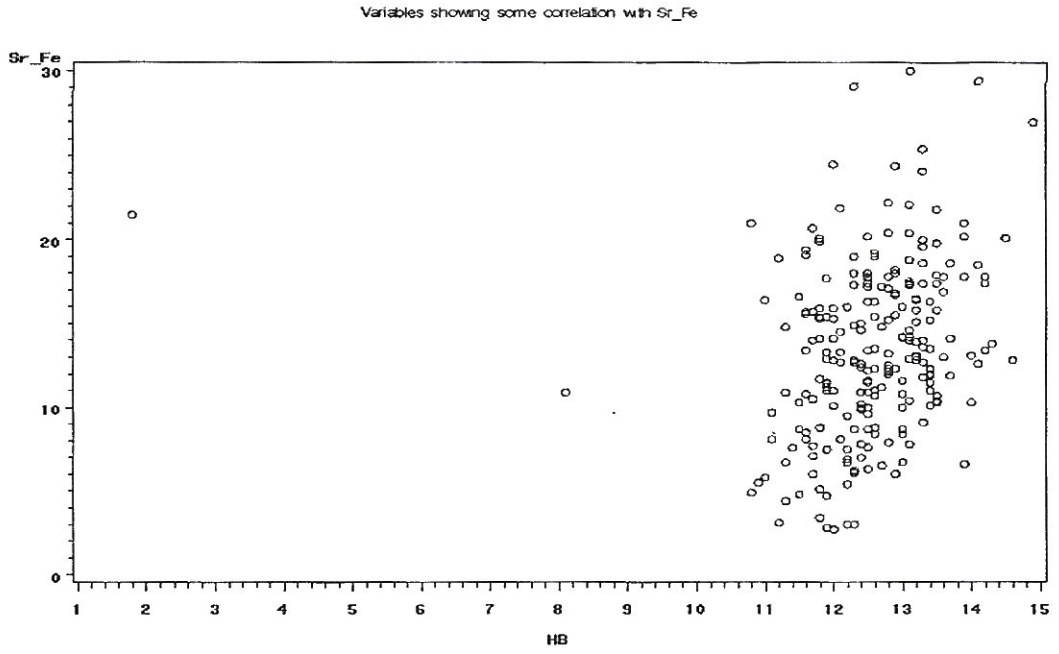
Other results showed no differences between male and female children on any individual iron measure or the proportion meeting criteria for iron deficiency, this result is a contrast to gender difference observed in some other studies [21,22,23]. Although the explanation for the differing finding is not readily available, iron food fortification might have played an important part.

Conclusions

Caution should be taken in drawing conclusion on the nutritional status (iron) of children, as

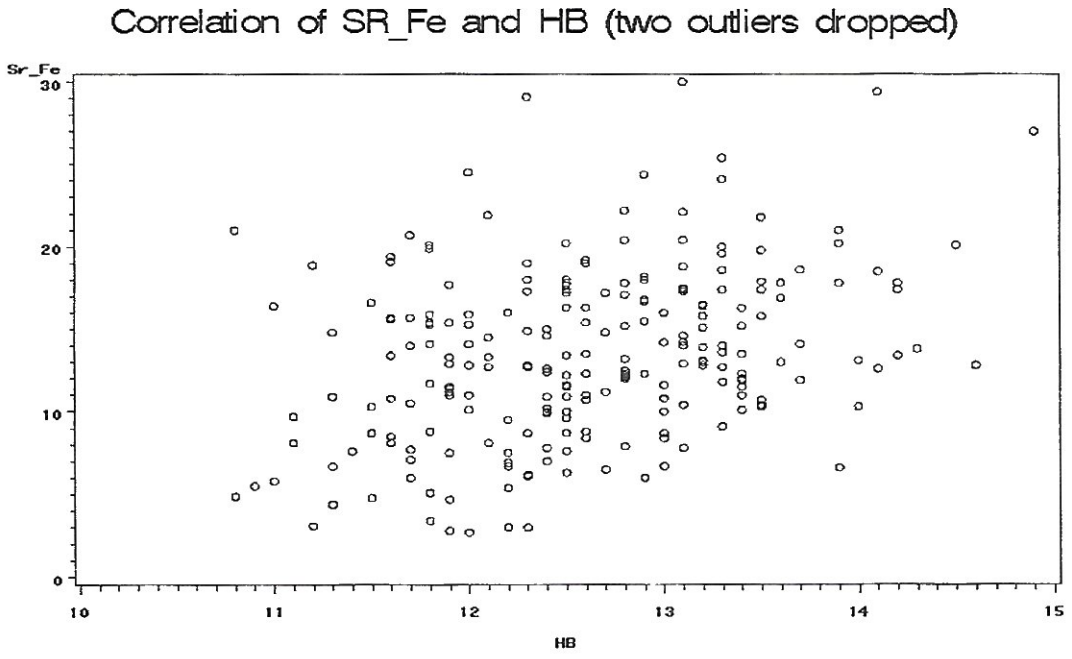
many factors can easily influence the low biochemical iron indicators. Children's immune systems are still developing and there are higher frequencies of sickness than in adults. Sub-clinical inflammation in apparently-healthy children, ethnic life style, and state of other nutrients can easily lead to misinterpretation of iron status and overestimation of those with deficiency.

Graph: 1



(11.5 ----------15.5g/dl)

Graph: 2



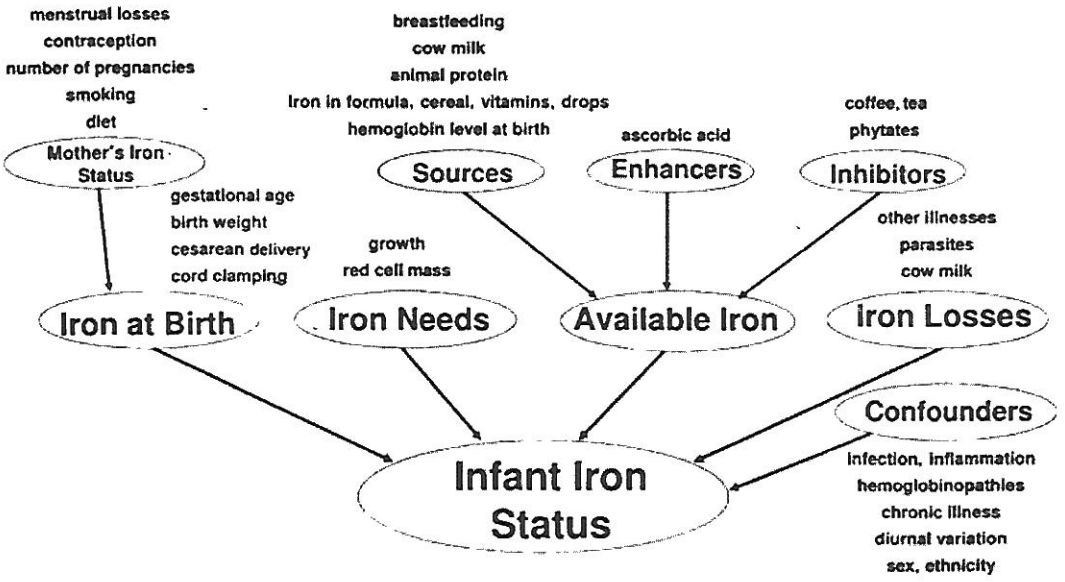
(11.5 ----------15.5g/dl)

These results had shown marginal iron deficiency in the children population and might have been influenced by non active infection (inflammation and worms). Hence, the need for more investigation on their involvements prior to treatment is very important as most direct intervention has not been effective. Iron deficiency can easily be corrected with supplementation and food rich in iron before it gets to a clinical deficiency state.

Acknowledgement
I acknowledge the following person for their immense contribution towards the successful completion of this paper; Prof. Rina Swart, Dr. David Fisher, staff and students of Human ecology and Dietetic department.

Graph 3.

PREDICTING IRON DEFICIENCY IN INFANTS



Estimated iron sources and deficiencies in infants (4)

REFERENCES

1. **UNICEF** In: *The progress of nations*, UNICEF, New York, p. 1998.
2. **Nwafia WC, Aneke JO, Okonji CU** Serum Iron and Total Iron Binding Capacity.
3. Levels among the ABO blood groups in Enugu, South Eastern Nigeria. Niger J. Physiol Sci. 2006 Jun-Dec; 21(1-2):9-14.
4. **Allen L. and Casterline-Sabel J.** Prevalence and causes of nutritional anemias. In: Ramakrishnan, U., Editor, 2001. *Nutritional anemia*, CRC Press, Boca Raton, pp. 7–21.
5. **Betsy Lozoff, Niko kaciroti and Tomas walter** Iron deficiency in infancy: applying a physiologic framework for prediction. Am J. Clin. Nutr. 2006 Dec., 84(6): 1412 – 1421.
6. **Margarita Diaz *et al.*** The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets; a field study using stable isotopes. The Am. J. of Cli. Nutr. Sept. 2003. Vol. 78, 436 – 440.
7. **Oti-Boateng P., Seshadri R., Petrick S., Gibson R. A., Simmer K.** Iron status and dietary iron intake of 6-24-month-old children in Adelaide. J. Paediatr Child Health. 1998 Jun; 34(3):250-3.
8. **Beard JL** Iron deficiency alters brain development and functioning. J. Nutr. (2003)133 suppl. 1, pp. 1468S-1472.

9. **Prpic-Majie D, Bobicc J, Simicc D, House DE, Otto DA, Jurasovicc J, Pizent A** Lead absorption and psychological function in Zagreb (Croatia) school children. Neurotoxicol Teratol (2002)22, pp. 347-356.
10. **David IT, Anne SWM, David L, Mwaniki and Arjan DW** Micronutrients in childhood and the influence of subclinical inflammation. Proceedings of the Nutrition Society (2005), 64, 502-509.
11. **RDA** Recommended Dietary Allowance. 10th Edition. National Academy Press, Washington DC. 1989.
12. **LSRO** (Life Science Research Office). Summary of a report on assessment of the iron nutritional status of the United State population. Am J. Clin. Nutri. (1985)42:1318-1330.
13. **Connie W. Bales, Jeanne H. freeland-Graves, Susan Askey, Fares Behmardi, Rebecca S, pobocik Jacqueline, and Patti Greenlee** Zinc, magnesium, copper and protein concentrations in human saliva: age and sex-related differences. Am J. Clin. Nutr. 1990; 51; 462-9.
14. **Tietz NW** Accuracy in clinical chemistry – Does anybody care. Clin chem. 1994 Jun; 40.6:859-61.
15. **Tietz NW, Rinker AD, Morrison SR** When is a serum iron really a serum iron? The status of serum iron measurement. Clin chem. 1994Apr. 40(4):546-51.
16. **Beckman Coulter, Inc.** (1998 – 2006). 4300N. Harbour Boulevard P. O. Box 3100. Fullerton CA 92834 – 3100 USA.
17. **Looker AC, Dallman P., Carroll MD, Gunter EW, Johnson CL** Prevalence of iron deficiency in the United States. *JAMA.* 1997; 277:973–976.
18. **National Committee for clinical laboratory standards** Procedures for the Handling and processing blood specimens; Approved Guideline. NCCLS, Publication H18.A Villanova. PA (1990).
19. **SAS Institute Inc.**, 1999. SAS/STATUser'sGuide, version 8, Cary, NC.
20. **United Nations**. Fourth Report on the World Nutrition Situation, January: Nutrition throughout the Life Cycle. Geneva: Administrative Committee on Coordination/Sub-Committee on Nutrition (ACC/SCN) in collaboration with IFPRI, 2000.
21. **WHO** (World Health Organization). 1968. Nutritional Anaemia. Report of a WHO Scientific Group. Who Technical Report Series No. 405. World Health Organization, Geneva.
22. **Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O** Sex differences in iron status during infancy. *Pediatrics.* 2002;110:545–552.
23. **Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G** Iron status at 12 months of age—Effects of body size, growth and diet in a population with high birth weight. *Eur J Clin Nutr.* 2003; 57:505–513.
24. **Hay G., Sandstad B., Whitelaw A., Borch-Iohnsen B.** Iron status in a group of Norwegian children aged 6–24 months. *Acta Paediatr.* 2004; 93:592–598.