Vitamin and Mineral Requirements in Infancy: How Can They Be Determined?

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There are many ways of determining the requirements for a particular nutrient. A time-honored and essentially practical one is to determine the intakes of free-living subjects who remain healthy; in early infancy the obvious diet is breast milk. In later infancy the clinical occurrence of specific nutritional disease has proved useful in determining requirements. This review discusses estimates of requirements in early and later infancy based on these two approaches, with particular regard to the problems that arise with respect to vitamin D and iron.

BREAST MILK AS A MODEL IN EARLY INFANCY

Breast milk as a model in early infancy has a long provenance. In recent years, as guidelines and directives have been issued for the composition of infant formulas, breast milk has invariably been used as the template (1–5). This has usually but not always been supported by other methods of assessment, in particular, anthropometry, sometimes by biochemical determination in blood and urine, and rarely by metabolic balance data (e.g., nitrogen).

Copper illustrates many of these points. Its concentration in mature breast milk is about 40 mg/dl and 60 mg/100 kcal (0.6 and 0.9 mmol, respectively) (6). Cow’s milk contains smaller amounts. Some formulas in the past have also contained small amounts because the addition of copper led to stability problems, particularly the oxidation (and then rancidity) of unsaturated fatty acids. As technology has improved, the amount present in breast milk has often become the recommended concentration for formulas, even though clinical evidence of copper deficiency has not been described in term babies fed breast milk or formula. However, there are various problems with this approach.
Physiological Variation

There are considerable variations in the composition of breast milk. One large study in Britain tried to standardize many factors. We arranged for a complete breastful of milk to be expressed, since hind- and foremilk have different compositions. Because of diurnal variations, the samples were taken midmorning. They were, moreover, taken only from women having term babies, since preterm milk differs from term milk. Because the milk composition also varies with the infant's age, the procedure was initiated 6 to 10 weeks postpartum. The result was a "standardized" breast milk—a standard for reference perhaps, but not what many babies actually receive. The composition of the "standard British breast milk" is included in Table 1, and for comparison other data from various sources are included, showing some of the variation with maturity of the pregnancy and the number of days postpartum. Picciano and her colleagues have provided extensive details of such variation (12,13).

Pathological Variation

If the mother is malnourished, breast milk may be a very unsuitable model. The best example is the thiamine-deficient breast milk produced by mothers living on a polished-rice diet in South East Asia. The babies thrive but develop cardiac or neurogenic beriberi.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Unit</th>
<th>Expressed per 100 ml</th>
<th>Expressed per 100 kcal</th>
<th>Drip per 100 ml 6-15 days</th>
<th>Drip per 100 ml 16-196 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>µg</td>
<td>60</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>µg</td>
<td>0.01</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-Linoleic acid</td>
<td>µg</td>
<td>0.35</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin K</td>
<td>µg</td>
<td>1.5</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine</td>
<td>µg</td>
<td>16</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>µg</td>
<td>31</td>
<td>44</td>
<td></td>
<td></td>
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<tr>
<td>Nicotinic acid</td>
<td>µg</td>
<td>230</td>
<td>329</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>µg</td>
<td>6</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B12</td>
<td>µg</td>
<td>0.01</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>µg</td>
<td>5.2</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>µg</td>
<td>260</td>
<td>371</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>µg</td>
<td>0.8</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inositol</td>
<td>mg</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>mg</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 1. (continued)

<table>
<thead>
<tr>
<th>Mineral (atomic weight)</th>
<th>Unit</th>
<th>Expressed per 100 ml</th>
<th>Drip per 100 kcal</th>
<th>Preterm milk (per 100 ml)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>Sodium (23) mg</td>
<td></td>
<td>15</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Potassium (39) mmol</td>
<td></td>
<td>0.6</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Chlorine (35) mg</td>
<td></td>
<td>60</td>
<td>86</td>
<td>63</td>
</tr>
<tr>
<td>Calcium (40) mmol</td>
<td></td>
<td>1.5</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Magnesium (24) mg</td>
<td></td>
<td>43</td>
<td>61</td>
<td>88</td>
</tr>
<tr>
<td>Phosphorus (31) mmol</td>
<td></td>
<td>35</td>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>Iron (56) μg</td>
<td></td>
<td>2.8</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>Copper (64) μmol</td>
<td></td>
<td>0.12</td>
<td>0.17</td>
<td>0.12</td>
</tr>
<tr>
<td>Zinc (65) μmol</td>
<td></td>
<td>15</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Manganese (55) μmol</td>
<td></td>
<td>76</td>
<td>109</td>
<td>78</td>
</tr>
<tr>
<td>Selenium (79) μmol</td>
<td></td>
<td>295</td>
<td>421</td>
<td>475</td>
</tr>
<tr>
<td>Iodine (127) μmol</td>
<td></td>
<td>2.0</td>
<td>2.8</td>
<td>0.61</td>
</tr>
<tr>
<td>Fluorine (19) μmol</td>
<td></td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Chromium (52) nmol</td>
<td></td>
<td>340–430</td>
<td>7–43</td>
<td>0.3–1.6</td>
</tr>
<tr>
<td>Molybdenum (96) μmol</td>
<td></td>
<td>0.5–25</td>
<td>0.005–0.26</td>
<td></td>
</tr>
</tbody>
</table>

* The data have been extracted mainly from the following sources, but some individual figures are from others:
  - Expressed breast milk [DHSS (6)].
  - Drip breast milk [Gibbs et al. (7)].
  - Preterm milk at 7 and 28 days [Gross et al. (8); see also Anderson et al. (9)].
  - Preterm B vitamins at 6–15 days and 16–196 days of mothers delivering at 29–34 weeks gestation [Ford et al. (10)].

Are Methods of Analysis Adequate?

This is a matter for analytical organic chemists. It is too obvious to say that breast milk is not a simple crystalline solution. Many compositional details are complex. Calcium and some other metals have a micellar relationship with casein; many vitamins have specific carrier proteins to which they are loosely bound. Physiologically, these relationships may ensure sta-
bility of the nutrient within the food, availability of the nutrient for intralu- 
minal digestion, and eventual presentation to the enterocyte for absorption. Analytically, these relationships may present technical problems and potentially misleading results.

In most cases, however, the analytical methods used some years ago have 
stood the test of time; e.g., most of the widely quoted figures of Macy et al. (14) were confirmed in the British study 27 years later. Indeed, a recent flurry of interest in a water-soluble vitamin D sulfate (15,16) is thought probably to be an analytical error (17,18), and even if it exists in breast milk it has little antirachitic activity (17–19).

A particular problem occurs with manganese. The British study reported 
2.0 mg/dl (0.04 mmol). This is broadly compatible with values found in United 
States breast milk collected during the first to thirty-first month of lactation 
(1.4 to 2.5 μg/dl), but is substantially more than the amounts found in Finland 
(median, 0.6 μg/dl at 2 weeks falling to 0.4 μg/dl at 2 months) (21). Are these 
true biological differences? If so, Why? Or are they methodological differ-
ences? No case of manganese deficiency has been demonstrated in infants, 
so perhaps these apparent variations are of little importance. The concen-
trations are higher in cow’s milk (e.g., 4.0 μg/dl), however, and very much 
higher in some cow’s-milk-based formulas from the United States and in 
soya-based formulas (concentrations about 100 μg/dl). If the breast milk 
model is valid, presumably these concentrations are unnecessarily high.

Breast Milk Lacks Certain Nutrients

Breast milk contains only small amounts of what are undoubtedly essential 
nutrients. Some of these are discussed in much more detail later in this book, 
but some comments here are pertinent.

Vitamin D

Breast milk does not contain vitamin D, nor does cow’s milk. It has been 
suggested that man probably evolved in the tropics and had little use for 
dietary vitamin D, since this was provided by sunlight. It has also been 
suggested that the persistence of intestinal lactase into adult life was a useful 
mutation for tropical man as he migrated to less sunny temperate climates; 
this enabled him to absorb calcium more effectively—calcium absorption is 
greater from diets containing more lactose (22). Surely if this were so, would 
not temperate man have also favored a mutation that supplied the infant 
with dietary vitamin D?
Iron

The “migration” argument cannot be applied to iron. Why do both human and cow’s milk contain such small amounts of iron? There is no other source of iron, even in the tropics, comparable to that of the sun for vitamin D. The young infant relies for iron on his endowment at birth plus the small amount in his diet. On the other hand, there is some evidence that dietary iron could inhibit various anti-infective defenses, and so there may be an evolutionary teleological argument against dietary iron for the young infant. (This is discussed later in this chapter.)

Vitamin K

It is well known that a few babies develop hemorrhagic disease of the newborn, due apparently to vitamin K deficiency. Limited stores at birth, insufficient gut synthesis because of only partial bacterial colonization, and small amounts in milk all contribute to the problem. Vitamin K deficiency leading to bleeding into the brain and elsewhere may occur beyond the neonatal period in exclusively breast-fed infants (23–27). Clearly, breast milk does not seem an ideal template for vitamin K requirements in the newborn.

Special Requirements of Low-Birthweight Babies

We have recently reviewed the requirements of preterm infants for all nutrients including vitamins and minerals (11), and many of these are discussed in later chapters; however, some general points may be made. Human milk has a larger proportion of its zinc content in the form of the citrate salt, which increases its bioavailability. Nevertheless, in the first week of life even healthy full-term infants are in negative zinc balance (28). Zinc deficiency with symptoms mimicking the genetic disease acrodermatitis enteropathica has been described in a number of very low birthweight babies receiving breast milk for 2 or more months (11); but, then, breast milk should not necessarily be seen as a food teleologically designed for preterm babies.

Copper deficiency has been described in only a small number of babies. Almost all were preterm (11), and all were receiving a cow’s milk formula. It therefore seems the breast milk template is suitable for copper, even in preterm infants. Extra vitamins are commonly given to low-birthweight babies receiving either formulas or breast milk. Is this a tacit assumption that the basic diet does not meet the requirements for these vitamins? Infants with severe respiratory distress syndrome have low concentrations of plasma retinol; extra vitamin E may prevent hemolytic anemia, retrolental fibroplasia, and bronchopulmonary dysplasia. Are these observations nutritional
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or pharmacological and is there really a difference between these two modes of action?

Breast milk may well meet the requirements for water-soluble vitamins, but heat processing, storage, and photodegradation when exposed during administration reduce the amounts of available vitamin in breast milk (11).

CLINICAL DEFICIENCIES IN LATER INFANCY

In later infancy, frank nutritional disease can tell us something about requirements. It is interesting that scurvy, once so common in older infants in Europe, has now almost completely disappeared. It is doubtful whether this has much to do with our policies for infant feeding. Infant formulas contain much more vitamin C than cow’s milk, and supplements containing vitamin C are freely available. Nevertheless, very few older infants, in Britain at least, receive these, and yet they do not get scurvy. I think this is because so many of the fruit juices, nectars, and “squashes” available for people of all ages have vitamin C added, and the older infants are given these. In effect, fortification of foods for the whole population has led to the disappearance of a disease from one small sector of the population.

Iron deficiency anemia and rickets are still with us, however, and there is concern about zinc. In central Birmingham we found that two-fifths of Asian toddlers were anemic, two-fifths had low plasma concentrations of 25-hydroxy vitamin D, and one-fifth had both deficiencies (29). Low concentrations of plasma zinc were not common (30), unlike reports on similar underprivileged immigrant groups in Denver, Colorado (32).

Iron-Deficiency Anemia

I will admit to a previous “cavalier” attitude with regard to mild iron deficiency. This changed when our study in Birmingham showed that the anemic children had a poorer psychomotor development than the nonanemic ones living in the same environment (29).

Others had suggested a causal relationship between iron deficiency and psychomotor delay. It had always seemed more likely that the underprivileged environment resulted in both anemia and psychomotor delay, i.e., merely association. Surprisingly, however, in a double-blind randomized controlled intervention study, it seemed that iron therapy could indeed improve the rates of both psychomotor development and weight gain. Table 2 summarizes the results of this and other intervention studies (32).

Rickets

The clinical disease of rickets, formerly known as die englische Krankheit (“English disease”), is less common now but low plasma concentrations of
VITAMINS AND MINERALS IN INFANCY

25-OH vitamin D are reported in both Asian and white children in Britain (33,34). Sunlight is thought to be a more important source of vitamin D than diet, but in Birmingham we find that the rise in vitamin D during the summer months is only modest in our Asian toddlers. They make poor use of our limited sunlight, and vitamin supplements have as much effect as the summer (Fig. 1).

Rickets occurs even in sunny countries. Probably this is because the children and their mothers are not exposed to the sun (36). Is this the whole story, however? The peak age incidence of rickets in cities as far apart as Cairo and Peking is before the age of 6 months, very different from the late-infancy onset seen in Europe and most Middle East countries. Why is this? Are other factors such as dietary calcium and its availability playing a role?

Growth Limitation Due to Zinc Deficiency

Zinc deficiency may complicate severe malnutrition such as kwashiorkor. It may occur much more frequently, however, as a mild deficiency that limits
<table>
<thead>
<tr>
<th>Criterion of abnormality to enter study</th>
<th>Aukett et al.</th>
<th>Lozoff et al.</th>
<th>Oski and Honig</th>
<th>Walter et al.</th>
<th>Oski et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>8–11 g/dl</td>
<td>Hemoglobin</td>
<td>Hemoglobin &lt; 10.5 g/dl, mean corpuscular volume ≤ 73 fl, serum iron &lt; 50 μg, and transferrin saturation ≤ 12%</td>
<td>(a) Hemoglobin &lt; 11 g/dl and two abnormal biochemical tests</td>
<td>Hemoglobin &gt; 11 g/dl and (a) normal mean corpuscular volume, electrophoresis, and ferritin</td>
</tr>
<tr>
<td>Age group (months) Treated group No</td>
<td>17–19</td>
<td>6–24</td>
<td>9–26</td>
<td>15</td>
<td>9–12</td>
</tr>
<tr>
<td></td>
<td>48 Anemic; iron deficiency</td>
<td>15 Anemic; iron deficiency</td>
<td>12 Anemic; iron deficiency</td>
<td>10 Anemic; iron deficiency</td>
<td>(a) 10 normal</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) 10 iron depleted</td>
<td>(b) 10 iron depleted</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) 10 iron deficient (biochemically)</td>
<td>(c) 10 iron deficient (biochemically)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) 8 iron deficient (biochemically and cellular)</td>
<td>(d) 8 iron deficient (biochemically and cellular)</td>
</tr>
<tr>
<td>Treatment/day</td>
<td>Oral iron 24 mg + vitamin C</td>
<td>Oral ferrous ascorbate 5 mg/kg plus ‘carrier’</td>
<td>Intramuscular iron dextran complex dose dependent on degree of anemia 5–8</td>
<td>Oral iron 3–4 mg/kg day</td>
<td>Intramuscular iron 50 mg (iron dextran)</td>
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<tr>
<td>Treatment period (days)</td>
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<tr>
<td>Control group</td>
<td></td>
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<tr>
<td>No</td>
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<tr>
<td>Treatment (if any)</td>
<td></td>
<td></td>
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<tr>
<td>Vitamin C 10 mg daily</td>
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<td></td>
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<tr>
<td>Psychomotor test Results: treated vs. controls</td>
<td>Denver</td>
<td>Bayley scales</td>
<td>Bayley scales</td>
<td>Bayley scales</td>
<td>Bayley scales</td>
</tr>
<tr>
<td></td>
<td>31% Treated children achieved six or more new skills vs 12% in controls ($p &lt; 0.05$); 37% of those whose hemoglobin rose &gt; 2 g did so vs 16% of those whose hemoglobin rose by &lt; 2 g ($p &lt; 0.05$); 42% effectively treated achieved six or more new skills vs 13% in controls ($p &lt; 0.02$)</td>
<td>Although scores improved in all groups, iron-treated anemia did not increase more than placebo treated, anemic, or nonanemic (treated or not) groups</td>
<td>Significant increase in mental development index ($p &lt; 0.05$); 66% of treated gained 10 or more points vs 25% of controls</td>
<td>Mental development index improved in treated anemic group ($p &lt; 0.05$). No change in treated controls. No change in treated nonanemic iron-deficient group</td>
<td>77% of treated iron deficient group increased 10 or more points vs 20% of treated non-iron-deficient group ($p &lt; 0.001$)</td>
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</tbody>
</table>

* For full references, see Aukett et al. (32).
growth and which can be corrected by zinc supplementation. The observations originally made in Denver are being joined by similar ones from other countries, but at present the exact place of zinc deficiency as a common problem in later infancy requires more studies in many centers (31).

Requirements

What do these clinical observations tell us about requirements for nutrition? Perhaps there are two points. First, requirements are likely to vary in different circumstances—the use made of available sunlight, availability from different foods, and so on. Second, the identification of deficiency and its effects is likely to be complex, often involving a clinical trial of the nutrient in question. Logistically this is a much more difficult way of determining a requirement than other methods, e.g., using a food model such as breast milk, or the factorial method. Perhaps, however, these clinical methods will prove the more significant ones in relating requirements to the health of the individual.

THE CONUNDRUM OF VITAMIN D AND IRON

To return to a point made earlier in this chapter, it is intriguing that breast milk contains only very small amounts of two nutrients that, only a few months after birth, are in such short supply as to result in frank nutritional disease.

Iron

In the case of iron there is some evidence of an advantage in avoiding its presence in early life. Only lactobacilli grow well without iron, and most pathogenic bacteria require iron, otherwise multiplication ceases (36). It may therefore be appropriate for the newborn baby to ensure that any potential pathogen is kept in an iron-deficient environment. This is first achieved by living on an “iron-deficient” diet, i.e., breast milk, and second by having an array of compounds with a high affinity for iron, which limit its availability to microorganisms (lactoferrin in the gut lumen, transferrin extracellularly, ferritin and hemoglobin within cells). One of these mechanisms (transferrin) is potentially compromised in some newborns because its plasma concentration is lower in preterm babies, and the degree of saturation is higher in all babies than in adults (37).

Clinical practice provides evidence of the potential infective danger from excessive “free” iron. Large doses of iron in children have been associated with episodes of meningitis, septicemia, and enteritis (38–41).
Are these clinical excesses of iron intake relevant to physiological life in infancy? Almost all infant formulas contain extra iron; guidelines for their composition allow concentrations up to 10 mg/liter. We have no evidence that this is dangerous. Indeed, there is considerable evidence of its value in later infancy. The use of iron-fortified infant formulas throughout infancy has been associated with normalization of hemoglobin distribution in infants in Chile and improvement in various measures of iron status even in affluent communities in the United States (42).

Nevertheless, all these apparent advantages of dietary iron apply to the older infant. Although we have not been able to demonstrate an adverse effect of modest amounts of dietary iron in early infancy, should we avoid it? My colleague, Dr. Susan Balmer, and I are at present studying aspects of this question.

**Vitamin D**

Although it is not possible to demonstrate an adverse effect of dietary iron in early infancy there are at least some theoretical microbiological objections to it. Vitamin D has many similarities to iron. Substantial stores exist at birth (iron in the "high" hemoglobin, vitamin D in the liver); breast milk contains very little, and clinical deficiency is widespread in later infancy. Yet there is not even a theoretical objection (unlike iron) to its presence in the diet in early life. Hypercalcemia is a well-known problem, and the British epidemic is a general warning about food fortification (43), although this occurred only following very high doses. Furthermore, while it is more or less unknown for a newborn to have clinical problems due to iron deficiency, this is not so for vitamin D deficiency. In our own hospital it was noted many years ago that Asian babies experienced late hypocalcemia more frequently than European infants; this was because of the poorer vitamin D status of their mothers (44). Three further studies have demonstrated the susceptibility of the newborn to vitamin D deficiency in their mothers (45–47).

Is it not strange, therefore, that newborn humans have evolved dependent on stores present at birth and on the sun, without the extra safety net of a dietary supply? Could it be that vitamin D has some, still unidentified, adverse effect in early infancy? It would be idle to deliberate on possible mechanisms, but the molecular similarity of vitamin D, bile acids, cholesterol, and various steroid hormones invites speculation.

**ACKNOWLEDGMENTS**

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REFERENCES