Iron Nutrition in Low-Birth-Weight Infants

Martti A. Siimes

Pediatric Hematology Division, Children's Hospital, University of Helsinki, SF-00290 Helsinki 29, Finland

Since the early 1920s pediatric textbooks have recognized that infants often become anemic a few months after a preterm birth. However, except for its greater severity, the anemia does not differ in any way from that of full-term infants. It can be divided into an early and a late anemia of prematurity. The early anemia represents the initial fall in concentration of hemoglobin which occurs during the first 2 months after birth. The degree of severity varies with the gestational age and the vitamin E nutrition of the premature. In contrast, the late anemia may start at any time from 2 months on, depending on the time when the premature infant’s iron supply is exhausted. Over the last 60 years, increasing doses of iron have been recommended for prematures in order to compensate for the rapid growth and to prevent the late anemia. Daily doses of 10 or 20 mg/kg body weight are currently being given by many pediatricians. These doses are far above what we currently consider to be optimal for these infants.

GROWTH AND IRON NEEDS

During the last 10 years, there has been a remarkable improvement in the survival ratio of very-low-birth-weight (VLBW) infants. Table 1 compares the body composition in iron of a VLBW infant with that of a full-term infant. The latter, who grows from 3.2 kg at birth to 10 kg at 1 year of age increases his total body iron content from 210 to 365 mg. Thus, the estimated increase in total body iron is about 50%, although the body weight triples during the same time. A VLBW infant with a birth weight of 0.8 kg may increase his weight 10-fold during the first year.

<table>
<thead>
<tr>
<th>Initial birth weight (kg)</th>
<th>Increments in 12 months</th>
<th>Ratio iron/weight (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (kg)</td>
<td>Body iron (mg)</td>
</tr>
<tr>
<td>0.8</td>
<td>7.2</td>
<td>210</td>
</tr>
<tr>
<td>1.6</td>
<td>7.9</td>
<td>215</td>
</tr>
<tr>
<td>3.2</td>
<td>6.8</td>
<td>155</td>
</tr>
</tbody>
</table>

75
The total body iron will increase from 50 mg of iron at birth to 260 mg at 1 year of age, representing a fivefold increase in total body iron. The need for iron in very rapidly growing low-birth-weight (LBW) infants is thus larger than one would estimate by the changes in body weight. Table 1 indicates that the changes in body iron over 12 months are similar in children with birth weights of 0.8 and 1.6 kg. However, the 0.8-kg infant eats less than the 1.6-kg infant; he therefore must obtain his iron from a considerably smaller volume of food, which may not be possible without supplementation with higher doses of iron.

**SERUM FERRITIN AND IRON STORES**

The role of iron stores is of particular importance in LBW infants since they are relatively large at the time of birth and will be used within a short period of time. For this reason the correlation between serum ferritin concentration and iron stores will be discussed here.

The first attempt to convert a serum ferritin value into a level of storage iron was published by Walters et al. in 1973 (1). They showed that 1 ng/ml of serum ferritin is equivalent to 8 mg of storage iron in adult tissues. Subsequently, a relationship of 1 ng/ml of serum ferritin to each 10 mg of storage iron has been suggested (2). Based on these observations, one might make a rough estimation that a serum ferritin level of 10 ng/ml would correspond to 100 mg of iron stored and a level of 100 ng/ml would correspond to 1,000 mg.

The concentration of serum ferritin is relatively high at the time of birth, about 200 to 250 ng/ml, which is similar to that of a healthy adult male (about 150–200 ng/ml) (3). This seems to indicate that the serum ferritin assay might be better correlated with the concentration of stored iron per kg of body weight which is similar in newborns (about 15 mg/kg) and adult males (about 10 mg/kg), than with the absolute quantities of stored iron, about 50 mg in the newborn and 700 mg in the adult male.

The correlation between serum ferritin and storage iron, however, presents some problems. Jacob et al. have shown that serum ferritin levels fall more rapidly when iron reserves are abundant than when they are reduced (4), indicating a curvilinear response in an individual subject. It is also possible that the relationship between iron stores and serum ferritin is different in adults and in infants or that serum ferritin is not directly related to the iron stores but rather reflects the status of some labile iron pool. A fairly sudden increase in iron stores when the destruction of red blood cells exceeds production, as during the early anemia of prematurity, may perhaps increase the ratio of serum ferritin to iron stores and thus leads to an overestimation of the changes in iron stores based on serum ferritin changes.

**IRON IN THE FETUS**

Several developmental changes occur in the erythropoiesis of the fetus. The site of red blood cell production is primarily in the liver after about 3 months of gestational age. The bone marrow takes over gradually, starting at around 5 months
of gestational age (Fig. 1). Usually in a full-term infant the bone marrow is almost exclusively responsible for erythropoiesis. In preterm newborns the liver plays a role in erythropoiesis at birth. There are also other developmental changes that occur simultaneously. The size of the red cell gradually becomes smaller during fetal life so that the red cell mean corpuscular volume (MCV) tends to be larger in prematures than in full-term infants. The concentration of hemoglobin seems to rise in the fetus during pregnancy, although the details of this development are not well known. Finally, the fetal hemoglobin is partially replaced by adult hemoglobin at term. Thus, the prematures have proportionally more hemoglobin-F than the full-term newborns.

It is well known that the fetus accumulates iron in tissue stores during pregnancy. It has been estimated that the amount of iron stored at birth is about 15 mg/kg. There seems to be a linear correlation between the body weight and quantity of total body iron (6). The concentration of serum ferritin, when estimated in cord blood, appears to be similar or lower in preterm infants at birth or in fetuses after operative abortion than in term infants (Fig. 2).

**FIG. 1.** The stages of hematoopoiesis in the developing embryo and fetus (5).

**FIG. 2.** Concentration of serum ferritin in cord blood serum from premature infants and fetuses aborted by laparotomy. The values are shown by gestational age.
A few fetal conditions or diseases result in low concentrations of serum ferritin at birth; these are maternal iron deficiency anemia and diabetes mellitus and occasionally conditions resulting in small-for-date newborns. In Fig. 3, storage iron has been calculated from the serum ferritin values and the respective body weights, assuming that the concentration of serum ferritin is proportional to the concentration of storage iron during this period of development. Figure 3 indicates that small-for-date infants tend to have less storage iron at birth than the controls.

We have very little information on any mechanism that regulates the accumulation of fetal iron stores. An interesting observation, that twins with separate placentas usually have similar concentrations of serum ferritin at birth (Fig. 4), may indicate that maternal iron rather than placental iron determines the accumulation of fetal iron stores. Against the placental regulation is also the finding that there is no correlation between the birth weight and the concentration of serum ferritin at birth in pairs of twins in conditions where one twin is growth retarded.

There seems to be little or no correlation between maternal hemoglobin or serum iron levels and the respective values in the newborn under normal conditions. Even in extreme cases of maternal iron deficiency anemia, the newborn can maintain a normal or only slightly decreased concentration of hemoglobin at birth (7). These observations indicate that fetal iron is well protected in practically all conditions, although the responsible mechanisms are not well understood.

**HUMAN MILK FEEDING**

In recent years there has been a return to the feeding of human milk to infants, after a period of several decades during which the use of proprietary formulas

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**FIG. 3.** Storage iron in small-for-date infants at birth. Storage iron has been calculated from the serum ferritin concentration and the body weight at birth.
predominated in most industrialized countries. In Finland, the formulas never gained widespread acceptance in the feeding of premature infants; human milk has been in almost continuous use. Thus, human milk banking has been carried out on a relatively large scale. About 5,000 liters per million individuals are collected annually and used for this purpose. The iron concentration of human milk gradually decreases from a mean value of around 0.6 mg/liter soon after the birth of a full-term infant to a mean value of about 0.3 mg/liter after 6 months of lactation (8). Thus, the average banked human milk contains less iron than the milk collected immediately after delivery since it is collected mostly from mothers 2 to 3 months after delivery. Thus, the prematures receiving banked human milk ingest less iron than breast-fed full-term infants soon after birth.

In Finland, about half of the mothers who deliver VLBW infants of less than 1,500 g at birth are able to lactate enough so that the infants can be fed with their own milk. This way of feeding prematures has some advantages over banked human milk, since the milk contains more protein than the milk after a normal pregnancy. The composition also seems to be different in other respects and meets the nutritional needs of the premature infant (9). On the average, the iron content of the milk of mothers of VLBW infants is greater than the iron content of banked human milk. It should be noted, however, that the iron content of the milk secreted by some mothers is unusually low, far below the average value. There is no practical way of identifying these mothers. A premature infant would, in such a case, get much less iron from his own mother than from pooled banked human milk and be more dependent on supplementary iron. It is not clear whether the iron concentration of human milk can be influenced by maternal iron supplementation during lactation. Such supplementation might potentially be a means to improve both the infant’s and mother’s iron nutrition.
HUMAN MILK IRON AVAILABILITY

McMillan et al. have studied a small group of infants who were exclusively fed with human milk without any iron supplementation for periods of over a year (10). These individuals had a normal concentration of hemoglobin and serum ferritin at the end of the study. More recently, a similar study of a group of 7 infants has been published (11). It was also concluded that the infants had no signs of iron deficiency even if they received no iron supplement. Under these conditions, the availability of human milk iron must be extremely high since the concentration of iron in human milk is relatively low and decreases with the length of lactation, as discussed earlier (8). Thus both studies appear to indicate that the absorption of iron from human milk is unique and that the magnitude of the absorption should be somewhere between 50 and 100%. It is interesting that similar conclusions have also been made in studies where the extrinsic tag method for the determination of absorption of iron from human milk has been used (12).

The absorption of iron from any milk is higher in prematures than in full-term infants (13,14). Järvenpää et al. have recently observed that exclusively human-milk-fed VLBW infants (body weight lower than 1,250 g) who were getting iron supplementation from 2 months of age on had a higher concentration of hemoglobin and serum ferritin at 4 months of age than another group that was formula fed and given supplemented iron from birth on (15) (Table 2). This observation indicates that supplementary iron is better absorbed from human milk than from a formula in small LBW infants (15).

TABLE 2. Mean (± SE) concentration of hemoglobin, serum ferritin, and free erythrocyte protoporphyrins in preterm infants (birth weight < 1,250 g) fed either human milk (n = 12 at 3 months, n = 9 at 4 months) or iron-supplemented formula (n = 32 at 3 months, n = 39 at 4 months)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human milk</td>
<td>10.9 ± 0.4</td>
<td>12.3 ± 0.2(^b)</td>
</tr>
<tr>
<td>Formula</td>
<td>10.2 ± 0.2</td>
<td>11.3 ± 0.2</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human milk</td>
<td>59 (50–69)</td>
<td>50 (39–65)(^c)</td>
</tr>
<tr>
<td>Formula</td>
<td>40 (35–46)</td>
<td>18 (16–20)</td>
</tr>
<tr>
<td>Protoporphyrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human milk</td>
<td>100 ± 23</td>
<td>64 ± 14</td>
</tr>
<tr>
<td>Formula</td>
<td>81 ± 6</td>
<td>81 ± 7</td>
</tr>
</tbody>
</table>

\(^a\)The formula contained 12 mg of iron/liter. Infants fed human milk received iron supplementation (2 mg/kg/day) from 2 months of age on (15).
\(^b\)P < 0.02.
\(^c\)P < 0.001.
The reasons for the high bioavailability of the human milk iron are not known. Recent studies have shown that the concentration of iron in milk varies during the day and with age (8).

Lactoferrin is a major iron-binding protein in milk. The degree of its iron saturation is reported to be low. Further, some reports indicate that this protein actually decreases absorption of iron from human milk (16). Schäfer et al. have shown that a considerable part of the milk iron is bound to fat (17). Other investigators have identified part of the iron in cow's milk in the membrane of fat globules.

The variations in concentrations of iron, fat, and lactoferrin during a single nursing are shown in Fig. 5. Lactoferrin concentration decreased while concentrations of fat and iron increased in human milk with the volume of milk expressed.

Fransson and Lönnerdal have shown that a very small fraction of the iron in human milk is bound to lactoferrin, and that the latter is very unsaturated (18) (Table 3). They therefore believed that iron concentration is not correlated with lactoferrin concentration. Rather it is a reflection of the metabolic activity of the mammary gland as both protein and trace minerals progressively decline over the course of lactation. Fransson and Lönnerdal's data indicate that the iron saturation of lactoferrin is very low, at the most 4%. This is considerably lower than values reported earlier, which ranged between 10 and 40%. The low saturation may be justified by the role that lactoferrin plays in the defense against infections. It has been suggested that increasing the iron saturation of lactoferrin may result in a decrease of its bacteriostatic properties.

![Graph showing the concentration of iron, fat, and lactoferrin during the course of a single nursing (18).](image-url)
TABLE 3. Iron saturation of lactoferrin

<table>
<thead>
<tr>
<th>Maximum percentage of Fe bound to lactoferrin</th>
<th>Maximum amount of Fe bound to lactoferrin (μg/ml)</th>
<th>Iron-binding capacity (μg/ml)</th>
<th>Maximum saturation of lactoferrin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (16–40)</td>
<td>0.10 (0.04–0.23)</td>
<td>5.4 (2.4–14.8)</td>
<td>2 (1–4)</td>
</tr>
</tbody>
</table>

*a100-(fat Fe)-(solids Fe)-(LMW Fe) %.

bMaximum % × Fe concentration (μg/ml).

cConcentration of lactoferrin (in moles) × 2 (each molecule binds 2 Fe) converted to μg/ml.

dMaximum amount of Fe bound to lactoferrin/iron-binding capacity.

Data from Fransson and Lönnertal (18).

TABLE 4. Percentage of iron distribution in human milk

<table>
<thead>
<tr>
<th>Fat</th>
<th>Solids and semisolids</th>
<th>Low-molecular-weight compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 (15–46)</td>
<td>9 (0–34)</td>
<td>36 (18–56)</td>
</tr>
</tbody>
</table>

*aData from 13–15 milk samples (18).

The unique studies of Fransson and Lönnertal further demonstrated, by use of ultrafiltration and gel filtration techniques, that a considerable fraction of the iron in human milk is bound to low molecular weight compounds (18) (Table 4). These compounds may have very high association constants for iron, which could explain why more iron is not bound to lactoferrin. On the other hand, Lönnertal et al. (19) have shown that citrate, which is present in a comparatively high concentration, binds a large part of the zinc in human milk, but the iron-binding compounds still have to be identified.

IRON SUPPLEMENTATION

Lundström et al. (20) have shown that there was no significant difference in hemoglobin concentration, MCV, or transferrin saturation between two groups of infants weighing at birth between 1 and 2 kg receiving no iron supplementation or 2 mg iron/kg/day between 2 weeks and 2 months of age (Figs. 6 and 7). At 2 months, however, the serum ferritin concentration was lower in the group receiving no iron supplement. This value (42 ng/ml) was still higher than the normal one for children, indicating that the unsupplemented infants were not iron deficient, even though the developmental low point in hemoglobin concentration occurs at this age. Only 7% of those who were not iron supplemented has a serum ferritin concentration below 10 ng/ml, a value which may indicate depleted iron stores and increased risk of developing iron deficiency anemia. Figures 6 and 7, however, clearly show that LBW infants cannot maintain optimal iron nutrition without iron supplementation after the age of 2 months. The hemoglobin as well as the MCV
FIG. 6. Hemoglobin concentration and mean corpuscular volume (MCV) in LBW infants who received no iron supplementation (open circles) or 2 mg iron/kg/day from 2 weeks of age on (closed circles) (20). Means ± SEM are indicated. Differences between the groups became significant at 3 months of age. The number of unsupplemented infants receiving iron supplementation because of anemia is shown within circles for each age.

of red blood cells, the transferrin iron saturation, and the serum ferritin were all significantly lower in the unsupplemented infants. The difference in hemoglobin averaged 1 to 2 g/dl during a follow-up period of up to 6 months. This difference would have been greater if many of the unsupplemented infants had not received iron once the diagnosis of anemia was made.

It is difficult to indicate what is the optimal dose of iron as supplement because individual needs for iron may vary with birth weight, neonatal sickness, and blood loss. Some data indicate that infants, whose birth weight ranged between 1 and 2 kg, who were given 2 mg of iron/kg/day did not show clinical iron deficiency (20). The dose was probably adequate and was certainly not excessive for the maintenance of iron stores. However, high frequency of borderline serum ferritin values were found although no anemia was documented (20). It could be argued that a higher dose of iron such as 3 mg/kg/day would provide a more comfortable margin of safety, especially in those infants with a birth weight between 1 and 1½ kg.
Some recent data indicate that 4 mg of iron/kg/day administered to a group of VLBW infants with a birth weight of less than 1,000 g prevent most laboratory signs of iron deficiency (21) (Figs. 8 and 9). There was, however, a marked decline in serum ferritin despite the large dose of iron administered.

**MAINTENANCE OF HEMOGLOBIN CONCENTRATION**

Preterm infants experience a fall in hemoglobin concentration during the first 2 months of life that is more marked than in term infants (Table 5). It seems that the fall varies with the birth weight, even if supposedly adequate iron supplementation is provided (Fig. 10). Thus the early anemia of prematurity, which results in low hemoglobin values at 2 months of age, cannot be prevented by iron supplementation and should therefore be considered as physiological.

Lundström and Siimes (22) have shown that despite the great demands of rapid growth and the rapid increase of blood volume, the LBW infants with a birth weight above 1 kg achieve an erythrocyte count, hemoglobin concentration, and red cell indices of term infants during the first half year of life when sufficient iron is administered.
Very-low-birth-weight infants with a birth weight less than 1 kg form an exceptional group among prematures. They achieve a remarkably rapid rate of postnatal growth (Fig. 11) and present unusually high nutritional requirements. In many cases the birth weight is increased by 10-fold or more during the 15 months following birth. However, there is an unusually large individual variation in weight gain. One might anticipate that the concentration of hemoglobin might be lowest in those infants with the most rapid rate of growth, but there is no significant relationship between these values (21) (Fig. 12). Even VLBW infants are apparently able to absorb the iron needed for their high requirements. The lack of a relationship between growth rate and hemoglobin concentration not only argues against the likelihood that iron is a limiting factor in hemoglobin production under these conditions, but also makes it less likely that a deficiency of other nutrients could play a major role in limiting hemoglobin production.
TABLE 5. Hemoglobin concentrations (g/dl) for preterm infants with serum ferritin levels ≥ 10 ng/ml (median and 95% range)

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>1,000-1,500 g</th>
<th>1,501-2,000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>16.3 (11.7-18.4)</td>
<td>14.8 (11.8-19.6)</td>
</tr>
<tr>
<td>1 mo</td>
<td>10.9 (8.7-15.2)</td>
<td>11.5 (8.2-15.0)</td>
</tr>
<tr>
<td>2 mo</td>
<td>8.8 (7.1-11.5)</td>
<td>9.4 (8.0-11.4)</td>
</tr>
<tr>
<td>3 mo</td>
<td>9.8 (8.9-11.2)</td>
<td>10.2 (9.3-11.8)</td>
</tr>
<tr>
<td>4 mo</td>
<td>11.3 (9.1-13.1)</td>
<td>11.3 (9.1-13.1)</td>
</tr>
<tr>
<td>5 mo</td>
<td>11.6 (10.2-14.3)</td>
<td>11.8 (10.4-13.0)</td>
</tr>
<tr>
<td>6 mo</td>
<td>12.0 (9.4-13.8)</td>
<td>11.8 (10.7-12.6)</td>
</tr>
</tbody>
</table>

Data from Lundström and Siimes (22).

FIG. 10. Concentration of hemoglobin in iron-supplemented infants after birth. A = VLBW infants with a birth weight less than 1,000 g who were given iron (4 mg/kg/day); B = LBW infants with a birth weight of about 1,500 g who were given 2 mg iron/kg/day; C = full-term infants with a birth weight over 3,000 g who were given 1 mg iron/kg/day.

It is interesting that the return to normal hemoglobin concentration takes a considerably longer time in premature infants with a birth weight lower than 1 kg than in larger premature infants (Fig. 10), although they were supplemented with 4 mg of iron/kg/day and there was little or no evidence of iron deficiency.

It is rare that an infant with a birth weight lower than 1 kg is treated without many laboratory studies, yet those who are compensate for such blood loss remarkably well (21). There are some infants who require no transfusion and maintain their subsequent hemoglobin concentration at levels similar to those of infants who are given transfusions (Table 6). This is another indication that regulation of iron absorption is well developed in these small infants and that provision of additional iron can effectively prevent the development of iron deficiency.
ROLE OF VITAMIN E AND SELENIUM

One of the roles of vitamin E is the protection of biological membranes against oxidative breakdown of lipids. Premature infants are born with low serum levels of vitamin E. Feeding these infants diets rich in polyunsaturated fatty acids, particularly when the diet is supplemented with iron, can produce a hemolytic anemia if the vitamin E deficiency is not corrected (Fig. 13). Melhorn and Gross (23) have demonstrated that in VLBW infants the daily administration of 7 to 10 mg of iron/kg/day accelerates the postnatal decline in the concentration of hemoglobin. Williams et al. (24) observed increased hemolysis and lower hemoglobin levels in small premature infants fed formulas with a high polyunsaturated fatty acid content, especially when the formulas were fortified with iron. Melhorn and
TABLE 6. Mean (± SE) hemoglobin concentration (g/dl) in 6 VLBW infants without and in 22 VLBW infants with blood transfusions during the first 2 months of life*.

<table>
<thead>
<tr>
<th>Age in months</th>
<th>Without transfusion</th>
<th>With transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>18.8 ± 1.1 [6]</td>
<td>16.0 ± 0.7 [21]</td>
</tr>
<tr>
<td>1</td>
<td>11.5 ± 1.0 [5]</td>
<td>11.8 ± 0.4 [21]</td>
</tr>
<tr>
<td>2</td>
<td>9.1 ± 0.5 [6]</td>
<td>9.5 ± 0.4 [22]</td>
</tr>
<tr>
<td>3</td>
<td>9.3 ± 0.3 [4]</td>
<td>8.8 ± 0.4 [20]</td>
</tr>
<tr>
<td>4</td>
<td>10.9 ± 0.3 [6]</td>
<td>10.1 ± 0.3 [19]</td>
</tr>
<tr>
<td>9</td>
<td>12.2 ± 0.1 [6]</td>
<td>12.2 ± 0.2 [20]</td>
</tr>
<tr>
<td>15</td>
<td>13.1 ± 0.4 [6]</td>
<td>13.0 ± 0.2 [18]</td>
</tr>
</tbody>
</table>

*The number of subjects is shown in brackets (21).

Gross have explored the relationship between gestational age and absorption of vitamin E (25). Their studies show that during the first 3 weeks of life, infants with a birth weight of less than 1,500 g and a gestational age of less than 32 weeks have decreased absorption of vitamin E. A subsequent study with a water soluble vitamin E preparation demonstrated better absorption, but the individual levels were not predictable. Greaber et al. (26) demonstrated that vitamin E adequacy, as defined by serum tocopherol levels and hydrogen peroxide hemolysis tests, can be achieved rapidly and safely by the intramuscular administration of DL-α-tocopherol. The results indicate that an intramuscular dose of vitamin E of 125 mg/kg administered over the first week of life is sufficient to maintain vitamin E adequacy during the first 6 weeks of life, even in the presence of intramuscular iron. Aside from mild erythema at the injection site, no detectable reaction could be related to vitamin E administration. In a recent article, Phelps has reviewed the potential dangers of vitamin E administration (27).

Rudolph et al. (28) have recently studied the role of selenium in LBW infants fed formulas with and without iron. Under the experimental conditions, there was no evidence of any association between selenium and early anemia of prematurity.

INTERRELATIONSHIP WITH COPPER

The metabolisms of copper and iron are linked together in many ways. The question is whether copper deficiency exists in infants after preterm delivery and, if so, whether it has any influence on iron metabolism. It has been shown that the serum concentration of copper is lower in preterm than in full-term infants during the first months after birth (29). However, it is difficult to estimate the significance of this finding. Any form of copper supplementation in formula-fed infants leads to another problem since the amount of copper ingested is primarily dependent on the copper concentration of the water that is added to the formula powder. The concentration in water varies between areas and even between houses within the same city.
FIG. 13. Concentrations of hemoglobin in premature infants with a birth weight of 1,000–1,500 g (25). All infants were fed formula without added iron and a multiple vitamin mixture containing vitamins A, C, and D. The infants were assigned to one of the four groups as follows: A = no additional supplement; B = ferrous sulphate, 8 mg elemental iron/kg/day up to 6 weeks of age, then 8 mg/liter of formula; C = a-tocopherol acetate 25 IU/day between 2 and 6 weeks of age; D = both ferrous sulphate and a-tocopherol acetate. For the sake of clarity, first three groups are shown. The highest hemoglobin concentrations were in the vitamin-E–supplemented infants.

MOBILIZATION OF STORAGE IRON

The high physiologic concentration of hemoglobin at birth decreases to a low value at 2 months of age. This decrease in hemoglobin concentration initially results in a rise in tissue iron stores, which are subsequently used during the next 12 months to maintain a normal level of hemoglobin. In each of the developmental changes of infancy, there is a considerable individual variation, including the ability to mobilize iron from tissue stores. Thus, some infants may develop iron deficiency anemia although they have iron stores (determined by their serum ferritin values) and they have no disease, such as infection or inflammation, to explain their inability to mobilize iron stores (30). A similar situation may also occur in adults, although only after some significant blood loss. In contrast, in infants it may be physiological.
In fact, in a large series of healthy infants who were followed carefully during the first year of life, those with mild iron deficiency anemia usually had serum ferritin concentrations within the normal range (30). The relative abundance of storage iron in these cases may effectively inhibit the availability of iron from other sources, namely from intestinal absorption. Thus both sources of iron for hemoglobin synthesis, from tissue stores and from food, would be inhibited and this coincidence would subsequently increase the risk of developing iron deficiency anemia. Lundström et al. have also shown (Figs. 6 and 7) that in preterm infants anemia commonly develops prior to the time when iron stores become exhausted (20). This latter finding also indicates that the rate at which iron can be mobilized from tissues may not be fast enough in rapidly growing infants.

REFERENCES

15. Järvenpää A-L, Räihä N, Gaull GE, Siimes MA. Human milk is superior to formula for iron nutrition also in preterm infants. (Submitted for publication.)
DISCUSSION

Dr. Hallberg: I have a comment about the relationship between iron stores and serum ferritin, which is used in the calculation of the changes in total body iron in infants. The relationship is based on four points: adult females, adult males, newborn infants, and infants 2 weeks old. The point used for adult males very probably overestimates the size of the iron stores. In these studies iron stores were measured by phlebotomy but no correction was made for the increased dietary iron absorption during the phlebotomy period. That gives a figure for iron stores which is about twice too high. (In 26 normal men the iron stores were 6.4 mg/kg body weight and serum ferritin 89 ng/ml.) These findings indicate that the relationship between iron stores and serum ferritin is different in adults and in infants. Another comment is that we don’t know how the increased destruction of red cells after birth affects the serum ferritin level. I think that it is very difficult to interpret serum ferritin levels in nonsteady state conditions. I would therefore suggest that iron absorption calculations based on changes in serum ferritin must be looked at with great caution.

Dr. Finch: I would like to also comment on ferritin. I agree that ferritin should be equated to body weight in kilograms. We have considered that 1 μg of ferritin per liter is equivalent to about 140 μg of storage iron per kilogram body weight. It would be interesting to compare the value for ferritin in the newborn and then later, when there has been a known decrease in circulating red cell mass with an increase in iron storage and see how this correction applies. It is also of interest that ferritin levels appear to be increased when body metabolism is increased. Perhaps this would apply to the infant. Finally, I suppose one can raise the question of some tissue damage or inflammation since these conditions would give an inappropriately high ferritin concentration.

Dr. Siimes: It is well documented that there is a correlation between the concentration of serum ferritin and the level of iron stores. Dr. Hallberg started a discussion of the details of the correlation for which there are surprisingly few studies. Our drawing indicates that the correlation is better if both serum ferritin and iron stores are expressed in a specific way; the former as log values and the latter per unit of body weight. After this manipulation,
the regression line is linear at all ages. Concerning the inflammation and infection, these conditions may raise the ferritin values and result in false high results, although chronic inflammations are very rare and acute infections relatively easy to recognize at this age.

Dr. Guesry: I was very interested by your comparison between human milk- and formula-fed babies because Royer in France who has published the same type of comparative study showed exactly the reverse: that babies fed with human milk from banks (and that is perhaps the difference) have a lower level of iron than babies receiving special formulas for premature babies. What was exactly the composition of the formula that your babies received?

Dr. Siimes: One difference may have been the birth weights: these were extremely low. The formula was a regular and humanized one containing the recommended amounts of vitamins and 12 mg of iron/liter.

Dr. Guesry: Did the babies who were not iron-deficient and were breast-fed up to the age of 18 months receive any iron supplement?

Dr. Siimes: The few infants recorded in the literature from the United States and Peru who were exclusively breast-fed received no iron supplementation. They developed no evidence of iron deficiency, although the number of cases was too small for conclusions. Nevertheless, I found it an interesting observation. In Finland, the pregnant mothers receive iron supplementation. It is my experience that the maternal iron supplementation during lactation does not influence the infants' iron status while they are exclusively breast-fed.

Dr. Chandra: What was the nature of the human milk fed to these babies? Was it their own mother's milk? We know that there are differences in the iron content of milk produced by mothers who deliver at preterm, compared with those who deliver at term, being almost twofold higher in the former.

Dr. Siimes: In this case it was banked milk.

Dr. Stekel: The implication of these figures is not only that they absorb the iron from breast milk but, probably more significantly so, that breast-fed infants absorb the supplementation iron better. Isn't that the implication?

Dr. Siimes: It could be there are other additional explanations, too. Possibly, chronic human milk ingestion decreases the physiological loss of iron through the intestinal tract.

Dr. Garby: You mentioned a study on iron isotope absorption from breast milk. I was wondering what is known about the isotopic exchange when you add labeled iron to breast milk.

Dr. Lönnerdal: We have been doing some studies on the distribution of an extrinsic tag in milk and compared this to the distribution of native iron. It doesn't seem that an extrinsic tag is distributed in the same manner as native iron in breast milk. The distribution of native and radioactive iron in cow's milk was more similar, but still not identical. In breast milk, an extrinsic tag almost exclusively binds to lactoferrin.

Dr. Garby: The distribution of labeled iron in the fractions must be related to the time of incubation, surely. There is no such thing as a distribution by itself; there is a distribution at a certain time.

Dr. Lönnerdal: It's true, but you can approach the problem from different angles. You can look at the starting point by incubating for an hour; the next thing you can do is to take a gastric aspirate from breast-fed infants and label it and look for the distribution. You can take tagged breast milk, incubate it at different pH, and look at the distribution again. It is not the true situation in the gastrointestinal tract, but you are approaching that situation at least. Both when we take the gastric aspirate and when we go down to pH 3 with HCl and when we are looking at it initially, a disproportionate part is definitely bound to lactoferrin. We do not see isotopic equilibrium in the breast milk.

Dr. Garby: I think you have misunderstood me. I do not want to imitate the conditions later on. One must be sure that one has incubated for sufficient time to have isotopic equilibrium. What I am saying is that, if after 1 hr of incubation at room temperature, the distribution is such and such, what is it after 2 hr, 24 hr, and so on?
Dr. Lönnerdal: This is not known as yet.

Dr. Cook: When the concept of the extrinsic tag was first presented, a number of investigators attempted to look at the distribution of radioiron incorporated biosynthetically as compared to the extrinsic tag. It was never possible to show that the distribution of the two tags in the food was similar. This does not necessarily argue against complete isotopic exchange of the extrinsic iron at some point in the gastrointestinal tract. One study that addresses the issue of isotopic exchange in milk is that of Schultz and Smith which showed that radioactive milk prepared by injecting a cow with radioiron was absorbed to the same extent as an extrinsic tag added to the milk. As far as I know, no one has prepared radioactive cow's milk since that time.

Dr. Fomon: In the study by Schulz and Smith [Am. J. Dis. Child. 95:109, 1958], iron absorption from cow's milk was studied in 10 subjects with the intrinsic tag and in 5 other subjects with the extrinsic tag. There was a wide range of ages (4-52 months) and, although none of the subjects was anemic, little information is available about iron nutritional status. I could not accept this study as a validation of the extrinsic tag method for determining iron absorption from cow's milk.

Dr. Cook: I agree completely. I think that this is the only observation that relates to the question.

Dr. Lönnerdal: I think we have to take account of the very specific properties of lactoferrin. First, the equilibrium of iron exchange between lactoferrin and other compounds is exceptionally slow, i.e., when you want to feed this solution it may not be fit for feeding anybody. So, coming back to Dr. Cook's comments, I think that there are a couple of factors that would speak against the fact that the conventionally assumed extrinsic-intrinsic iron exchange would happen. You have to go below pH 2 to release iron from lactoferrin. It is a very strong iron-binding protein. We are talking about a stomach of an infant where we do not even get down to that pH. The second point: lactoferrin is comparatively resistant to proteolysis. Both these points counteract the assumption that the extrinsic tag would behave as an intrinsic tag. I think in most cases that is true, but this may be an exception when it does not hold true.

Dr. Chandra: The large quantity of lactoferrin in human milk may permit the isotope label to be disproportionately tagged to it. Studies on this question must have at least two additional controls: human milk which has been depleted of lactoferrin using an immunoabsorbent column, and purified lactoferrin.

Dr. Dallman: From the discussion over the last 15 min, there seem to be some doubts about the reliability of the breast-milk iron absorption data. Even though there may be uncertainty about the exact values for iron absorption from breast milk, we should not lose sight of the very strong evidence that the percentage of iron absorbed from breast milk is much greater than from cow's milk or cow's milk formula.

Dr. Lönnerdal: I agree that there is a lot of support that it certainly is more available. I think we must exercise a little caution here about what is the absorption number. Dr. Siimes has indicated that in some cases you can almost calculate it to be 100%—in your case you got absorption of about 49 to 50%—maybe that was for the lactoferrin compartment which is just one part of total iron, maybe some of the other parts are better absorbed; the low molecular weight iron can have a very high availability and there is very little low molecular weight iron in the cow's milk, for example.

Dr. Siimes: I agree that the extrinsic tag method may result in underestimation rather than overestimation of iron absorption.

Dr. Guesry: I would like to come back to a suggestion made by Dr. Siimes that the difference between breast milk and formula is mainly due to protein allergy and there is now good information that the T helper cells are not mature in very-low-birth-weight infants and that there is little probability that this protein allergy would occur in a very-low-birth-weight infant.
Dr. Fomon: That is certainly possible. It is important to keep in mind that we do not yet have data on relative amounts of blood lost from the gastrointestinal tract by breast-fed and formula-fed infants.

Dr. Siimes: I completely agree and would like to add that there are at least two mechanisms through which iron is lost: one is blood lost through the gastrointestinal tract and the other is the loss through epithelial cells, which is reported to be increased in some gastrointestinal conditions.

Dr. Stekel: Coming back to the question of the high serum ferritin levels in premature infants, some of the data that Dr. Dallman showed us indicate that infants that one would have predicted to have used all their iron stores also had high serum ferritins.