

# Iron Status of Infants and Toddlers Age 6 to 18 Months and Association with Type of Milk Consumed from DNSIYC Secondary Analysis

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## Abstract

This study compares the intake of different types of milk, iron, haem iron, non-haem iron, and Vitamin C intake among infant and children age 6-18 months of age from Diet and Nutrition Survey of Infants and Young Children (DNSIYC). Approach SACN 2010 recommendation, the Reference Nutrient Intakes (RNI) of iron and Vitamin C for infant and children age 6-18 months was investigated in this study. Moreover, Ferritin and haemoglobin investigated as well to assess iron status, subjects who donated bloods were included in this study to investigate their iron status, 473 subjects for haemoglobin, and 443 for ferritin. Different factors investigated on 2567 subjects, include age groups, sex, ethnicity, socioeconomic level, maternal education level, and different types of milk consumed. This study found significant difference for ferritin and haemoglobin in different levels, across different types of milk consumed, haem-iron, Vitamin C, age groups and maternal education level. While there were no significant differences across sex, ethnicity and socioeconomic level in all age groups.

**Keywords:** Infants; Vitamin; Nutrition; Health; Gastroenterology

## Introduction

This dissertation examines the association between the type of milk consumed and iron status of infants and toddlers ages 6-18 months living in the United Kingdom (UK). It is based on a secondary analysis of the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) commissioned by the Department of Health and the Food Standards Agency [1,2]. The literature review establishes the importance of this relationship in application to recommendations about feeding cow's milk to infants and toddlers in this age group. Using current standards adopted by the World Health Organisation (WHO), it defines Iron Deficiency Anaemia (IDA) in children under the age of 5 years, as determined by indicators including haemoglobin and ferritin plasma concentrations. It then reviews current evidence and guidelines for breast-feeding and cow's milk intake for infants and toddlers with a focus on the relationship of type of milk intake to iron status. Although some discussion of infants under the age of 6 months is necessary to establish an understanding of the increased dietary needs for iron that occur after the age of 6 months, the focus of the review is on infants aged 6-18 months. This will set the context for the present study undertaken, which is expected to add to the current evidence base for future dietary recommendations in this area.

Breastfed infants of normal birth weight under the age of 6 months have a low prevalence of IDA and do not require iron supplementation; however iron supplements of 1-3 mg per day are thought to improve neurodevelopment for low birth weight infants less than 6 months [3]. For formula-fed infants under the age of six months, iron-fortified formula prevents IDA and may improve neurodevelopment [4]. For infants ages 6-12 months, follow-on formulas with iron fortification prevents IDA, but evidence regarding neurodevelopment is contradictory [4]. In infants ages 4-12 months, IDA is prevented by feeding iron-rich complementary foods and avoiding unmodified cow's milk; iron supplements prevents IDA in populations with high prevalence of IDA but does not benefits populations with low prevalence of IDA [4]. For toddlers 12-36 months of age, there is a dearth of studies, but there is moderate evidence that IDA may be prevented by feeding iron-rich complementary foods and restricting unmodified cow's-milk intake to less than 500 mL per day [4].

Consistent with these findings, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition, which had previously failed to make recommendations for iron intake, now make the following recommendations regarding infants and toddlers above the age of 6 months [4]: "Follow-on formulas should be iron fortified; however, there is not enough evidence to determine the optimal iron concentration in follow-on formula.

From the age of 6 months, all infants and toddlers should receive iron-rich (complementary) foods, including meat products and/or iron-fortified foods.

Unmodified cow's milk should not be fed as the main milk drink to infants before the age of 12 months and intake should be limited to <500 mL daily in toddlers."

Woldu et al. [5] concur with these recommendations, concluding from their review that the best prevention against IDA is to replace unmodified cow's milk with iron supplemented formula; avoid feeding infants unmodified cow's milk until the age of one year; and provide breastfed infants with iron supplements or iron-fortified cereal.

## Methodology

### Sampling

In January 2010 the Cambridge shire 4 Research Ethics Committee approved the DNSIYC as a portfolio adopted study, as most of the clinics were NHS Trust sites [2,3] Stage 1 of the DNSIYC primarily involved the selection of subjects. A sample of infants and young children 4 months up to 18 months were drawn from across the

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UK Child Benefit (CB) Records, with the addition of a Healthy Start boost sample using a multi-stage random probability design to ensure the sample was stratified by “Government). A major limitation of the DNSIYC was that the number of subjects included in the study, exceeded the number of participants who donated blood; this affects the statistical power of the results Office Region, Index of Multiple Deprivation (IMD) scores and population density” [2].

### Data preparation

The DNSIYC collected information on infants starting at age 4 months. Although this study interested in iron status from ages 6-18 months, they were also interested in the effects of complementary feeding which many parents follow earlier than 6 months, contrary to recommended practice [4]. However, the present dissertation loaded its dataset from UK data archive, and limits its analysis to the population of interest, which is the infants and toddlers age 6-18 months. Therefore, the worksheet for analysis was first limited by excluding any infants younger than 6 months, for a total of 2683 participants. The original main worksheet file contained 2683 participants and 1861 variables. When children under the age of 6 months were excluded, the number of subjects in the edited worksheet contained 2567 subjects. SPSS program was used to compare means of Ferritin and Haemoglobin in two age groups in these variables: Recode age group, sex, ethnic groups, socioeconomic group, education group, and drink of milk group, Vitamin C including supplements, total iron including supplements, haem iron including supplements and non-haem iron including supplements.

The present studies only looks at Haemoglobin (Hgb) and ferritin frequency statistics and compare means with all above recoded variables, to test the null hypothesis that the means of Ferritin and Haemoglobin for all age groups, sex, mother education, socioeconomic level and type of milk are equal.

The significance and relationships between hemoglobin, ferritin and Vitamin C tested accordingly in the following variables, SPSS software used for data analysis:

1. Age;
2. Gender;
3. Ethnic group;
4. Socioeconomic level;
5. Maternal education level;
6. Type of milk used (breast milk, infant’s formula, follow-on formula or cow’s milk).

Ferritin variable was divided into 2 groups According to WHO [6] Ferritin cut-off point:

- Normal ferritin above 12 µg/L.
- Low ferritin below 12 µg/L.

Haemoglobin variable was divided into 2 groups (non-anaemic) and (anaemic which include; mild/moderate/severe anaemia), according to the WHO [7] Haemoglobin cut-off point:

- Non anaemia (11 g/dL).
- Mild anaemia (10–10.0 g/dL).
- Moderate anaemia (7.0–9.9 g/dL).
- Severe anaemia ( $\leq 7.0$  g/dL).

An independent samples t-test is the statistical method used to compare the means of a continuous dependent variable between two unrelated groups, and assess whether there is at least a 95% probability that the difference found does not arise by chance.

Independent sample T-test, to test the null hypothesis that the means of two groups, group of Ferritin and group of Haemoglobin are equal in different variables: Recode age groups, sex, Vitamin C including supplements, total iron including supplements, haem iron including supplements and non-haem iron including supplements, in the two age groups. While the independent samples t-test is used to compare two groups, the one-way Analysis of Variance Test (ANOVA) compares two groups and above.

One way anova, to test the null hypothesis that the means of Ferritin and Haemoglobin in all population in the groups are equal in the these variables: Group of education, group of socioeconomic level, group of ethnic and group of drink milk.

### Results

The study included infants aged 6-18 months. Though the original sample consisted of 2,567 subjects, subjects with missing information relating to any aspect of the study were excluded from the sample. Therefore, number of subjects was 473 for haemoglobin-related tests, and 444 for ferritin-related tests (Figure 1).

#### Age

An independent sample T-test was conducted in order to compare Haemoglobin levels in infants aged 6-9 months and infants aged 10-18 months.

The test was unable to find a significant difference in the haemoglobin level for the two age groups  $p=0.297$  (Figure 2). While there was a statistically significant difference in the Ferritin level for infants the two aged groups  $p=0.013$  (Figure 3).

#### Sex

An independent sample T-test was conducted in order to compare Haemoglobin levels in males and females. The test was unable to find a significant difference in the scores for males and females  $p=0.509$ . For

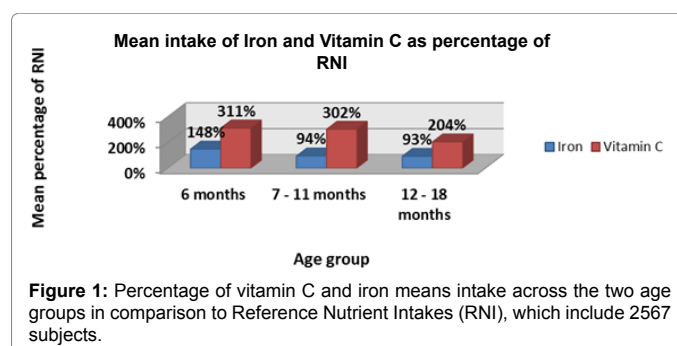


Figure 1: Percentage of vitamin C and iron means intake across the two age groups in comparison to Reference Nutrient Intakes (RNI), which include 2567 subjects.

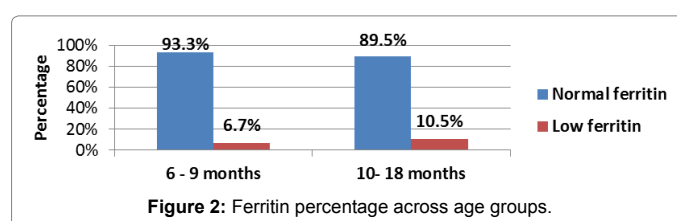


Figure 2: Ferritin percentage across age groups.

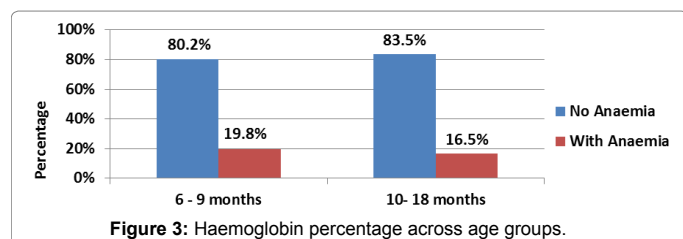


Figure 3: Haemoglobin percentage across age groups.

Ferritin, the test was unable to find a significant difference in the scores for males  $p=0.104$ .

### Vitamin C

An independent samples T-test was conducted in order to compare Vitamin C levels in anaemic and non-anaemic subjects.

The test was unable to find a significant difference in the scores for anaemic subjects  $p=0.236$ , though the difference was close to being statistically significant. While for Ferritin, there was a statistically significant difference in the scores for low ferritin subjects and normal ferritin subjects  $p=0.022$ .

There was no statistically significant difference between the anaemic and non-anaemic subjects in levels of vitamin C, but there was a significant difference between subjects with low ferritin and normal ferritin; subjects with normal ferritin levels had significantly higher levels of vitamin C.

### Dietary iron intake

An independent samples T-test was conducted in order to compare iron levels in anaemic and non-anaemic subjects.

The test was unable to find a significant difference in the scores for anaemic subjects and non-anaemic subjects  $P=0.796$ . There was no significant difference in the scores for low ferritin subjects and normal ferritin subjects  $p=0.310$ .

### Dietary intake: Haem iron

An independent samples T-test was conducted in order to compare haem iron dietary intake in anaemic and non-anaemic subjects.

The test was find a significant difference in the scores for anaemic subjects and non-anaemic subjects  $p=0.014$ . A significant difference in the scores for low ferritin and normal ferritin subjects  $p=0.017$ .

### Dietary intake: Non-haem Iron

An independent samples T-test was conducted in order to compare non-hem iron levels in anaemic and non-anaemic subjects.

The test was unable to find a significant difference in the scores for anaemic subjects and non-anaemic subjects  $p=0.861$ . There was no significant difference in the scores for low ferritin subjects and normal ferritin subjects  $p=0.26$ .

### Ethnicity

A one-way analysis between subjects Analysis of Variance (ANOVA) was conducted in order to compare the effect of ethnicity on haemoglobin rates, comparing three groups: White, South Asian and other.

The test was unable to find a statistically significant effect of ethnicity on haemoglobin at the  $p<0.05$  level;  $p=0.730$ . Moreover, ferritin at the  $p<0.05$  level  $p=0.921$ . Ethnicity was not able to adequately explain the differences between subjects.

### Maternal level of education

A one-way analysis between subjects ANOVA was conducted in order to compare the effect of parents' level of education on haemoglobin rates, comparing three groups: Degree level or above, qualification below degree level and no qualifications.

There was a statistically significant effect of parents' education on haemoglobin rates at the  $p<0.05$  level;  $p=0.036$ . For ferritin, the test was unable to find a statistically significant effect of parents' educational level on ferritin at the  $p<0.05$  level;  $p=0.574$ .

### Socioeconomic group

A one-way analysis between subjects ANOVA was conducted in order to compare the effect of socioeconomic status on haemoglobin rates, comparing four groups: Managerial and professional occupations, intermediate occupations, routine and manual occupations, and not classifiable.

The test was unable to find a statistically significant effect of socioeconomic level on haemoglobin at the  $p<0.05$  level,  $p=0.667$ . The test was unable to find a statistically significant effect of socioeconomic level on ferritin as well at the  $p<0.05$  level  $p=0.932$ .

### Milk consumption

A one-way between subjects ANOVA was conducted in order to compare the effect of consumption of different types of milk on haemoglobin rates, comparing five groups: Breast Milk, Infant Formula, and Follow on Milk, Cow's Milk or other.

There was a statistically significant effect of type of milk consumed on haemoglobin rates at the  $p<0.05$  level;  $p=0.001$ . Moreover, there was a statistically significant effect of type of milk consumed on ferritin rates at the  $p<0.05$  level;  $p=0.000$  (Figures 4-7).

### Discussion

Studies have shown that, during the first six months, infants who have been exclusively breastfed exhibited low iron intake, since human

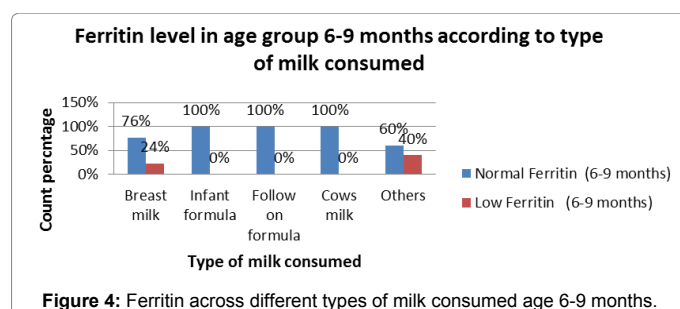


Figure 4: Ferritin across different types of milk consumed age 6-9 months.

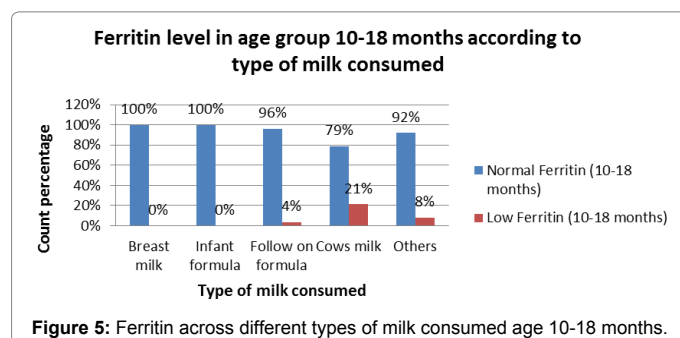


Figure 5: Ferritin across different types of milk consumed age 10-18 months.

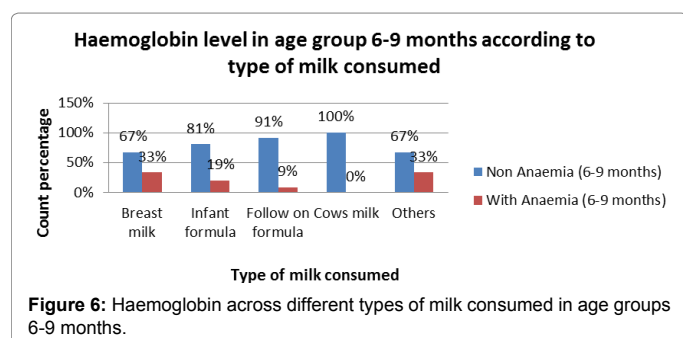


Figure 6: Haemoglobin across different types of milk consumed in age groups 6-9 months.

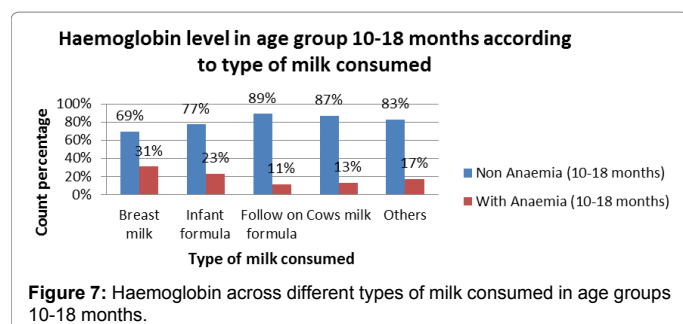


Figure 7: Haemoglobin across different types of milk consumed in age groups 10-18 months.

milk is relatively low in iron. At the age of 6 months, however, many infants are already receiving other forms of food, including specialised baby food and the beginning of solid food intake [7]. In addition, iron absorption undergoes developmental changes during infancy and early toddlerhood. Healthy adults have internal “regulators”, such as dietary regulation, based on recent dietary iron intake, iron stores and other activity within the body. These regulators may not function at the same level or in the same way during very early infancy [8,9]. For instance, an iron-supplementation study of infants aged 4-9 months, in Honduras and Sweden, found that, at age 4 months, iron supplements were able to increase haemoglobin, regardless of the haemoglobin levels prior to the supplement intake. However, at the age of 6 months, the haemoglobin response was affected by the iron levels prior to supplement intake [10]. While these findings are compelling, the current study did not find a difference in haemoglobin levels, but rather it identified differences in ferritin levels, as younger infants had a higher level of ferritin. The current literature does not refer to this phenomenon.

In regards to gender differences, the current literature is inconclusive. Some studies have found that male infants may be more susceptible to anaemia, which is reflected in low haemoglobin levels, as well as lower rates of ferritin. For instance, one study conducted in an Asian population found that the risk for anaemia was 1.6 times higher for males than for females, and the risk for ferritin-deficiency anaemia was 3.3 times higher than for females [11]. However, other studies have failed to find any difference between male and female infants [12] the current study have approved the same finding, male and female have no difference in susceptible to anaemia.

Comparison of vitamin C and iron levels between the two levels of either haemoglobin or ferritin provides information regarding the possible association between levels of haemoglobin or ferritin and of vitamin C and iron. The study found an association between ferritin levels and vitamin C, in that higher ferritin levels were associated with higher vitamin C, but not with the haemoglobin.

Vitamin C plays an important role in facilitating the metabolism of

iron, as well as the utilisation of iron for forming red blood cells. It has been posited that vitamin C may facilitate and stimulate the release of iron from ferritin-deposit storage [13] which may explain the positive association found in this study between vitamin C and ferritin.

There were no associations between iron, non-haem iron and ferritin or haemoglobin. Thus, haem-iron is the factor that is associated with ferritin and haemoglobin levels, and therefore this should be the focus of iron-enhancing interventions.

To this extent, studies have linked low serum ferritin concentrations to iron deficiency [14] and this may account for the association found in this research between iron levels and ferritin levels. The current study did not identify any significant association between total iron intake and haemoglobin or ferritin levels, though other studies have indicated that iron fortification of foods contributed to an increase in ferritin and haemoglobin alike [15], but this study has approved there is a significant association between haem iron intake and haemoglobin or ferritin levels. Therefore, future interventions intended to increase iron should focus on sources of haem iron rather than general sources of iron, seeing as non-haem iron has no effect on haemoglobin and ferritin.

This study shows that infant at age 7-11 months, mean intake of iron was 7.4 mg, 94% of recommended nutritional intakes RNI [16] 7.8 mg of iron, while infants at age of 6 months, mean consumption of iron was 6.4 mg, which exceeds RNI [16] 4.3 mg at 148%. Within this study, these research findings explains why infants who received either exclusive breast feeding or infant formula, at age 6 months met and exceed the iron RNI, while infants above the age of 6 months, which is the age of complementary feeding, failed to meet the iron RNI [16,17].

For Vitamin C, the mean intake for all age groups exceeds the RNI. Subjects, who consumed higher Vitamin C, have higher ferritin level than those who consumed less Vitamin C.

Significant differences between groups were found when grouping according to level of maternal education for haemoglobin, but not for ferritin, and no difference was found across ethnic groups. Upon further investigation, the study found that the difference between mothers with degree level education and those with below degree level education accounted for the statistically significant difference in haemoglobin rates, as infants whose mothers had a below degree level exhibited higher haemoglobin levels. These findings may indicate the involvement of a third factor that mediates this connection. Some of the possible explanations include the existence of a relationship between work demands and the feeding habits among mothers of infants, as well as an association between educational level and the child’s care during the day. Studies have found that infants attending day care are less likely to be anaemic, which is attributed to their dietary intake and an increased likelihood that they consume iron-supplemented food [18-21].

When subjects were grouped according to the type of milk they consumed, statistically significant differences emerged in both haemoglobin and ferritin rates. Subjects consuming cow’s milk were significantly lower levels of haemoglobin and ferritin levels (mean levels were 86 and 81, respectively) and those consuming milk other than the types listed exhibited the highest rates of haemoglobin and ferritin (mean levels were 110 and 105, respectively). Infant formula accounted for differences in the ferritin but not haemoglobin levels (for ferritin infant formula was the second highest, and for haemoglobin it was in the middle), and breast milk and follow on milk accounted for differences in the haemoglobin, but not ferritin levels.

Interestingly, the association between ferritin levels and type of milk consumed differed between the two age groups; among the younger group (aged 6-9 months) 24% of the infants consuming breast milk had low ferritin levels, while none of the older infants (aged 10-18 months) consuming breast milk exhibited low ferritin rates. Infants consuming infant formula in both age groups did not exhibit low ferritin, and among the infants consuming follow on milk, 4% of infants aged 10-18 months exhibited low ferritin while all infants aged 6-9 months exhibited normal ferritin rates. Among infants consuming cow's milk, there were no individuals in the younger age group with low ferritin levels, but among the older group 21% exhibited low ferritin. Finally, among infants consuming other types of milk, 40% of the infants in the younger age group who consumed other types of milk had low ferritin levels, but among the older group only 8% exhibited low ferritin.

Therefore, the main question guiding this research, with reference to the effect of the type of milk consumed on iron levels, was answered by isolating the types of milk that affected haemoglobin and ferritin in a unique manner. Researchers, health professionals and nutritionists alike agree that unmodified cow's milk should not be the primary milk drink for infants under the age of 12 months, and that general intake should be limited [22]. This coincides with the findings in this research according to which cow's milk accounted for differences in haemoglobin as well as ferritin.

Moreover, the general recommendation, regarding infants whose main source of nutrition is infant formula, is that this formula should be iron-fortified and contain at least 4-8 mg/L of iron [23,24]. This may explain the findings in the current research according to which infants receiving infant formula had different levels of ferritin, but not haemoglobin, compared to infants fed other forms of milk [25].

Iron content in breast milk has a higher bio-availability than iron-fortified formulas (12-49% absorption), as stated above, this level is low compared to the infants' needs [26]. During the first 6 months of an infant's life, the iron needs are mainly supplied by iron stored at birth, seeing as infants born full term and at adequate weight the endowed iron stores that can make up for the deficient breast milk for 6 months. After this time it is recommended to supplement the infant's nutrition with iron-rich or iron-fortified food [27].

Genetic factors may also play a part in iron deficiency, especially those related to red blood cells or the metabolism, such as sickle cell, and therefore should also be considered [28].

This dissertation examines the association between the type of milk consumed and iron status of infants and toddlers ages 6-18 months living in the United Kingdom (UK). It is based on a secondary analysis of the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) commissioned by the Department of Health and the Food Standards Agency. The literature review establishes the importance of this relationship in application to recommendations about feeding cow's milk to infants and toddlers in this age group. Using current standards adopted by the World Health Organisation (WHO), it defines Iron Deficiency Anaemia (IDA) in children under the age of 5 years, as determined by indicators including haemoglobin and ferritin plasma concentrations. It then reviews current evidence and guidelines for breast-feeding and cow's milk intake for infants and toddlers with a focus on the relationship of type of milk intake to iron status. Although some discussion of infants under the age of 6 months is necessary to establish an understanding of the increased dietary needs for iron that occur after the age of 6 months, the focus of the review is on infants aged 6-18 months. This will set the context for the present study undertaken,

which is expected to add to the current evidence base for future dietary recommendations in this area.

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