

# **NUTRIENT ADEQUACY OF EXCLUSIVE BREASTFEEDING FOR THE TERM INFANT DURING THE FIRST SIX MONTHS OF LIFE**



DEPARTMENT OF NUTRITION FOR HEALTH AND DEVELOPMENT  
DEPARTMENT OF CHILD AND ADOLESCENT HEALTH AND DEVELOPMENT  
**WORLD HEALTH ORGANIZATION**

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# **NUTRIENT ADEQUACY OF EXCLUSIVE BREASTFEEDING FOR THE TERM INFANT DURING THE FIRST SIX MONTHS OF LIFE**

**NANCY F. BUTTE, PHD**

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics,  
Baylor College of Medicine, Houston, TX, USA

**MARDIA G. LOPEZ-ALARCON, MD, PHD**

Nutrition Investigation Unit, Pediatric Hospital, CMN, Mexico City, Mexico

**CUTBERTO GARZA, MD, PHD**

Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA



**GENEVA  
WORLD HEALTH ORGANIZATION  
2002**

WHO Library Cataloguing-in-Publication Data

Butte, Nancy F.

Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life / Nancy F. Butte, Mardia G. Lopez-Alarcon, Cutberto Garza.

1.Breastfeeding 2.Milk, Human – chemistry 3.Nutritive value 4.Nutritional requirements 5.Infant I.Lopez-Alarcon, Mardia G. II.Garza, Cutberto III.Expert Consultation on the Optimal Duration of Exclusive Breastfeeding (2001 : Geneva, Switzerland) IV.Title.

ISBN 92 4 156211 0

(NLM Classification: WS 125)

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Designed by minimum graphics  
Printed in France

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# Contents

Abbreviations & acronyms	v
Foreword	vii
<b>Executive summary</b>	<b>1</b>
<b>1. Conceptual framework</b>	<b>3</b>
1.1 Introduction	3
1.2 Using ad libitum intakes to assess adequate nutrient levels	3
1.3 Factorial approaches	4
1.4 Balance methods	5
1.5 Other issues	6
1.5.1 Morbidity patterns	6
1.5.2 Non-continuous growth	6
1.5.3 Estimating the proportion of a group at risk for specific nutrient deficiencies	6
1.5.4 Summary	7
<b>2. Human-milk intake during exclusive breastfeeding in the first year of life</b>	<b>8</b>
2.1 Human-milk intakes	8
2.2 Nutrient intakes of exclusively breastfed infants	8
2.3 Duration of exclusive breastfeeding	8
2.4 Summary	14
<b>3. Energy and specific nutrients</b>	<b>15</b>
3.1 Energy	15
3.1.1 Energy content of human milk	15
3.1.2 Estimates of energy requirements	15
3.1.3 Summary	15
3.2 Proteins	16
3.2.1 Dietary proteins	16
3.2.2 Protein composition of human milk	16
3.2.3 Total nitrogen content of human milk	17
3.2.4 Approaches used to estimate protein requirements	17
3.2.5 Protein intake and growth	20
3.2.6 Plasma amino acids	21
3.2.7 Immune function	21
3.2.8 Infant behaviour	22
3.2.9 Summary	22

3.3	Vitamin A	22
3.3.1	Introduction	22
3.3.2	Vitamin A in human milk	22
3.3.3	Estimates of vitamin A requirements	23
3.3.4	Plasma retinol	23
3.3.5	Functional end-points	24
3.3.6	Summary	26
3.4	Vitamin D	26
3.4.1	Introduction	26
3.4.2	Factors influencing the vitamin D content of human milk	26
3.4.3	Estimates of vitamin D requirements	27
3.4.4	Vitamin D status and rickets	29
3.4.5	Vitamin D and growth in young infants	29
3.4.6	Vitamin D and growth in older infants	30
3.4.7	Summary	30
3.5	Vitamin B6	30
3.5.1	Introduction	30
3.5.2	Vitamin B6 content in human milk	30
3.5.3	Approaches used to estimate vitamin B6 requirements	31
3.5.4	Estimates of requirements	31
3.5.5	Vitamin B6 status of breastfed infants and lactating women	31
3.5.6	Growth of breastfed infants in relation to vitamin B6 status	32
3.5.7	Summary	32
3.6	Calcium	32
3.6.1	Human milk composition	32
3.6.2	Estimates of calcium requirements	32
3.6.3	Summary	33
3.7	Iron	34
3.7.1	Human milk composition	34
3.7.2	Estimates of iron requirements	34
3.7.3	Summary	35
3.8	Zinc	35
3.8.1	Human milk composition	35
3.8.2	Estimates of zinc requirements	35
3.8.3	Summary	37
	<b>References</b>	<b>38</b>

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# Abbreviations & acronyms

AI	Adequate intake
BMD	Bone mineral density
BMC	Bone mineral content
CDC	Centers for Disease Control and Prevention (USA)
DPT	Triple vaccine against diphtheria, pertussis and tetanus
DXA	Dual-energy X-ray absorptiometry
EAR	Estimated average requirement
EAST	Erythrocyte aspartate transaminase
EPLP	Erythrocyte pyridoxal phosphate
ESPGAN	European Society of Paediatric Gastroenterology
FAO	Food and Agriculture Organization of the United Nations
IDECG	International Dietary Energy Consultative Group
IU	International units
NCHS	National Center for Health Statistics (USA)
NPN	Non-protein nitrogen
PLP	Pyridoxal phosphate
PMP	Pyridoxamine phosphate
PNP	Pyridoxine phosphate
PTH	Parathyroid hormone
RE	Retinol equivalents
SD	Standard deviation
SDS	Standard deviation score
UNICEF	United Nations Children's Fund
UNU	United Nations University
WHO	World Health Organization

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# Foreword

This review, which was prepared as part of the background documentation for a WHO expert consultation,<sup>1</sup> evaluates the nutrient adequacy of exclusive breastfeeding for term infants during the first 6 months of life. Nutrient intakes provided by human milk are compared with infant nutrient requirements. To avoid circular arguments, biochemical and physiological methods, independent of human milk, are used to define these requirements.

The review focuses on human-milk nutrients, which may become growth limiting, and on nutrients for which there is a high prevalence of maternal dietary deficiency in some parts of the world; it assesses the adequacy of energy, protein, calcium, iron, zinc, and vitamins A, B6, and D. This task is confounded by the fact that the physiological needs for vitamins A and D, iron, zinc – and possibly other nutrients – are met by the combined availability of nutrients in human milk and endogenous nutrient stores.

In evaluating the nutrient adequacy of exclusive breastfeeding, infant nutrient requirements are assessed in terms of relevant functional outcomes. Nutrient

adequacy is most commonly evaluated in terms of growth, but other functional outcomes, e.g. immune response and neurodevelopment, are also considered to the extent that available data permit.

This review is limited to the nutrient needs of infants. It does not evaluate functional outcomes that depend on other bioactive factors in human milk, or behaviours and practices that are inseparable from breastfeeding, nor does it consider consequences for mothers. In determining the optimal duration of exclusive breastfeeding in specific contexts, it is important that functional outcomes, e.g. infant morbidity and mortality, also are taken into consideration.

The authors would like to thank the World Health Organization for the opportunity to participate in the expert consultation;<sup>1</sup> and Nancy Krebs, Kim Michaelson, Sean Lynch, Donald McCormick, Paul Pencharz, Mary Frances Picciano, Ann Prentice, Bonny Specker and Barbara Underwood for reviewing the draft manuscript. They also express special appreciation for the financial support provided by the United Nations University.

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<sup>1</sup> Expert consultation on the optimal duration of exclusive breastfeeding, Geneva, World Health Organization, 28–30 March 2001.



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# Executive summary

In this review nutrient adequacy of exclusive breastfeeding is most commonly evaluated in terms of growth. Other functional outcomes, e.g. immune response and neurodevelopment, are considered when data are available. The dual dependency on exogenous dietary sources and endogenous stores for meeting requirements is also considered in evaluating human milk's nutrient adequacy. When evaluating the nutrient adequacy of human milk, it is essential to recognize the incomplete knowledge of infant nutrient requirements in terms of relevant functional outcomes. Particularly evident is the inadequacy of crucial data for evaluating the nutrient adequacy of exclusive breastfeeding for the first 4 to 6 months.

Mean intakes of human milk provide sufficient *energy* and *protein* to meet mean requirements during the first 6 months of infancy. Since infant growth potential drives milk production, the distribution of intakes likely matches the distribution of energy and protein requirements.

The adequacy of *vitamin A* and *vitamin B6* in human milk is highly dependent upon maternal diet and nutritional status. In well-nourished populations the amounts of vitamins A and B6 in human milk are adequate to meet the requirements for infants during the first 6 months of life. In populations deficient in vitamins A and B6, the amount of these vitamins in human milk will be sub-optimal and corrective measures are called for, either through maternal and/or infant supplementation, or complementary feeding for infants.

The *vitamin D* content of human milk is insufficient to meet infant requirements. Infants depend on sunlight exposure or exogenous intakes of vitamin D; if these are inadequate, the risk of vitamin D deficiency rises with age as stores become depleted in the exclusively breastfed infant.

The *calcium* content of human milk is fairly constant throughout lactation and is not influenced by maternal diet. Based on the estimated calcium intakes of exclusively breastfed infants and an estimated absorption efficiency of > 70%, human milk meets the calcium requirements of infants during the first 6 months of life.

The dual dependency on exogenous dietary sources and endogenous stores to meet requirements needs to be borne in mind particularly when assessing the adequacy of *iron* and *zinc* in human milk. Human milk, which is a poor source of iron and zinc, cannot be altered by maternal supplementation with these two nutrients. It is clear that the estimated iron requirements of infants cannot be met by human milk alone at any stage of infancy. The iron endowment at birth meets the iron needs of the breastfed infant in the first half of infancy, i.e. 0 to 6 months. If an exogenous source of iron is not provided, exclusively breastfed infants are at risk of becoming iron deficient during the second half of infancy. Net zinc absorption from human milk falls short of zinc needs, which appear to be subsidized by prenatal stores.

In the absence of studies specifically designed to evaluate the time at which prenatal stores become depleted, circumstantial evidence has to be used. Available evidence suggests that the older the exclusively breastfed infant the greater the risk of specific nutrient deficiencies.

The inability to estimate the proportion of exclusively breastfed infants at risk of specific deficiencies is a major drawback in terms of developing appropriate public health policies. Conventional methodologies require that a nutrient's average dietary requirement and its distribution are known along with the mean and distribution of intakes and endogenous stores.

Moreover, exclusive breastfeeding at 6 months is not a common practice in developed countries, and it is rarer still in developing countries. There is a serious lack of measurement, which impedes evaluation, of the human-milk intakes of 6-month-old exclusively breastfed infants from developing countries. The marked attrition rates in exclusive breastfeeding through 6 months postpartum, even among women who are both well nourished and highly motivated, is a major gap in our understanding of the biological, cultural and social determinants of the duration of exclusive breastfeeding. A limitation to promoting exclusive breastfeeding for the first 6 months of life is our lack of understanding of the reasons for the attrition rates. Improved understanding of the biological, socioeconomic and

cultural factors influencing the timing of supplementation of the breastfed infant's diet is an important part of advocating a globally uniform infant-feeding policy that accurately weighs both this policy's benefits and possible negative outcomes.

It is important to recognize that this review is limited to the nutrient needs of infants. No attempt has been made to evaluate functional outcomes that depend on other bioactive factors in human milk, or behaviours and practices that are inseparable from breastfeeding. Neither have the consequences, positive or negative,

for mothers been considered. It is important that functional outcomes, e.g. infant morbidity and mortality, be taken carefully into account in determining the optimal duration of exclusive breastfeeding in specific environments.

This review was prepared parallel to, but separate from, a systematic review of the scientific literature on the optimal duration of exclusive breastfeeding.<sup>1</sup> These assessments served as the basis for discussion during an expert consultation (Geneva, 28–30 March 2001), whose report is found elsewhere.<sup>2</sup>

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<sup>1</sup> Kramer MS, Kakuma R. *The optimal duration of exclusive breastfeeding: a systematic review*. Geneva, World Health Organization, document WHO/NHD/01.08–WHO/FCH/CAH/01.23, 2001.

<sup>2</sup> *The optimal duration of exclusive breastfeeding: report of an expert consultation*. Geneva, World Health Organization, document WHO/NHD/01.09–WHO/FCH/CAH/01.24, 2001.

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# 1. Conceptual framework

## 1.1 Introduction

Dietary surveys of presumably healthy populations, factorial approaches (summing needs imposed by growth and maintenance requirements), and balance techniques (measuring “inputs and outputs”) are the methods used most often to estimate nutrient requirements. None are particularly satisfactory because they seldom adequately address growing concerns that nutrient intakes support long-term health and optimal functional capacities rather than just avoid acute deficiency states. These concerns are most evident when considering the nutrient needs of infants because of the paucity of data for estimating most nutrient requirements and the limited number of functionally relevant outcome measures for this age group. As these limitations apply to nearly all the sections that follow, they will not be repeated.

Growth is the most commonly used functional outcome measure of nutrient adequacy. This outcome is particularly useful for screening purposes because the normal progression of growth is dependent on many needs being met and many physiological processes proceeding normally. However, this strength also betrays this outcome’s principal weakness since abnormal growth is highly non-specific. The single or multiple etiologies of abnormal growth are usually difficult to ascertain confidently. This is most apparent in the differential diagnosis of failure to thrive found in most standard paediatric texts (1). Yet, this outcome is key to present approaches for interpreting dietary surveys, calculating factorial estimates and evaluating outcomes of balance studies. Specific issues, which relate to dependence on growth for estimating nutrient needs by each of the above-listed methods, are considered in most of the sections that follow.

Another problem that is almost unique to infancy (possible exceptions may be found in specific processes during pregnancy and lactation) is that the normal progression of growth and development during this life stage likely relies on both exogenous sources and endogenous stores of nutrients. For exclusively breastfed infants, these are met by human milk and endogenous nutrient stores transferred to the infant from the mother during gestation (see sections below on iron, vitamins

A and D, and zinc). It is becoming increasingly clear that this is likely the case for iron, zinc and possibly calcium. Calcium is included because the physiological significance of the transient lower bone mineral content observed in breastfed infants, compared to their formula-fed counterparts, is not understood. Assessing nutrient needs without acknowledging this dual dependency likely leads to faulty conclusions.

To make matters yet more complicated, it is clear that there is a range between clear deficiency and “optimal” adequacy within which humans adapt. The closer one is to deficiency within that range, the more vulnerable one is to common stresses (e.g. infections) and the less one is able to meet increased physiological demands (e.g. growth spurts). Perhaps the best examples of the conceptual difficulties that arise due to the capacity of humans to “adapt” to a range of intakes are debates that swirl around the “small is beautiful” proposition and the “adaptation to lower energy intakes” viewpoint. The former has been discredited fairly conclusively while the latter has been abandoned in recent estimates of energy needs; this is in recognition of the fact that humans can adapt to a range of energy intakes, but at a cost whenever there are sustained deviations from requirement levels (2, 3). Thus, energy requirements are estimated on the basis of multiples of basal metabolic rate to ensure that needs are met for both maintenance and socially acceptable and necessary levels of physical activity (3).

## 1.2 Using ad libitum intakes to assess adequate nutrient levels

The paucity of available functional measures of optimal intakes compared to functional measures of deficiency leads most investigators interested in assessing infant nutrient requirements to base their estimates on data concerning nutrient intakes by presumably healthy, exclusively breastfed infants, i.e. those with no overt evidence of deficiency. This exercise generally relies on estimates of intake volumes and human milk nutrient composition. For some studies, estimates of both have been obtained in the same infant-mother dyad. In most cases, either milk volume or milk composition is

assumed. Data on day-to-day variability for either measure are available for only a few studies. The most notable exceptions to these generalities are requirement estimates for energy (4), protein (5) and iron (6). Factorial approaches are used most commonly to estimate average requirements for energy and these two nutrients.

Generally speaking, estimates of nutrient requirements for the first year of life are based on measured intakes of human milk during the first 6 months. Estimated needs during the second 6 months are sometimes determined by extrapolating from these intake measures. The reasons for selecting the first 6 months appear arbitrary. One can offer physiological milestones as a reason for selecting this age, e.g. changes in growth velocities, stability in nutrient concentrations in human milk, disappearance of the extrusion reflex, teething, and enhanced chewing capabilities. However, the variability in the ages at which these milestones are reached is far greater than the specificity that the cut-off suggests.

As noted above, growth may be used to justify selecting the first 6 months as a basis for estimating nutrient requirements, although its use this way has severe limitations. Waterlow & Thomson (7), for example, concluded that exclusive breastfeeding sustained normal growth for only approximately 3 months. WHO and others have questioned the present international reference used to reach this and other conclusions related to the maintenance of adequate growth (8). At present, there is no universally accepted reference or standard that is used for assessing the normality of either attained growth or growth velocity in infants. In the absence of such a reference or standard, rationales used in this review that rely on growth are based on WHO data (8) for attained growth and growth velocity.

The composition of human milk changes dramatically in the postpartum period as secretions evolve from colostrum to mature milk. The stages of lactation correspond roughly to the following times postpartum: colostrum (0–5 days), transitional milk (6–14 days), and mature milk (15–30 days). Changes in human-milk composition are summarized in Table 1. The first 3 to 4 months of lactation appear to be the period of most rapid change in the concentrations of most nutrients. After that period nutrient concentrations appear to be fairly stable as long as mammary gland involution has not begun (9, 10). However, few studies assess the dietary and physiological factors that determine either the rate of change in nutrient concentrations or inter-individual variability. Intake data appearing in subsequent sections are presented in monthly intervals.

All intake estimates are derived from nutrient concentrations and human-milk volumes obtained in studies of self-selected or opportunistic populations. In no case are randomly representative data available for these types of assessments. When data are available, variability of milk volume and composition are estimated by pooled weighted variances of specific studies cited for each nutrient. Unless otherwise stated only studies of “exclusively” or “predominantly” breastfed infants were used to make these estimates.

To the extent possible no cross-sectional data of milk volumes and milk composition have been used in subsequent sections in order to minimize self-selection biases that such data present (11). However, it should be noted that most longitudinally designed studies have significant attrition rates as lactation progresses. Thus, these data also present special problems that are difficult to overcome.

### 1.3 Factorial approaches

Factorial approaches are generally based on estimates of maintenance needs, nutrient accretion that accompanies growth, measures of digestibility and/or absorption (bioavailability), and utilization efficiency. The sum of maintenance needs and accretion could be used to estimate requirement levels if dietary nutrients were absorbed and utilized with 100% efficiency. Since this does not occur, however, the sum is corrected to account for absorption rates and utilization efficiency.

Generally speaking, with the exception of protein, only maintenance, bioavailability and accretion rates will be of concern in the application of factorial approaches that target nutrient needs of exclusively human-milk fed infants. Thus, again with the exception of protein, in the sections that follow the efficiency of utilization of absorbed nutrients will be assumed to be 100%. The utilization of absorbed nutrients is determined by the nutrient’s biological value, which relates to the efficiency with which a target nutrient (e.g. protein) is assimilated or converted to some functionally active form (e.g. efficiency of use of  $\beta$ -carotene compared to retinol).

Maintenance needs reflect endogenous losses related to cellular turnover (e.g. skin desquamation and intestinal epithelial shedding) and unavoidable metabolic inefficiency (e.g. endogenous urinary and biliary losses) of endogenous nutrient sources. Maintenance needs for young infants are known with greatest certainty where energy is concerned. Basal and resting metabolic rates generally are accepted as the best

**Table 1. Human milk composition**

Age (months)	Energy (kcal <sub>inh</sub> /g) <sup>a</sup>	Protein (g/l) <sup>a</sup>	Vitamin A (μmol/l) <sup>b</sup>	Vitamin D (ng/l) <sup>c</sup>	Vitamin B6 (mg/l) <sup>d</sup>	Calcium (mg/l) <sup>a</sup>	Iron (mg/l) <sup>a</sup>	Zinc (mg/l) <sup>a</sup>
1	0.67	11	1.7	645	0.13	266	0.5	2.1
2	0.67	9	1.7	645	0.13	259	0.4	2
3	0.67	9	1.7	645	0.13	253	0.4	1.5
4	0.67	8	1.7	645	0.13	247	0.35	1.2
5	0.67	8	1.7	645	0.13	241	0.35	1
6	0.67	8	1.7	645	0.13	234	0.3	1
7	0.67	8	1.7	645	0.13	228	0.3	0.75
8	0.67	8	1.7	645	0.13	222	0.3	0.75
9	0.67	8	1.7	645	0.13	215	0.3	0.75
10	0.67	8	1.7	645	0.13	209	0.3	0.5
11	0.67	8	1.7	645	0.13	203	0.3	0.5
12	0.67	8	1.7	645	0.13	197	0.3	0.5

<sup>a</sup> Reference 40.<sup>b</sup> Reference 6.<sup>c</sup> Reference 122.<sup>d</sup> Reference 150.

measure of energy maintenance needs. There are no unassailable estimates of protein maintenance needs of infants, whether or not breastfed, nor, for that matter, are there reliable estimates for any other nutrient. In adults, endogenous losses are estimated from data collected under conditions that limit the target nutrient's content in the diet to approximately zero.

Accretion rates are related to nutrient accumulations that accompany growth. In infancy, these rates are estimated from measured growth velocities and estimates of the composition of tissues gained as part of growth.

Bioavailability generally relates to the availability of nutrients for intestinal absorption (e.g. of ferric versus ferrous iron and the various forms of calcium commonly found in foodstuffs). The determinants of absorption are too nutrient-specific to be considered in this general introduction. Generally, the host's physiological state and the physical characteristics of nutrients as consumed are among the principal determinants of absorption.

In addition to a nutrient's obligatory losses that occur even when the target nutrient level falls to approximately zero, unavoidable losses are expected to increase as intake levels rise substantially above zero to meet physiological needs. This inefficiency is considered inconsistently in applications of factorial approaches, especially where the nutrient needs of infants are concerned. In the segments that follow, no allowance is made for this highly probable inefficiency other than in consideration of protein needs, and to the extent that

iron absorption rates are affected by the status of iron stores. For iron and other minerals, endogenous or unavoidable losses and the bioavailability of dietary sources are measurable simultaneously by multiple-tracer stable-isotope methods. Because these measurements are made at nutrient intakes above zero, estimates of bioavailability and endogenous losses include the unavoidable inefficiencies in both absorption and utilization that are incurred as intakes rise.

#### 1.4 Balance methods

Balance methodologies also have been used to estimate nutrient needs and utilization. The general strengths and weaknesses of balance methods have been reviewed extensively and thus will not be repeated (12). For present purposes it is sufficient to acknowledge two characteristics of balance methods. The first is that their interpretation often relies heavily on estimates derived by factorial approaches, that is the appropriateness of retained quantities of target nutrients is determined by comparison with expected retention based on estimates derived by factorial methods. Thus, estimates of growth velocity and tissue composition are key to interpreting balance results. The second characteristic is that balance results are complicated by the unidirectional biases that are inherent in the method. These biases always favour overestimation of retention for two reasons. Firstly, intakes are generally overestimated (i.e. even if balance experiments are carefully carried out, it is much easier to miss "spills" than it is to "overfeed") and, secondly,

underestimating losses is much likelier than overestimating them (i.e. it is easier to under- than to over-collect urine, faeces and skin losses).

## 1.5 Other issues

### 1.5.1 Morbidity patterns

Three other issues should also be considered, the first of which is the estimation of common morbidity patterns. Although estimates of nutrient requirements reflect needs during health, it is increasingly recognized that accumulated deficits resulting from infections – due to decreased intakes and increased metabolic needs and losses – must be replenished during convalescence. Thus, it is generally important to consider safety margins in estimating nutrient needs. In the case of exclusive breastfeeding, the estimates presented below assume that infants will demand additional milk to redress accumulated energy deficits, that the nursing mother is able to respond to these increased demands, and that the increased micronutrient and protein intakes accompanying transient increases in total milk intake correct shortfalls accumulated during periods of illness. These assumptions are based on the generally recognized well-being of successfully breastfed infants, who experience occasional infections and live under favourable conditions. We recognize that no direct data are available to evaluate these assumptions under less favourable circumstances and that not enough is known to estimate the effects of possible constraints on maternal abilities to respond to transient increased demands by infants or constraints imposed by inadequate nutrient stores.

### 1.5.2 Non-continuous growth

The second issue is the possibility of non-continuous growth evaluated by Lampl, Veldhuis & Johnson (13). Estimates of nutrient needs based on factorial approaches assume steady, continuous growth. The literature reports observations in support of the possibility that growth occurs in spurts during infancy. Non-continuous growth's potential demands on nutrient stores and/or exogenous intakes have not been examined sufficiently, and thus no allowance for “non-continuous” growth needs is made in these assessments.

### 1.5.3 Estimating the proportion of a group at risk for specific nutrient deficiencies

The third issue relates to the challenges of estimating the proportion of exclusively breastfed infants at risk of specific nutrient deficiencies using either the “probability approach” (14) or the simplified estimated average requirement (EAR) cut-point method described by Beaton (15). The probability approach estimates the proportion of a target group at risk for a specific nutrient deficiency/inadequacy based on the distributions of the target group's average estimated nutrient requirement and the group's ad libitum intake of the nutrient of interest. To use this approach, intakes and requirements should not be correlated and the distributions of requirements and intakes should be known. The EAR cut-point method is a simplified application of the probability approach; it can be used to estimate the proportion of a population at risk when ad libitum intakes and requirements are not correlated, inter-individual variation in the EAR is symmetrically distributed around the mean, and variance of intakes is substantially greater than the variance of the EAR. The dependence of both approaches on a lack of correlation between intakes and requirements presents some difficulties to the extent that the energy intakes, nutrient requirements and ad libitum milk intakes of exclusively breastfed infants are related to each other. This difficulty arises because milk production is driven by the infant's energy demands and by maternal abilities to meet them. Thus, as energy requirements rise, so should the intakes of all human-milk constituents.

The nature of the expected correlation can be illustrated by interrelationships between milk composition and energy and protein requirements imposed by growth. The protein-to-energy ratio of mature human milk is approximately 0.013 g protein/kcal<sub>th</sub> (16).<sup>1</sup> The energy cost of growth is approximately 19 kcal<sub>th</sub>/kg, 12 kcal<sub>th</sub>/kg, 9 kcal<sub>th</sub>/kg and 5 kcal<sub>th</sub>/kg for the age intervals 3–4 months, 4–5 months, 5–6 months and 6–9 months, respectively (4). To the degree that increased energy requirements imposed by growth drive increased human-milk consumption, the corresponding increase in protein intakes will be, respectively, 0.25, 0.15, 0.12 and 0.06 g protein/kg for the four above-mentioned age intervals. These values will increase to the extent that non-protein nitrogen (NPN) in human milk is utilizable (see section 3.2.3). The protein deposited per kg of body weight appears fairly stable, approximately 0.24 g/kg from 4 to 9 months of age (4). If we assume a net absorption rate of 0.85 for human-milk protein and an efficiency of dietary protein utilization of 0.73, the mean dietary protein requirement for growth is approximately

<sup>1</sup> 1000 kcal<sub>th</sub> is equivalent to 4.18 MJ.

0.39 g protein/kg (see section 3.2.3). Thus, although increased energy needs imposed by growth should simultaneously drive protein intakes upward, human milk becomes less likely to meet the infant's need for protein unless energy requirements for activity increase in a manner that corrects the asynchrony described above. In the absence of such an adjustment, as long as human milk remains the only source of protein the growing infant becomes increasingly dependent upon stable or enhanced efficiencies in protein utilization. These types of correlations can be dealt with, in part, by suitable statistical techniques, as was demonstrated in the report of the International Dietary Energy Consultative Group (IDECG) evaluating protein and energy requirements (4, 5).

However, the challenges presented by relationships among milk intakes and micronutrient requirements and intakes are more problematic. Theoretically, the same type of relationship exists among energy and micronutrient intakes and requirements as described above for protein but with an added complication. As will be evident in the sections that follow, it is clear that physiological needs for vitamin A, vitamin D, iron, zinc and possibly other nutrients are met by the combined availability of nutrients from human milk and nutrient stores transferred from mother to infant during late gestation. Thus, dietary nutrient requirements vary with the adequacy of those stores. As a consequence there is inadequate information to estimate "true" physiological requirements (i.e. the optimal amounts of a nutrient that should be derived from human milk and from stores accumulated during gestation). We therefore have inadequate information to estimate what the dietary EAR is for any of the nutrients for which

there is a co-dependency on stores and an exogenous supply to meet physiological needs. Arriving at an EAR for specific nutrients based on the intakes of healthy breastfed infants assumes, by definition, "optimal" nutrient stores. However, this assumption grows progressively more precarious as the nutritional status of pregnant women becomes increasingly questionable.

#### 1.5.4 Summary

None of the available methods for assessing the nutrient needs of infants are entirely satisfactory because they address only short-term outcomes rather than short- and longer-term consequences for health. Of particular concern is the heavy dependence of most methods on growth in the absence of acceptable references/standards of normal attained growth and velocity, and their normal variability. A similar observation can be made regarding the paucity of information on the causes of the high attrition occurring in nearly all longitudinal studies of exclusive breastfeeding in the period of interest, i.e. beyond the first 4 months of life. Similarly, poor understanding of the determinants of inter-individual variability in the nutrient content of human milk creates significant problems in assessing key questions related to the assessment of present methods for estimating nutrient requirements in the first year of life. The infant's co-dependence on nutrient stores acquired during gestation and nutrients from human milk further complicates estimation of nutrient requirements. This is particularly vexing in applying methods for assessing population rates of inadequacy that require estimates of average nutrient requirements.

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## 2. Human-milk intake during exclusive breastfeeding in the first year of life

### 2.1 Human-milk intakes

Human-milk intakes of exclusively and partially breastfed infants during the first year of life in developed and developing countries are presented in Table 2 and Table 3, respectively. Studies conducted in presumably well-nourished populations from developed countries and in under-privileged populations from developing countries in the 1980s–1990s were compiled. In most of these studies, human-milk intake was assessed using the 24-hour test-weighing method. However, the 12-hour test-weighing method (17, 18) and the deuterium dilution method (19–21) were also used in a few cases. If details were not provided in the publication regarding the exclusivity of feeding, partial breastfeeding was assumed. The overall mean human-milk intakes were weighted for sample sizes and a pooled standard deviation (SD) was calculated across studies.

Mean human milk intake of exclusively breastfed infants, reared under favourable environmental conditions, increases gradually throughout infancy from 699 g/day at 1 month, to 854 g/day at 6 months and to 910 g/day at 11 months of age. The mean coefficient of variation across all ages was 16% in exclusively breastfed infants compared to 34% in partially breastfed infants. Milk intakes among the partially breastfed hovered around 675 g/day in the first 6 months of life and 530 g/day in the second 6 months.

There is a notable decrease in sample size in studies encompassing the transitional period from exclusive breastfeeding to partial breastfeeding (22–27).

### 2.2 Nutrient intakes of exclusively breastfed infants

Nutrient intakes derived from human milk were calculated (Table 4) based on the mean milk intakes of exclusively breastfed infants from developed countries (Table 2) and human milk composition from well-nourished women (Table 1). The small samples of exclusively breastfed infants between 7 and 12 months of age limit the general applicability of these calculations for older breastfed infants.

### 2.3 Duration of exclusive breastfeeding

Although reasons for supplementation are not always discernible from the literature, evidence to date clearly indicates that few women exclusively breastfeed beyond 4 months. Numerous socioeconomic and cultural factors influence the decision to supplement human milk, including medical advice, maternal work demands, family pressures and commercial advertising. Biological factors including infant size, sex, development, interest/desire, growth rate, appetite, physical activity and maternal lactational capacity also determine the need and timing of complementary feeding. However, neither socioeconomic nor cultural nor biological factors have received adequate systematic attention.

In a longitudinal study in the USA, human-milk intake of infants was measured from 4 to 9 months through the transitional feeding period (26). Complementary feeding was started at the discretion of the mother in consultation with the child's paediatrician. Forty-two per cent (19/45) of the infants were exclusively breastfed until 5 months of age, 40% (18/45) until 6 months, and 18% (8/45) until 7 months.

In a Finnish study (25), 198 women intended to breastfeed for 10 months. The number of exclusively breastfed infants was 116 (58%) at 6 months, 71 (36%) at 7.5 months, 36 (18%) at 9 months, and 7 (4%) at 12 months. The reason given for introducing complementary feeding before the age of 4 to 6 months was the infant's demand appeared greater than the supply of human milk. This was decided by the mother in 77 cases and by the investigators in 7 cases. Complementary feeding reversed the progressive decline in the standard deviation score (SDS) for length from  $-0.52$  to  $-0.32$  ( $p=0.07$ ) during the 6 to 9-month period. These authors concluded that, although some infants can thrive on exclusive breastfeeding until 9 to 12 months of age, on a population level prolonged exclusive breastfeeding carries a risk of nutritional deficiency even in privileged populations.

In a study in the USA of growth and intakes of energy and zinc in infants fed human milk, despite intentions to exclusively breastfeed for 5 months, 23% of mothers added solids to their infant's diet at 4.5 months; 55%



**Table 2. Human-milk intake of infants from developed countries**

Reference	Country	Age (months)																	
		1			2			3			4			5			6		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Exclusively breastfed infants</b>																			
Butte et al. (19)	USA							691	141	8	724	117	14						
Butte et al. (16)	USA	751	130	37	725	131	40	723	114	37	740	128	41						
Chandra (22)	Canada										793	71	33	856	99	31	925	112	28
Dewey & Lönnerdal (23)	USA	673	192	16	756	170	19	782	172	16	810	142	13	805	117	11	896	122	11
Dewey et al. (29)	USA (boys)							856	129	34									
Dewey et al. (29)	USA (girls)							775	125	39									
Goldberg et al. (212)	UK	802	179	10				792	177	10									
Hofvander et al. (213)	Sweden	656		25	773		25	776		25									
Janas et al. (214)	USA				701		11	709		11									
Krebs et al. (28)	USA							690	110	71									
Köhler et al. (215)	Sweden				746	101	26	726	143	21									
Lönnerdal et al. (216)	Sweden	724	117	11	752	177	12							756	140	12			
Michaelsen et al. (40)	Denmark				754	167	60				827	139	36						
Neville et al. (24)	USA	668	117	12	694	98	12	734	114	10	711	100	12	838	134	12	820	79	9
Pao et al. (217)	USA	600	159	11				833		2							682		1
Picciano et al. (218)	USA	606	135	26	601	123	26	626	117	26									
Rattigan et al. (219)	Australia	1187	217	5				1238	168	5									
Salmenperä et al. (61)	Finland										790	140	12				800	120	31
Stuff et al. (220)	USA													735	65	9			
Stuff & Nichols (26)	USA										792	111	19						
Stuff & Nichols (26)	USA										792	111	19						
Stuff & Nichols (26)	USA										734	150	18	729	165	18			
Stuff & Nichols (26)	USA										792	189	8	769	198	8	818	166	8
van Raaij et al. (221)	Netherlands	692	122	16	718	122	16												
van Raaij et al. (221)	Netherlands				745	131	40												
Whitehead & Paul (27)	UK (boys)				791	116	27	820	187	23	829	168	18	790	113	5	922		1
Whitehead & Paul (27)	UK (girls)				677	87	20	742	119	17	775	138	14	814	113	6	838	88	4
Wood et al. (222)	USA	688	137	17	729	178	20	758	201	21	793	215	19	789	195	19			
<b>Mean, weighted for sample size</b>		<b>699</b>			<b>731</b>			<b>751</b>			<b>780</b>			<b>796</b>			<b>854</b>		
<b>Pooled SD</b>		<b>134</b>			<b>132</b>			<b>130</b>			<b>138</b>			<b>141</b>			<b>118</b>		
<b>N</b>		<b>186</b>			<b>354</b>			<b>376</b>			<b>257</b>			<b>131</b>			<b>93</b>		
<b>Number of study groups</b>		<b>11</b>			<b>14</b>			<b>17</b>			<b>13</b>			<b>10</b>			<b>8</b>		

**Table 2. Human-milk intake of infants from developed countries (continued)**

Reference	Country	Age (months)																			
		1			2			3			4			5			6				
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N		
<b>Partially breastfed Infants</b>																					
Dewey et al. (29)	USA (boys)																		814	183	27
Dewey et al. (29)	USA (girls)																		733	155	33
Köhler et al. (215)	Sweden													722	114	13			689	120	12
Krebs et al. (28)	USA													720	130	16					
Michaelsen et al. (40)	Denmark				488	232	16					531	277	26							
Pao et al. (217)	USA	485	79	4				467	100	11									395	175	6
Paul et al. (223)	UK				787	157	28	824	176	28	813	168	28	717	192	25			593	207	26
Paul et al. (223)	UK				676	87	20	728	141	19	741	182	20	716	233	17			572	225	19
Prentice et al. (224)	UK				741	142	48	785	168	47	783	176	48	717	207	42			588	206	45
Rattigan et al. (219)	Australia																		11282	16.9	5
Stuff et al. (220)	USA																		640	94	17
Stuff & Nichols (26)	USA													703	156	19			595	181	19
Stuff & Nichols (26)	USA																		648	196	18
van Raaij et al. (221)	Netherlands							746	175	16											
Whitehead & Paul (27)	UK (boys)				648		1	833	123	5	787	172	10	699	204	20			587	188	25
Whitehead & Paul (27)	UK (girls)							601		2	664	258	6	662	267	11			500	194	15
WHO (225)	Hungary	607	123	84	673	144	86	681	147	85	631	168	85						539	150	85
WHO (225)	Sweden	642	149	28	745	148	28	776	95	28	791	131	28						560	208	28

<b>Mean, weighted for sample size</b>	<b>611</b>	<b>697</b>	<b>730</b>	<b>704</b>	<b>710</b>	<b>612</b>
<b>Pooled SD</b>	<b>129</b>	<b>150</b>	<b>149</b>	<b>184</b>	<b>194</b>	<b>180</b>
<b>N</b>	<b>116</b>	<b>227</b>	<b>241</b>	<b>251</b>	<b>163</b>	<b>380</b>
<b>Number of study groups</b>	<b>3</b>	<b>7</b>	<b>9</b>	<b>8</b>	<b>8</b>	<b>15</b>

Reference	Country	Age (months)																	
		7			8			9			10			11			12		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Exclusively breastfed Infants</b>																			
Chandra (22)	Canada	872	126	27	815	97	24												
Neville et al. (24)	USA	848	63	6	818	158	3												
Salmenperä et al. (67)	Finland							890	140	16				910	133	10			
Whitehead & Paul (27)	UK	854		1															

<b>Mean, weighted for sample size</b>	<b>867</b>	<b>815</b>	<b>890</b>		<b>910</b>
<b>Pooled SD</b>	<b>118</b>	<b>103</b>	<b>140</b>		<b>133</b>
<b>N</b>	<b>34</b>	<b>27</b>	<b>16</b>		<b>10</b>
<b>Number of study groups</b>	<b>3</b>	<b>2</b>	<b>1</b>		<b>1</b>

**Table 2. Human-milk intake of infants from developed countries (continued)**

Reference	Country	Age (months)																	
		7			8			9			10			11			12		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Partially breastfed infants</b>																			
Dewey et al. (43)	USA	875	142	8	834	99	7	774	180	5	691	233	5	516	215	6	759	28	2
Dewey et al. (29)	USA (boys)							687	233	25							499	270	20
Dewey et al. (29)	USA (girls)							605	197	25							402	228	22
Krebs et al. (28)	USA	640	150	71															
Michaelsen et al. (40)	Denmark							318	201	18									
Pao et al. (217)	USA							554		3									
Paul et al. (223)	UK	484	182	21	340	206	18	251	274	12									
Paul et al. (223)	UK	506	255	16	367	266	12	443	319	7									
Prentice et al. (224)	UK	493	216	38	342	228	31	328	292	19									
Rattigan et al. (219)	Australia							884	252	4							880	74	4
Stuff & Nichols (26)	USA	551	142	19															
Stuff & Nichols (26)	USA	602	186	18	522	246	18												
Stuff & Nichols (26)	USA	677	242	8	645	250	8	565	164	8									
van Raaij et al. (221)	Netherlands	573	187	16															
Whitehead & Paul (27)	UK (boys)	484	181	21	342	203	18												
Whitehead & Paul (27)	UK (girls)	481	246	15	329	242	11												
WHO (225)	Sweden							452	301	28									
<b>Mean, weighted for sample size</b>		<b>569</b>			<b>417</b>			<b>497</b>			<b>691</b>			<b>516</b>			<b>497</b>		
<b>Pooled SD</b>		<b>188</b>			<b>226</b>			<b>249</b>			<b>233</b>			<b>215</b>			<b>238</b>		
<b>N</b>		<b>251</b>			<b>123</b>			<b>154</b>			<b>5</b>			<b>6</b>			<b>48</b>		
<b>Number of study groups</b>		<b>11</b>			<b>8</b>			<b>11</b>			<b>1</b>			<b>1</b>			<b>4</b>		

**Table 3. Human-milk intake of infants from developing countries**

Reference	Country	Age (months)																	
		1			2			3			4			5			6		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Exclusively breastfed infants</b>																			
Butte et al. (20)	Mexico										885	146	15						
Cohen et al. (30)	Honduras										806		50	824		50	823		50
Gonzalez-Cossio et al. (226)	Guatemala	661	135	27	749	143	27							776	153	27			
Naing & Co (18)	Myanmar	423	20	29	480	20	29	556	30	29	616	16	24	655	27	17	751	15	6
van Steenberg et al. (227)	Indonesia	828	41	5	862	184	6	732	90	5	768	109	6	728	101	3	727	224	8
<b>Mean, weighted for sample size</b>		<b>562</b>			<b>634</b>			<b>582</b>			<b>768</b>			<b>778</b>			<b>804</b>		
<b>Pooled SD</b>		<b>92</b>			<b>110</b>			<b>42</b>			<b>63</b>			<b>83</b>			<b>76</b>		
<b>N</b>		<b>61</b>			<b>62</b>			<b>34</b>			<b>95</b>			<b>97</b>			<b>64</b>		
<b>Number of study groups</b>		<b>3</b>			<b>3</b>			<b>2</b>			<b>4</b>			<b>4</b>			<b>3</b>		

**Table 3. Human-milk intake of infants from developing countries (continued)**

Reference	Country	Age (months)																		
		1			2			3			4			5			6			
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	
<b>Partially breastfed infants</b>																				
Butte et al. (20)	Mexico																	869	150	15
Cohen et al. (30)	Honduras									799	47		688	47				699		47
Cohen et al. (30)	Honduras									787	44		731	44				725		44
Coward et al. (21)	Papua New Guinea				670	190	17													
de Kanashiro et al. (17)	Peru				685	245	129					690	240	126				655	226	113
Frigerio et al. (228)	Gambia				738	47	16													
Gonzalez-Cossio et al. (226)	Guatemala	655	198	26	726	153	26							721	166	26		720	165	26
Gonzalez-Cossio et al. (226)	Guatemala	719	138	22	789	112	22							804	128	22		776	121	22
Gonzalez-Cossio et al. (226)	Guatemala	887	125	27	727	113	27							769	128	27		771	117	27
Hennart & Vis (229)	Central Africa	517	169	8				605	78	22								525	95	29
Prentice et al. (224)	Gambia	649	113	7	705	183	8	782	168	6	582	169	10	643	149	17				
van Steenberg et al. (228)	Kenya	778	180	7				619	197	13				573	208	9				
van Steenberg et al. (227)	Indonesia	693	138	32	691	117	31	712	118	29	725	131	30	691	97	31	664	109	26	
WHO (225)	Guatemala (urban)	524	246	32	561	222	30	653	255	28										
WHO (225)	Philippines (urban)	336	191	34	404	242	25	320	200	20	344	244	10					374	117	16
WHO (225)	Guatemala (urban)	519	186	28				548	173	30								586	185	28
WHO (225)	Philippines (urban)	502	176	32	577	154	23	693	117	32	586	167	27					597	214	30
WHO (225)	Guatemala (rural)	543	131	28				686	151	27								588	142	28
WHO (225)	Philippines (rural)	571	187	27	689	216	30	622	221	28	613	201	23					589	136	29
WHO (225)	Zaire (urban)	609	244	135	656	256	156	588	202	99	607	185	58					641	198	115
WHO (225)	Zaire (rural)	338	159	52	355	132	50	356	173	57	368	147	66					357	170	99

<b>Mean, weighted for sample size</b>	<b>568</b>	<b>636</b>	<b>574</b>	<b>634</b>	<b>714</b>	<b>611</b>
<b>Pooled SD</b>	<b>196</b>	<b>212</b>	<b>182</b>	<b>177</b>	<b>107</b>	<b>166</b>
<b>N</b>	<b>497</b>	<b>590</b>	<b>391</b>	<b>441</b>	<b>223</b>	<b>694</b>
<b>Number of study groups</b>	<b>15</b>	<b>14</b>	<b>12</b>	<b>10</b>	<b>8</b>	<b>16</b>

Reference	Country	Age (months)																	
		7			8			9			10			11			12		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Exclusively breastfed infants</b>																			
van Steenberg et al. (227)	Indonesia	740	7	2	691	143	6												

<b>Mean, weighted for sample size</b>	<b>740</b>	<b>691</b>			
<b>Pooled SD</b>	<b>7</b>	<b>143</b>			
<b>N</b>	<b>2</b>	<b>6</b>			
<b>Number of study groups</b>	<b>1</b>	<b>1</b>			

**Table 3. Human-milk intake of infants from developing countries (continued)**

Reference	Country	Age (months)																		
		7			8			9			10			11			12			
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	
<b>Partially breastfed Infants</b>																				
Coward et al. (21)	Papua New Guinea	936	173	8																
de Kanashiro et al. (17)	Peru				624	219	110						565	208	100					
Hennart & Vis (229)	Central Africa							580	73	39								582	55	43
van Steenberg et al. (227)	Indonesia	617	80	28	635	149	23													
WHO (225)	Philippines (urban)							321	156	16										
WHO (225)	Philippines (urban)							558	183	31								548	158	29
WHO (225)	Guatemala (urban)							587	186	28										
WHO (225)	Zaire (urban)							613	193	72								593	192	60
WHO (225)	Guatemala (rural)							602	187	28										
WHO (225)	Philippines (rural)							534	176	32								502	185	26
WHO (225)	Zaire (rural)							378	153	91								407	174	85
<b>Mean, weighted for sample size</b>		<b>688</b>			<b>635</b>			<b>516</b>						<b>565</b>			<b>511</b>			
<b>Pooled SD</b>		<b>106</b>			<b>149</b>			<b>167</b>						<b>208</b>			<b>164</b>			
<b>N</b>		<b>36</b>			<b>23</b>			<b>337</b>						<b>100</b>			<b>243</b>			
<b>Number of study groups</b>		<b>2</b>			<b>1</b>			<b>8</b>						<b>1</b>			<b>5</b>			

**Table 4. Nutrient intakes derived from human milk<sup>a</sup>**

Age (month)	Human milk intake (g/day)	Human milk intake, corrected for IWL <sup>b</sup> (g/day)	Energy (kcal <sub>th</sub> /day)	Protein (g/day)	Vitamin A (µmol/day)	Vitamin D (ng/day)	Vitamin B6 (mg/day)	Calcium (mg/day)	Iron (mg/day)	Zinc (mg/day)
1	699	734	492	8.1	1.25	473	0.1	195	0.37	1.54
2	731	768	514	6.9	1.3	495	0.1	199	0.31	1.54
3	751	803	538	7.2	1.37	518	0.1	203	0.32	1.20
4	780	819	549	6.6	1.39	528	0.11	202	0.29	0.98
5	796	836	560	6.7	1.42	539	0.11	201	0.29	0.84
6	854	897	601	7.2	1.52	578	0.12	210	0.27	0.90
7	867	910	610	7.3	1.55	587	0.12	208	0.27	0.68
8	815	856	573	6.8	1.45	552	0.11	190	0.26	0.64
9	890	935	626	7.5	1.59	603	0.12	201	0.28	0.70
10	900	945	633	7.6	1.61	610	0.12	198	0.28	0.47
11	910	956	640	7.6	1.62	616	0.12	194	0.29	0.48

<sup>a</sup> Nutrient intakes calculated based on the mean milk intakes of exclusively breastfed infants from developed countries (Table 2) and human milk composition from well-nourished women (Table 1).

<sup>b</sup> IWL = insensible water losses.

added solids at 6 months and 93% added solids at 7 months (28).

In a Canadian study, the growth performance of 36 exclusively breastfed infants was monitored (22). The number (percent) of children displaying growth faltering – defined as below the NCHS 10th weight-for-age percentile – increased from 3 (8.3%) at 4 months to 5 (13.6%) at 5 months, 8 (22.2%) at 6 months, 9 (25%) at 7 months, and 12 (33.3%) at 8 months. Even in well-nourished women, exclusive breastfeeding did not sustain growth beyond 4 months of age according to the 1977 growth curves; furthermore, growth faltering was associated with higher rates of infectious morbidity.

Breastfed boys consistently consumed more human milk than breastfed girls did (29, 27). Girls tended to be exclusively breastfed longer than boys were; complementary foods were offered to boys at 4.1 months and to girls at 4.9 months (27). In the same study, after 4 months only 20% of the boys and 35% of the girls were exclusively breastfed. Complementary feeding resulted in some increase in total energy intake in boys but not in girls.

Since exclusive breastfeeding is rare in developing countries, the number of observational studies on human-milk intakes of exclusively breastfed infants is limited. An intervention study was conducted in Honduras where one group ( $n=50$ ) was required to breastfeed exclusively for 6 months (30). Although this is an important study, it may not be totally representative of all mothers and infants in that community. Sixty-four women were ineligible to participate because they did not maintain exclusive breastfeeding through 16 weeks for the following reasons: insufficient milk ( $n=26$ ), personal choice ( $n=16$ ), maternal health ( $n=12$ ), and family pressure not to breastfeed exclusively ( $n=10$ ). Weight gain ( $1092 \pm 356$  g) in the exclusively breastfed group was similar to the supplemented groups; however, the SD ( $\pm 409$  g) of weight gain of exclusively breastfed infants of mothers with low BMI was greater than the supplemented infants in both groups. It is unclear whether all infants were growing satisfactorily. Based on this limited number of studies, intakes of exclusively breastfed infants were, on average, similar to those of infants between 4 and 6 months of age from developed countries.

More recently, encouraging results have accrued from community-based breastfeeding promotion programmes in developing countries. For example, an intervention conducted in Mexico to promote exclusive breastfeeding succeeded in increasing rates of predominant breastfeeding above controls at 3 months postpartum from

12% in controls to 50% and 67% in the experimental groups (31). Rates of exclusive breastfeeding were 12% in controls and 38–50% in experimental groups. Although the programme succeeded in promoting exclusive breastfeeding, it did not approach the goal of exclusive breastfeeding for 6 months.

Meanwhile, in Dhaka, Bangladesh, counsellors – local mothers who received 10 days' training – paid 15 home-based counselling visits (2 in the last trimester of pregnancy, 3 early postpartum, and fortnightly until infants were 5 months old) in the intervention group (32). For the primary outcome, the prevalence of exclusive breastfeeding at 5 months was 202/228 (70%) for the intervention group and 17/285 (6%) for the control group. For the secondary outcomes, mothers in the intervention group initiated breastfeeding earlier than control mothers and were less likely to give prelacteal and postlacteal foods. At day 4, significantly more mothers in the intervention group breastfed exclusively than controls.

## 2.4 Summary

Longitudinal studies conducted among well-nourished women indicate that, during exclusive breastfeeding, human-milk production rates gradually increase from ~700 g/day to 850 g/day at 6 months. Because of the high attrition rates in these studies, the corresponding milk-production rates represent only a select group of women and thus do not reflect the population variability in milk production and infant nutrient requirements.

Exclusive breastfeeding at 6 months is not a common practice in developed countries and appears to be rarer still in developing countries. Moreover, there is a serious lack of documentation and evaluation of human-milk intakes of 6-month-old exclusively breastfed infants from developing countries. A limitation to the uniform recommendation of exclusive breastfeeding for the first 6 months of life is the lack of understanding of reasons for the marked attrition rates in exclusive breastfeeding, even among highly motivated women, in the lactation period of interest.

The limited relevant evidence suggests that sufficiency of exclusive breastfeeding is infant-specific (e.g. based on sex, size and growth potential), in addition to being linked to maternal lactational capacity and environmental factors that may affect an infant's nutritional needs and a mother's ability to respond to them. Nevertheless, recent intervention studies suggest that these variables are amenable to improvement in the presence of adequate support.

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## 3. Energy and specific nutrients

### 3.1 Energy

#### 3.1.1 Energy content of human milk

Proteins, carbohydrates and lipids are the major contributors to the energy content of human milk (33). Protein and carbohydrate concentrations change with duration of lactation, but they are relatively invariable between women at any given stage of lactation. In contrast, lipid concentrations vary significantly between both individual women and populations, which accounts for the variation observed in the energy content of human milk.

Differences in milk sampling and analytical methods also contribute to the variation in milk energy (34, 35). Within-day, within-feeding, and between-breast variations in milk composition; interference with milk “let-down”; and individual feeding patterns affect the energy content of human milk. In the present context, two milk-sampling approaches have been used to estimate the energy content of human milk – expression of the entire contents of one or both breasts at a specific time or for a 24-hour period, and collection of small aliquots of milk at different intervals during a feed. Human milk’s energy content was determined directly from its heat of combustion measured in an adiabatic calorimeter, or indirectly from the application of physiological fuel values to the proximate analysis of milk protein, lactose and fat.

The mean energy content of human milk ranges from 0.62 kcal<sub>th</sub>/g to 0.80 kcal<sub>th</sub>/g (33). For present purposes, a value of 0.67 kcal<sub>th</sub>/g has been assumed.

#### 3.1.2 Estimates of energy requirements

The energy requirements of infants may be derived from total energy expenditure and energy deposition (4). Total energy expenditure was measured by using the doubly labelled water method and energy deposition from protein and fat accretion in breastfed and formula-fed infants at 3, 6, 9, 12, 18 and 24 months of age (36). In this study, the mean coefficient of variation for total energy expenditure (TEE) and total energy requirements were 18% and 17%, respectively, across all ages.

Total energy requirements of breastfed infants (Table 5) were estimated using weight at the 50th percentile of the WHO pooled breastfed data set (8). An allowance for growth was derived from the weight gains at the 50th percentile of the WHO pooled breastfed data set (8), the rates of fat and protein accretion, and the energy equivalents of protein and fat deposition taken as 5.65 kcal<sub>th</sub>/g and 9.25 kcal<sub>th</sub>/g, respectively (37). The TEE of breastfed infants (36) was predicted at monthly intervals using the equation  $TEE \text{ (kcal}_{th}/\text{day}) = 92.8 * \text{Weight (kg)} - 151.7$ .

Energy intakes based on the mean milk intakes of exclusively breastfed infants appeared to meet mean energy requirements during the first 6 months of life. Since infant size and growth potential drive energy intake, it is reasonable to assume a positive relationship between energy intake and energy requirements. Positive correlations between energy intake and infant weight, and energy intake and weight gain, have been reported (37–39). The matching of intake to requirements for energy is unique in this regard. Thus, it is likely that infant energy needs can be met for 6 months, and possibly longer, by women wishing to breastfeed exclusively this long. The major shortcoming appears to be the marked attrition rates in exclusive breastfeeding, even among women who seem to be highly motivated and who have presumably good support networks. There is a major gap in our understanding of the role – and the relative positive or negative contribution – of biological and social determinants of observed attrition rates.

#### 3.1.3 Summary

Energy requirements derived from the sum of total energy expenditure and energy deposition were used to evaluate the adequacy of human milk to support the energy needs of exclusively breastfed infants. Energy intakes based on the mean milk intakes of exclusively breastfed infants appear to meet mean energy requirements during the first 6 months of life. Since infant growth potential drives milk production, it is likely that the distribution of energy intakes matches the distribution of energy requirements. Women who

**Table 5. Energy requirements of breastfed Infants**

	Weight (kg) <sup>a</sup>	Weight velocity (g/day) <sup>a</sup>	Total energy expenditure (kcal <sub>th</sub> /day) <sup>b</sup>	Energy deposition (kcal <sub>th</sub> /day) <sup>c</sup>	Energy requirement (kcal <sub>th</sub> /day)
<b>Boys</b>					
1	4.58	35.2	273	211	485
2	5.50	30.4	359	183	541
3	6.28	23.2	431	139	570
4	6.94	19.1	492	53	546
5	7.48	16.1	542	45	588
6	7.93	12.8	584	36	620
7	8.3	11	619	17	635
8	8.62	10.4	648	16	664
9	8.89	9	673	14	687
10	9.13	7.9	696	21	717
11	9.37	7.7	718	21	739
12	9.62	8.2	741	22	763
<b>Girls</b>					
1	4.35	28.3	252	178	430
2	5.14	25.5	325	161	486
3	5.82	21.2	388	134	522
4	6.41	18.4	443	68	511
5	6.92	15.5	490	57	548
6	7.35	12.8	530	47	578
7	7.71	11	564	20	584
8	8.03	9.2	593	17	610
9	8.31	8.4	619	15	635
10	8.55	7.7	642	18	660
11	8.73	6.6	663	15	678
12	9	6.3	684	14	698

<sup>a</sup> Reference 8.<sup>b</sup> Reference 36.<sup>c</sup> Reference 37.

wish to breastfeed exclusively can meet their infants' energy needs for 6 months.

## 3.2 Proteins

### 3.2.1 Dietary proteins

Dietary proteins provide approximately 8% of the exclusively breastfed infant's energy requirements and the essential amino acids necessary for protein synthesis. Thus, the quantity and quality of proteins are both important. Because protein may serve as a source of energy, failure to meet energy needs decreases the efficiency of protein utilization for tissue accretion and other metabolic functions. Protein undernutrition produces long-term negative effects on growth and neurodevelopment.

### 3.2.2 Protein composition of human milk

The protein content of mature human milk is approximately 8–10 g/l (33). The concentration of protein changes as lactation progresses. By the second week postpartum, when the transition from colostrum to mature milk is nearly complete, the concentration of protein is approximately 12.7 g/l (40). This value drops to 9 g/l by the second month, and to 8 g/l by the fourth month where it appears to remain until well into the weaning process when milk volumes fall substantially. At this point protein concentrations increase as involution of the mammary gland progresses. The inter-individual variation of the protein content of human milk, whose basis is unknown (41), is approximately 15%.

Several methods have been used to analyse the protein content of human milk and each has yielded different results with implications for the physiology and



nutrition of the breastfed infant (42). Direct analyses include the determination of total nitrogen by the Kjeldahl method and total amino-acid analysis. To derive the protein nitrogen content by the Kjeldahl method, the NPN fraction is separated by acid precipitation. Indirect analyses based on the protein molecule's characteristics include the Biuret method (peptide bond), Coomassie-Blue/BioRad, BCA method (dye-binding sites) and the Lowry method (tyrosine and phenylalanine content). The Biuret method, whose results conflict with the BCA method, is not recommended for use in human milk because of high background interference. The Lowry method, although efficient, is subject to technical difficulties (e.g. spectrophotometric interference by lipids and cells, differential reaction of proteins in human milk with the colour reagent, and appropriate protein standard representative of complex, changing mixture). The protein content of mature human milk is approximately 9 g/l by the Kjeldahl method (33), and approximately 12–14 g/l by the Lowry and BCA methods (43, 23, 44). The 25% higher values obtained by the Lowry method have been attributed to using bovine serum albumin (BSA), which has fewer aromatic amino acids than human milk, as the standard. As a result, some investigators have adjusted milk-protein concentrations determined by the Lowry method (45).

Although it is known that the stage of lactation influences the content and relative amounts of protein in human milk, the physiological mechanisms that regulate their levels have not been identified nor has the role of diet been well defined. Based on field studies, human milk's total protein concentration does not appear to differ among populations at distinct levels of nutritional risk. However, difficulties arise in interpreting published data because total protein content often has been estimated from measurements of total nitrogen. This presents problems because in well-nourished populations approximately 25% of nitrogen is not bound to protein. However, in contrast to conclusions reached in field studies, when dietary protein was increased from 8 to 20% of energy consumption in metabolically controlled studies, protein N concentrations increased by approximately 8%, and 24-hour outputs of protein N increased by approximately 21%, in the milk of well-nourished women (46).

Extrapolation from metabolically controlled studies to free-living subjects requires caution. Results from field studies may reflect chronic adaptations; those from shorter-term laboratory studies may represent acute responses to dietary change. There also is a lack of

consensus in the literature as to whether low-protein diets result in reduced milk volumes, and therefore in reduced protein outputs (47, 46, 48). Longer-term studies are needed in diverse populations to help resolve these gaps in knowledge.

### 3.2.3 Total nitrogen content of human milk

Human milk's total nitrogen content, which appears to depend on the stage of lactation and dietary intakes, ranges from 1700 to 3700 mg/l. Eighteen to 30% of the total nitrogen in milk is non-protein nitrogen (NPN). Approximately 30% of NPN are amino acids (5, 49) and thus should be fully available to the infant. As much as 50% of NPN may be bound to urea (5, 49) and the remaining approximately 20% is found in a wide range of compounds such as nitrogen-containing carbohydrates, choline, nucleotides and creatinine (50). Changes in the relative composition of non-protein nitrogen, as lactation progresses, are not well described. From the limited information available, NPN appears to decrease by approximately 30% over the first 3 months of lactation (51). If this nitrogen fraction behaves similarly to protein, it should remain stable thereafter until possibly weaning is well under way.

### 3.2.4 Approaches used to estimate protein requirements

Several approaches have been used to estimate protein requirements for infants and children. At present the protein intake of breastfed infants from 0 to 6 months of age is considered the standard for reasons reviewed by the 1994 IDECG report on protein and energy requirements (5). However, two other approaches also have been used to assess the protein requirements of infants – balance methods and factorial estimations.

The 1985 FAO/WHO/UNU Report on Energy and Protein Requirements (52) states the rationale for using the protein intakes of exclusively breastfed infants from 0 to 6 months of age to estimate requirements: "The protein needs of an infant will be met if its energy needs are met and the food providing the energy contains protein in quantity and quality equivalent to that of breast milk." This assumes that decreases in the protein content of human milk are synchronous with decreases in energy requirements expressed per kg of body weight from 0 to 6 months of age, and that the apparently high efficiency of protein utilization in early infancy is sustained at and beyond 6 months of age. There is no scientific evidence that seriously questions these assumptions in relation to utilization efficiency (see

**Table 6. Efficiency of protein utilization: growth and body composition of breastfed infants and infants consuming infant formula with varying protein concentrations**

Reference	N	Age (months)	Type of feeding	Growth	LBM	Efficiency of protein utilization
Butte & Garza (58)	40	0–4	BF	60th percentile W/L		
Heinig et al. (45)	71 46	3–12 FF	BF <sup>a</sup>	Similar to FF	Higher in FF	Higher in BF
Motil et al. (237)	10 10	1.5–6	BF FF	60 pct Similar to FF	Similar to FF	Similar to FF
Salmenperä et al. (61)	202	4–12	BF FF			
Åkeson et al. (62)	27 <sup>b</sup> 10 9 8	6	BF FF-13 FF-15 FF-18	Similar among groups		
Nielsen et al. (232)	339	10	BF > 7 months BF < 7 months	13.7 g/day 12.5 g/day		
Butte et al. (20)	15 15	4 6	BF	Drop growth velocity		
Dewey et al. (63)	50 91	5–6	BF Partial BF	Similar weight and length gain		

**Abbreviations:**

W/L: weight-for-length percentile of the NCHS reference, 1977

LBM: lean body mass

BF: breastfed

FF: formula-fed

<sup>a</sup> Breastfeeding and solids after 6 months.<sup>b</sup> Sample size varies because breastfed infants were changed to formula.

Table 6), but changes in energy and protein requirements for growth do not appear to be proportionately synchronous. Evolutionary arguments presented for or against the adequacy of exclusive breastfeeding are equally unconvincing because of their basic teleological character. As will be evident below, the absence of sounder physiological data makes the use of human milk intakes during this age interval the best available choice.

The 1996 IDECG report on energy and protein requirements (5) reviewed the flaws in the 1985 FAO/WHO/UNU protein requirement estimates for infants (52). These are the assumption that at 1 month of lactation protein concentrations in milk are sustained (indeed, as discussed above, they fall); possible underestimation of milk-intake volumes (because some investigators decided not to measure insensible water

losses when milk intakes are determined by test-weighing techniques, although this probably represents a trivial source of error); and failures to account for either the non-protein component of human milk or the possible under-utilization of some of the milk's protein constituents because of their resistance to digestion. The following reasons are posited for these inaccurate estimates.

Discomfort with reliance on intake data collected mostly under "opportunistic" situations has led to comparing estimates based on ad libitum intakes with nitrogen balance data, and "armchair estimates" based on the factorial approach. Of the two bases for comparisons, balance data are less satisfactory. Many of the difficulties with balance data arise because often they have been obtained from undernourished infants during repletion, or from premature infants. In either case these infants'

physiological condition renders difficult extrapolation to healthy term infants. Moreover, the complexities imposed by relationships between energy intake and efficiencies of protein utilization, and by differences in utilization efficiencies due to the varying biological values and amounts of proteins fed in balance studies, significantly lessen the value of balance results for the purpose of directly estimating protein requirements for healthy term infants.

Thus, the factorial approach, which requires estimating maintenance needs, protein accreted during growth and efficiency of utilization, appears more attractive than balance methods. Maintenance needs are based on obligatory losses and the progressive loss of efficiency in protein utilization as levels of protein increase. Utilization efficiency is believed to be maximal below requirement levels and to become progressively less efficient as requirement levels are approached and surpassed.

The 1996 IDECG report used results from multiple studies to estimate maintenance needs (5). This estimate was calculated by extrapolating relationships between nitrogen intake and retention to a y intercept of 10 mg N/kg per day to account for integumental losses, and by adjusting relationships between intake and retention to an assumed slope of 0.73. In the report the maintenance requirement was estimated to be 90 mg N/kg per day. An alternative approach, which requires fewer assumptions and less manipulation of experimental data, is the use of basal metabolism to estimate obligatory losses (53). Although this approach was abandoned in the 1985 report because of inconsistent ratios across several ages, it appears reasonably consistent in the age range of interest, i.e. the range of values in published studies of children 4 to 15 months of age is 1.2 to 1.5 mg N per "basal" kcal<sub>th</sub> (53). For 1- and 4-month-old exclusively breastfed infants, minimal observable energy expenditure rates are approximately 45 kcal<sub>th</sub>/kg per day (54). If one uses 1.5 mg N per "basal" kcal<sub>th</sub> as a conservative estimate, obligatory losses are 68 mg N/kg per day and extrapolations of this value to 6 or 8 months present no substantial problems since major changes in basal metabolism are not anticipated at these ages. The mean protein gain between 4 and 8 months of age for exclusively breastfed infants is 0.24 g protein/kg of body weight/day or 38 mg N/kg per day. The sum of nitrogen needs for maintenance and growth is 106 mg N/kg per day.

However, this sum must be corrected for the absorption rate of human-milk proteins and the rate of protein

utilization for growth in the intake range of interest. Most studies that have examined the absorption of human-milk nitrogen and specific human milk-protein components have been performed among premature infants (55, 56). In examining this issue, Donovan et al. (55) reported apparent absorption rates of 85%, which confirmed earlier data published by Schanler et al. (56). These rates of absorption are remarkably similar to those summarized by Fomon (57) for infants fed various types of cow's milk-based formulas. These estimates all include losses of both dietary and endogenous nitrogen, thus available data likely underestimate "true" dietary absorption rates. If we nevertheless accept the value for purposes of estimating dietary N requirements, the figure adjusted for absorption is 125 mg N/kg per day.

Taking this "conservative" approach, however, is not as unbalanced as it may first appear. The absorption of human milk's immunological components has been a major concern because of their functional role and putative resistance to digestion. Studies examining this issue also have been performed principally in preterm infants (55, 56). Analyses by Donovan et al. (55) for specific components suggested a maximum absorption rate of 75% for SIgA and 91% for lactoferrin. The apparent absorption rates for lactoferrin reported by these investigators agree with the earlier studies published by Schanler et al. (56). However, the SIgA values in the two studies are quite different. Schanler et al. (56) reported total apparent SIgA absorption rates of 91% compared to the mean of 75% by Donovan et al. (55). This disparity likely reflects the different analytical methods used for measuring SIgA.

The estimated requirement for efficiency of utilization must also be corrected. Once again, the best data have been published from studies of premature infants. If we accept the efficiency of utilization of 0.73 adopted by the IDECG group, the N needs of infants in this age range are approximately 171 mg N/kg per day.

This estimate compares well with the mean protein N intakes reported by Butte et al. (16) for breastfed infants at 1 and 2 months of age. By 3 months of age the sum of the mean protein N intake and 30% of the mean NPN (assuming that this fraction consists of free amino acids) is 178 mg. By 4 months of age this sum is 161 mg N/kg, still reasonably close to the mean estimated requirement.

This leaves us with the remainder of the NPN unaccounted for in terms of its potential utilization. Rates of NPN utilization vary greatly from approximately 10% to almost 50% (5). Given the very incomplete know-

**Table 7. Protein intake of breastfed and formula-fed infants**

Reference	N	Type of feeding	Protein intake (g/kg per day)						Growth	
			1	2	3	4	6	9		12
Butte & Garza (58)	40	BF	1.6 ± 0.3	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.2				60th percentile W/L
Heinig et al. (45)	71	BF			1.09 ± 0.2		1.06 ± 0.3	1.67 ± 0.89	2.45 ± 1.1	Similar
	46	FF			1.81 ± 0.3		1.76 ± 0.3	2.03 ± 0.4	2.48 ± 0.6	
Motil et al. (231) <sup>a</sup>	10	BF	22 ± 3		14 ± 2	12 ± 3				Similar
	10	FF	29 ± 5		25 ± 6	27 ± 10				
Åkeson et al. (62)	27 <sup>b</sup>	BF					1.39 ± 0.2	1.67 ± 0.9	2.45 ± 1.1	Similar
	10	FF-13					1.87 ± 0.2	2.01 ± 0.3	2.48 ± 0.4	
	9	FF-15					2.0 ± 0.2	2.18 ± 0.4	2.63 ± 0.5	
	8	FF-18					2.3 ± 0.2	2.32 ± 0.5	2.73 ± 0.3	
Butte et al. (20)	15	BF				1.2 ± 0.3				Drop growth velocity
	15						1.1 ± 0.3			
Dewey et al. (63) <sup>c</sup>	50	BF					0.98 ± 0.2			Similar
	91	Partial BF					1.18 ± 0.2			

**Abbreviations:**

BF: breastfed

FF: Formula-fed

W/L: weight-for-length percentile of the NCHS reference, 1977

<sup>a</sup> Proteins are in mmol/kg per day.<sup>b</sup> Sample size varies because breastfed infants were changed to formula.<sup>c</sup> Breastfeeding and solids after 6 months.

ledge of factors that account for this five-fold range in utilization rates and the variability of this component in human milk, the presumption of its use and significance to infant nutrition appears tenuous. The decision was thus taken not to include it further in the above calculations.

It is possible to estimate the prevalence of inadequacy from these data using the probability approach that was taken in the 1996 IDECG report. A requirement of approximately 170 mg N/kg, which is close to the report's "Model C", yielded a population inadequacy prevalence of approximately 8%.

### 3.2.5 Protein intake and growth

Butte et al. examined the adequacy of protein intake from human milk by determining protein intakes and growth of exclusively breastfed infants from middle to upper economic groups in Houston, TX (16, 58). Protein intake was 1.6 ± 0.3 g/kg per day at 1 month and 0.9 ± 0.2 g/kg per day at 4 months of age. The mean

Z-scores of these infants' weights and lengths were consistently greater than zero (based on the WHO pooled breastfed data set) (Table 7) until the fourth month when the mean declined to slightly below zero. Later, Heinig et al. (45) evaluated a sample of breastfed infants from 0 to 12 months of age enrolled in the DARLING Study. Protein intakes of breastfed infants at 3 months were comparable to those reported by Butte et al. (1.1 ± 0.22 g/kg per day), and they remained at approximately 1.1 ± 0.3 g/kg per day through 6 months of exclusive breastfeeding. Weight-for-age Z-scores were between 0.5 and 0 for the first 6 months of life (59).

Two other studies, also conducted in developed countries, reported that after the first 2 to 3 months breastfed infants gained weight less rapidly than formula-fed infants (60, 61). In both studies infants were not exclusively breastfed and there was a significant drop (17.5 to 45%) in sample size over time. The unstable anthropometric Z-scores in both studies are thus difficult to evaluate.

Similarly, results from developing countries are inconsistent. Although protein intakes of exclusively breastfed Mexican infants were comparable to those of American infants ( $1.2 \pm 0.3$  g protein per kg/day at 4 months and  $1.1 \pm 0.3$  g/kg per day at 6 months), their weight and length velocities were significantly lower than those observed in American infants by 6 months of age (20). Weight velocity declined from  $16.1 \pm 3.2$  g/day at 4 months ( $Z =$  approximately 0.2, using boys) to  $8 \pm 3.5$  g/day ( $Z =$  approximately  $-1.25$ , using boys) at 6 months, and length velocities from  $1.92 \pm 0.22$  cm/month ( $Z = -0.25$ , using boys) to  $1.02 \pm 0.34$  cm/month ( $Z = -0.75$ , using boys) (20).

In contrast, a sample in Honduras of exclusively breastfed infants were assigned randomly at 4 months either to continue exclusive breastfeeding until 6 months or to receive a high-protein complementary food from 4 to 6 months. After 4 months of age, the mean protein intake (g/kg per day) in the group of infants who received solid food was 20% higher than that of the exclusively breastfed group. Despite these differences in protein intake, no differences from 4 to 6 months in weight or length gain were noted between feeding groups. Furthermore, 20 infants with the highest protein intakes were matched to 20 exclusively breastfed infants with similar energy intakes. Although protein intake was 33% higher in the non-exclusively breastfed group, growth rates were similar (5). These negative findings should be interpreted cautiously because of the precision of the balances used (accurate to 100 g) relative to the changes in weight observed between 4 and 6 months; and because earlier findings by the same group documented a positive correlation between weight gain and protein intake (39) when intakes and weight gain of both breast- and bottle-fed infants were examined but no such correlation when only breastfed infants were considered.

In an experimental study (62), the growth of infants after 6 months receiving formulas with varying protein concentrations (13, 15 and 18 g/l) was compared to the growth of exclusively breastfed infants from 0 to 6 months. Breastfed infants from the DARLING Study were used as the comparison group (59). Although energy intakes were similar in all groups, protein intakes were significantly lower at 6 months of age in the breastfed group compared with those of the three formula-fed groups. Increments in weight and length between 4 and 8 months were similar in the formula-fed and breastfed groups (62).

It thus appears that human milk meets the protein needs for growth of infants between 0 and 6 months. There

are no data to evaluate the protein adequacy of exclusive breastfeeding at later ages (45, 62), and one may well ask whether any of the published studies have sufficient power to detect physiologically relevant differences in growth. However, the formula study described above suggests that protein should not be the limiting factor.

Some concerns may be raised by the seemingly conflicting data of Butte et al. from Mexico (20) and Dewey et al. from Honduras (63). Each of the reports is based on infants from low socioeconomic status settings. Data from Butte et al. may reflect the insufficiency of exclusive or predominant breastfeeding for sustaining normal growth rates in harsh settings. On the other hand, data from Dewey et al. may reflect the reality that, under the circumstances, exclusive breastfeeding is "as good as" what is achievable in terms of growth. However, the period over which weight gain is calculated may influence this conclusion. Dewey et al. (63) reported a weight gain of 1017 g – or the equivalent of approximately 14.5 g/day – in the exclusively breastfed group, and 1004 g, or 14.3 g/day, in the supplemented breastfed group between 16 and 26 weeks of age. Although weight gains over the entire period were not discernibly different between groups, weekly weight gains cannot be calculated or assessed. In contrast Butte et al. evaluated specific weight gains (16.7, 12.3 and 7.8 g/day at 4, 5 and 6 months, respectively) and noted a downward trend in predominantly breastfed infants (20).

### 3.2.6 Plasma amino acids

Postprandial concentrations of plasma amino acids also have been used as an index of the adequacy of protein intakes (64). We were unable to find any data evaluating changes in plasma amino-acid patterns in exclusively versus partially breastfed infants during the first year of life.

### 3.2.7 Immune function

Protein undernutrition adversely affects immune function. Protein-deficient infants present impaired immune responses that, in turn, increase their risk of infectious episodes (65).

Two papers have been published regarding the association between protein intake and immune function in breastfed infants. In one study infants were classified at birth as breastfed or formula-fed according to maternal choice (66). Formula-fed infants were assigned randomly to either a low- or high-soy protein formula,

or to a cow's milk-based formula adapted to European Society of Paediatric Gastroenterology (ESPGAN) recommendations (i.e. a whey:casein ratio of 50:50). Infants received the polio immunization and the triple vaccine against diphtheria, pertussis and tetanus (DPT) at 2 and 4 months of age. Blood antibodies were analysed at 5 and 8 months. Results were consistent with those reported in the previous study, i.e. infants fed on the low-protein cow's milk- and soy-based formulas presented poorer antibody responses than did infants who received the high-protein cow's milk-based formula. Infants consuming the adapted formula had a higher initial antibody response, which was not sustained. Five-month-old exclusively breastfed infants presented sustained antibody responses that were similar to those of the high-protein group (66). In another study, breastfed infants from Sweden presented significantly higher faecal titres of both IgA and IgM antibodies, as well as the secretory component to poliovirus and diphtheria and to tetanus toxoid than did infants receiving a formula with 1.1 or 1.5 g protein per 100 ml (67).

The interpretation of the functional significance of these observations remains difficult without robust comparisons of morbidity in exclusively, predominantly and partially breastfed infants 4 to 12 months old in diverse settings.

### 3.2.8 Infant behaviour

Several authors have reported better cognitive development and intelligence quotients in breastfed infants compared with those who are formula-fed (68, 69). A review by Pollitt et al. (70) amply demonstrates the complexities related to this issue and the difficulties presented by available studies because of their inability to distinguish among competing hypotheses. No studies were found that assess the behavioural outcomes of feeding healthy term infants diverse levels of protein during the first year of life.

### 3.2.9 Summary

Based on factorial and balance studies, infants' mean protein requirements are approximately 1.1 g/kg per day from 3 to 6 months of age. "True protein" provided by human milk is sufficient to meet the mean protein requirements of infants for the first 2 months of life, and "true protein" intake plus free amino acids and other forms of NPN are likely sufficient to meet the needs of most, though not all, infants after 4 months. A more precise estimate of the proportion of infants whose needs

are met at all ages requires improved understanding of the efficiency of human milk nitrogen utilization (both protein and NPN), improved methods for estimating obligatory needs and better functional measures of nitrogen adequacy.

## 3.3 Vitamin A

### 3.3.1 Introduction

Vitamin A is a generic term for a group of retinoids with similar biological activity. The term includes retinal, retinol, retinoic acid and substances considered to be pro-vitamin A because they can be transformed into retinol. Among the pro-vitamin A compounds,  $\beta$ -carotene has the highest potential vitamin A activity. Recent recommendations by the United States Food and Nutrition Board re-evaluated conversion equivalency and recommended use of 1/12 retinol equivalents (RE) from a mixed diet.

Retinols are stored in the liver as esters, and storage increases in the fetal liver during late gestation. The placenta regulates the passage of a sufficient amount of vitamin A from mother to fetus to meet physiological requirements but not to build up a substantial body reserve. This tight regulation is believed to result in low hepatic reserves of vitamin A at birth, even in infants born to well-nourished mothers, compared to levels achieved in later life stages (71, 72). After birth, vitamin A is transferred to the infant through human milk.

The vitamin A content of human milk depends on maternal vitamin A status. Infants of women with inadequate vitamin A status are born with low reserves of vitamin A, and thus their vitamin A status is likely to be protected for shorter periods than the status of infants born with higher reserves. Since most vitamin A for tissue reserves is transferred late in gestation, preterm infants have lower stores than full-term infants. In populations that are at risk of vitamin A deficiency, the age at which a deficiency occurs is related to the age of weaning, i.e. the shorter the duration of breastfeeding, the earlier the onset of deficiency (73). This is likely due to the combined effect of the consumption of complementary foods that are low in vitamin A and higher vitamin A utilization rates imposed by more frequent infections.

### 3.3.2 Vitamin A in human milk

The mature milk of well-nourished mothers contains approximately 1.7 moles/l vitamin A (6). In addition,

human milk contains carotene that may contribute to the vitamin A transferred to the infant (57) and bile salt-stimulated lipase, which facilitates the infant's absorption of vitamin A and precursor carotenoids (74).

Because the vitamin A content of human milk is strongly influenced by maternal nutritional status, it is not surprising to find lower amounts of vitamin A in human milk in regions where undernutrition is widespread and mothers consume vitamin A-containing foods less frequently than women in privileged environments. Consequently, the concentration of vitamin A in mature milk of women in underprivileged countries may be extremely low. For example, Muhilal et al. (75) reported baseline values of  $0.60 \pm 0.29$  moles/l in studies conducted in Indonesia.

Vitamin A concentrations vary with the stage of lactation. In a cross-sectional study in Guatemala, vitamin A concentrations in milk of low-income women decreased from 1.40  $\mu$ moles/l at 6 months of lactation to 1.33 and 1.26  $\mu$ moles/l at 9 and 15 months, respectively (76). In the Philippines concentrations decreased from 1.26  $\mu$ moles/l at 3 months to 0.88  $\mu$ moles/l at 9 months of lactation (76). Similarly, lactating Ethiopian mothers presented vitamin A concentrations of  $1.16 \pm 0.52$   $\mu$ moles/l at 1.5–3.5 months of lactation, and  $0.74 \pm 0.25$   $\mu$ moles/l at 11.5–23.5 months of lactation (77).

According to Stoltzfus & Underwood (73), the best evidence that vitamin A levels in human milk correspond to maternal vitamin A status is the improved concentrations in milk after maternal supplementation in areas where vitamin A deficiency is endemic (75, 78–80).

### 3.3.3 Estimates of vitamin A requirements

The vitamin A requirements of infants are difficult to estimate accurately because of the lack of a sensitive index of vitamin A status. Plasma retinol levels are insensitive to the adequacy of intake until hepatic stores are severely depleted. Other methods that have been considered to assess vitamin A status include adaptation to darkness, the pupillary response test, total liver reserves by isotope dilution, relative dose response/modified relative dose response, conjunctival impression cytology and immune function (6). However, none of these methods is completely suitable for assessing the vitamin A requirements of infants.

Intestinal absorption is among the dietary factors that influence vitamin A requirements. In turn, dietary fat, infections, the food matrix and food processing all affect intestinal absorption (6). However, none of these factors

is relevant for breastfed infants since the bioavailability of preformed vitamin A from human milk is likely to be greater than 90%, and breastfed infants are protected against infection, particularly gastrointestinal infections. The potential vitamin A activity of vitamin A precursors in human milk is not known. Thus, in children younger than 1 year, retinoid requirements have been based on the estimated intakes of this vitamin by breastfed infants (6). However, the dependence on maternal diets of human milk vitamin A levels makes accurate estimates of requirements difficult to calculate from milk composition data alone.

The recommended vitamin A intake level for infants 0 to 6 months was set at 1.4  $\mu$ moles/day and 1.75  $\mu$ moles/day for infants 6 to 12 months based on the intakes of breastfed infants of well-nourished women (6). A deficiency state has been defined as stores that are insufficient to maintain optimal vitamin A concentrations in target tissues. This is generally observed when body stores fall below 0.07 moles/g liver (81). Serum retinol levels and the relative dose-response test have been used to assess hepatic vitamin A stores, but other functional measures of vitamin A status, e.g. ocular manifestations, are used more often in practice. Less specific measures of vitamin A status such as growth retardation, increased susceptibility to infections and greater mortality risk are also occasionally used (82, 83).

### 3.3.4 Plasma retinol

Serum retinol levels in individuals are tightly regulated; for the reasons outlined above, however, they are indicative of vitamin A status in individuals only when body reserves are depleted or surpassed. Fortunately, serum vitamin A distribution curves and population dietary intake patterns can be used to assess and compare the vitamin A status of populations (83). However, interpretation of serum retinol levels can be confounded by stress, which reduces levels on an acute basis, resulting in abnormally high prevalence rates of poor vitamin A status.

At birth, serum vitamin A concentrations in term infants of well-nourished mothers are approximately 0.70  $\mu$ moles/l or greater (84, 85). In contrast, serum vitamin A levels of infants born to mothers with marginal vitamin A status are reported at approximately 0.49  $\mu$ moles/l (86). In Indonesia more infants whose mothers had vitamin A concentrations below 1.4  $\mu$ mol/l in their milk had evidence of depleted liver stores and had lower serum retinol concentrations than did infants whose mothers' vitamin A milk concentrations were above this value (80).

WHO and UNICEF use a mean population value of  $0.7 \mu\text{mol/l}$  to identify subclinical vitamin A deficiency in populations, but they caution that this value may not identify deficient individuals (87). None the less, this value is commonly used to describe the status of both populations and individuals and to characterize responses to interventions designed to improve vitamin A status. For example, the mean serum retinol levels of 6-month-old exclusively breastfed Bangladeshi infants was  $0.77 \pm 0.21 \mu\text{mol/l}$ . Thirty-four per cent presented levels below the  $0.70 \mu\text{mol/l}$  cut-off (88). Serum levels were  $0.84 \pm 0.23 \mu\text{mol/l}$  (89) in a similar group of infants whose mothers were supplemented postnatally with vitamin A, but 25% of the infants in this group were reported to have serum values below  $0.7 \mu\text{mol/l}$ . The vitamin A content of human milk in these populations was  $0.87 \pm 0.61 \mu\text{mol/l}$  in unsupplemented women and  $0.85 \pm 0.53 \mu\text{mol/l}$  in women supplemented with vitamin A.

A mean serum retinol concentration of  $0.67 \mu\text{mol/l}$  was reported in 1.5-month-old infants in a multicentre trial conducted in Ghana, India and Peru. Levels below  $0.70 \mu\text{mol/l}$  were reported for 63% of those infants (90). The administration of 25 000 IU of vitamin A with each of the first three doses of DPT/poliomyelitis immunizations resulted in mean serum vitamin A levels of  $0.84 \mu\text{mol/l}$  in these groups at 6 months of age, and the percentage of infants with retinol levels below  $0.70 \mu\text{mol/l}$  decreased to 30% by 6 months. However, the average retinol concentration  $0.80 \mu\text{mol/l}$  and the percentage of infants with retinol levels below  $0.7 \mu\text{mol/l}$  also dropped (37%) in the placebo group included in that trial (90). Of 339 infants 77% had abnormal relative dose response tests at 1.5 months of age. The percentages with abnormal tests declined to 43%, 38% and 28% at, respectively, 6, 9 and 12 months following the administration of vitamin A. Parallel declines were observed in the placebo group (90). The relative vitamin A concentrations in milk were similar for both the treatment and placebo groups.

### 3.3.5 Functional end-points

#### *Growth and vitamin status*

Associations between linear growth retardation and vitamin A deficiency have been found in some, but not all, studies. In a community-based study conducted in Indonesia, 466 children were identified as vitamin A-deficient (presence of night blindness, Bitot's spots or xerophthalmia). Age-specific paired comparisons showed a lower height-for-age, weight-for-height, mid-

upper arm circumference and triceps skin-fold in children under 3 years of age with xerophthalmia than in controls. Vitamin A-deficient children consumed almost half the amount of vitamin A-containing foods (dark-green leafy vegetables and milk) than controls (91). In another study, also conducted in Indonesia by the same group, children who spontaneously recovered from xerophthalmia were compared to a group of children who did not recover spontaneously and to a group of healthy children. Weight and height were evaluated at 3-month intervals. Infants who recovered spontaneously gained weight at the same rate as healthy infants, but their height deficits persisted. Infants who did not recover, and those who became xerophthalmic during the follow-up period, presented the greatest weight and height deficits (92). Although such descriptive data are interesting, they are difficult to interpret because it is likely that all subjects suffered from multiple nutrient deficiencies.

However, intervention trials that examined the effect of vitamin A intake on growth also present difficulties. In a randomized, placebo-controlled study conducted in India, supplementing infants for 1 year with weekly doses of 2500  $\mu\text{g}$  (8.8 moles) vitamin A and 20 mg vitamin E failed to improve growth (93). This occurred despite a higher mortality rate in the placebo group, suggesting a beneficial effect of vitamin A supplementation. Failure to see a growth response suggests that either the level of vitamin A provided was insufficient to achieve normal growth or other nutrient levels were more limiting with respect to growth. In another randomized study conducted in Indonesia, children received 60 000  $\mu\text{g}$  (210 moles) retinol or a placebo on two occasions within 1 year. Supplementation improved weight gain only in males 24 to 60 months of age and had no effect in males or females younger than 24 months (94). Similarly, in another Indonesian trial, commercial monosodium glutamate was fortified with vitamin A. Fortification resulted in improved linear growth, but this time only in children 12 to 24 months old (75).

A more recent trial by the same group in Indonesia controlled for baseline vitamin A status. Children were supplemented randomly with vitamin A (103 000 to 206 000 IU, according to age) or a placebo. Results were adjusted by the children's pre-treatment vitamin A status. An improvement in linear growth (0.16 cm), but not in weight, was observed after supplementation in children older than 24 months. After adjusting for pre-treatment vitamin A status, supplemented children with serum retinol  $< 0.35 \mu\text{mol/l}$  at baseline grew 0.39 cm and gained 152 g more weight in a period of



16 weeks than children in the placebo group. Weight and height gains were not different between treatment groups in children with retinol concentrations above 0.35  $\mu\text{mol/l}$  (95). Levels of vitamin A in human milk for the various groups were not reported.

Thus, an effect of supplementation with vitamin A on growth may be observed in a group of children with a high likelihood of deficiency, but apparently not in infants, i.e. 0 to 12 months of age. The above-mentioned multicentre randomized and placebo-controlled study conducted in Ghana, India and Peru followed infants until 12 months of age. No difference in weight and length gain or Z-scores was observed between supplemented and placebo groups despite the high percentage of infants with vitamin A deficiency as determined by serum retinol and relative dose response (90). Thus, it is possible that factors other than vitamin A status are more sensitive determinants of growth in infants in this age group. Indeed, in a study conducted in Egypt, the growth of sick and healthy infants was compared after supplementing their mothers with vitamin A. Milk retinol levels correlated significantly with the growth of healthy infants, but not with those who were ill (96).

### ***Ocular manifestations***

In Malawi 152 children with xerophthalmia were compared to 151 age-matched children without visual manifestations of vitamin A deficiency. Weaning was initiated and breastfeeding stopped significantly earlier for children with xerophthalmia than for healthy children (97). However, in another study conducted in Indonesia more than 90% of the children, with or without xerophthalmia, were breastfed for at least 12 months, and the duration of breastfeeding was similar between infants with xerophthalmia and controls. Although the age of introduction of solid foods was not investigated, infants with xerophthalmia received significantly less vitamin A-rich foods than did controls. This suggests that in some settings the quality of complementary foods plays as important a role as the duration of breastfeeding (91).

### ***Morbidity and mortality***

High morbidity and mortality rates due to infectious diseases are associated with clinical and subclinical vitamin A deficiency. Moreover, it has been reported that during infectious episodes vitamin A is excreted in urine at higher levels than usual (98). Thus, the risk of increased frequency and severity of infections is

greater in vitamin A-deficient infants and their requirements for vitamin A are higher.

Evidence for these associations comes from different types of studies. For example, in South Africa 10-month-old infants hospitalized with complicated measles and supplemented with a single dose of 400 000 IU vitamin A recovered more rapidly from pneumonia and diarrhoea than the placebo group (99). In Bangladesh, infants of vitamin A-supplemented mothers had significantly shorter episodes of respiratory tract infections and fewer febrile episodes in the first 9 months postpartum than infants of unsupplemented mothers (79). In contrast, after controlling for age and nutritional status, smaller but frequent doses of vitamin A supplements provided Indian infants 6 to 60 months of age (2500  $\mu\text{g}$  weekly) had no effect on the incidence, severity or duration of diarrhoea or respiratory tract infections (93). Similarly, in the above-mentioned multicentre study conducted in Ghana, India and Peru no differences in the prevalence of diarrhoea and acute lower-respiratory infections were noted between placebo and supplemented groups (90). In Indonesia, a placebo-controlled trial was conducted in 2067 neonates who received either 50 000 IU vitamin A orally or a placebo on the first day of life. The vitamin A supplement reduced the infant mortality rate and the prevalence of severe respiratory infection (100). Vitamin A supplementation at birth reduced the risk of pneumococcal colonization in South Indian infants (101).

The effect of vitamin A supplementation on mortality rates is also somewhat inconsistent. A recent review by Villamoore & Fawzi (102) summarized various issues related to vitamin A sufficiency. The authors reviewed the inconsistent results of community-based trials targeting infants older and younger than 6 months. Although supplementation of deficient populations generally appears to decrease mortality, this outcome has not always been observed. The inconsistency in results is ascribed to multiple factors that include interactions among multiple but variable nutrient deficiencies, other population-specific characteristics, and magnitude and frequency of supplementation (102).

Mortality risks have been reported to be 30–60% higher in children with keratomalacia and xerophthalmia than in healthy populations (103). Most studies have reported reductions in mortality rates in preschool children after vitamin A supplementation (99, 75, 104, 103). However, other supplementation studies have failed to observe protective effects (105, 106). Two meta-analyses that included the above-cited studies

concluded that supplementation reduced mortality in preschool children (107, 108). However, more recent studies failed to observe similar protective effects. In Nepal, differences in mortality rates were not noted in infants, from 0–5 months of age, who received a single dose of 15 000 to 30 000 IU of vitamin A or a placebo (109). Cumulative morbidity and mortality rates were similar between supplemented and placebo groups from 1 to 12 months of age in the Ghanaian, Indian and Peruvian multicentre trial (90).

Studies of this type suggest several possibilities. Either present cut-offs used to assess vitamin A adequacy should not be used as proxies for increased risk of morbidity and mortality in all infants; or inadequate vitamin A status of young infants in some study populations is not a major risk to increased morbidity or mortality; or inadequate vitamin A status acts in concert with other factors in a way that requires their simultaneous correction before expected benefits can be achieved.

### 3.3.6 Summary

The absence of any evidence of vitamin A deficiency in well-nourished populations suggests that the vitamin A content of human milk is adequate to meet the vitamin A requirements for infants during the first 6 months of life when mothers are well nourished. However, it is important to recall that there are no population assessments of the vitamin A status of exclusively breastfed infants beyond this age. Human milk is the primary source of vitamin A in environments where vitamin A deficiency is prevalent, and in these settings the population of breastfed infants with deficient or marginal vitamin A stores appears to be significant from a public health perspective. None the less, the lower risk of xerophthalmia and mortality observed in breastfed infants compared to their non-breastfed counterparts argues strongly in favour of continued breastfeeding. This difference is likely to be the result of inappropriate complementary foods and heightened vitamin A requirements due to high rates of infection in prematurely weaned infants. Also, based on the available evidence, it is not possible to make a case for exclusive over predominant breastfeeding unless one argues that all supplementary feeding decreases milk intake and thus, in these settings, also diminishes vitamin A intakes.

## 3.4 Vitamin D

### 3.4.1 Introduction

Vitamin D is a fat-soluble vitamin that is synthesized in the skin and may be obtained from the diet. There are two forms: vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol). Vitamin D<sub>2</sub> originates from ergosterol, a plant sterol, and is obtained through the diet; vitamin D<sub>3</sub> originates from 7-dehydrocholesterol, a precursor of cholesterol. Both vitamins D<sub>2</sub> and D<sub>3</sub> require one hydroxylation in the liver to 25-hydroxy-vitamin D and another in the kidney to form the biologically active hormone, 1,25-dihydroxyvitamin D (1,25 (OH)<sub>2</sub>D).

Receptors for 1,25 (OH)<sub>2</sub> D are found in the small intestine and other tissues such as the brain, pancreas and heart. Anti-proliferation and pro-differentiation functions have been suggested for vitamin D (110). Receptors for 1,25 (OH)<sub>2</sub> D have been detected in the small intestine and colon of the human fetus (110), which suggests that vitamin D has an important role in cell differentiation during gestation (111). In postnatal life, the most widely recognized functions of vitamin D are related to calcium and phosphate metabolism.

### 3.4.2 Factors influencing the vitamin D content of human milk

It is widely accepted that human milk contains very low levels of vitamin D (Table 8). Vitamin D concentrations in human milk depend on maternal vitamin D status (112). Factors affecting vitamin D status include skin pigmentation, season and latitude (113). Increased skin melanin concentration reduces the efficiency of vitamin D synthesis in the skin. Thus, individuals with dark skin and limited sun exposure are at greater risk of inadequate vitamin D synthesis than those with less skin pigment. Although the vitamin D<sub>2</sub> and D<sub>3</sub> content in the milk of dark-skinned women may be lower than that of light-skinned women, maternal serum 25(OH)D levels can nevertheless be similar in both groups (114). However, these findings are not consistent with an earlier report by Specker et al. (115) of lower 25(OH)D in the milk and maternal serum of dark-skinned than light-skinned women and a significant correlation between maternal 25(OH)D in serum and milk. The vitamin D in milk of mothers who deliver in the late autumn or winter at or above 40°N latitude or below 40°S latitude comes only from dietary sources or stores because there is hardly any synthesis in the skin at these times of the year (116). Thus, the vitamin D content of the milk of women living at these latitudes can be reduced.

**Table 8. Vitamin D content of human milk**

Reference	N	Country	Vitamin D status	Milk concentration ( $\mu\text{g/l}$ )	Vitamin D activity (IU)	Stage of lactation	Milk sample
Hollis et al. (120)	5	Canada	Normal (NS)	$0.39 \pm 9$	25	1–21 days	Whole milk
Leerbeck & Sondergaard (233)	2	Europe			15	Pooled	Lipid fraction
Reeve et al. (234)	3	USA	Normal (S)	0.16	53	Mid-lactation	
Bawnik et al. (119)	5	Israel	Unknown (NS)	$0.37 \pm 0.03$ $0.35 \pm 0.07$	15 15	3–21 days pooled	Whole milk
Zoeren-Grobbe et al. (235)	8	Netherlands	Healthy (NS)	$1.5 \pm 6$ NA		1–8 months	Lipid fraction

**Abbreviations:**

NS = non-supplemented

S = supplemented with vitamin D

NA = not available.

Since it is not easy to obtain preformed vitamin D from the diet – it is found in only a few food sources such as egg yolk, liver and fatty fish – maternal vitamin D status is most often a function of sun exposure. The consequences of this relationship can be highly significant. For example, 70% of the women of upper socioeconomic class and their exclusively or predominantly breastfed infants studied in Pakistan were reported to be vitamin D deficient (117). These findings suggest that vitamin D activity in human milk can be increased by maternal supplementation with preformed vitamin D (118–121, 112). Ala-Houhala et al. (118) reported that the administration of 2000 IU of vitamin D to breastfeeding women normalized their infants' 25(OH)D levels. A lower dose of 1000 IU was not effective in this regard. Yet, it is difficult to understand the rationale for supplementing women to correct their infants' vitamin D status, unless maternal supplementation is also used as a strategy to increase maternal vitamin D stores in preparation for a subsequent pregnancy, or to correct or avoid other maternal vitamin D abnormalities.

**3.4.3 Estimates of vitamin D requirements**

The United States Food and Nutrition Board (122) recommends 5 g of vitamin D for infants 0 to 6 months of age, although it also acknowledges that breastfed infants “with habitual small doses of sunshine” do not require supplemental vitamin D. Infants in far northern latitudes or those with minimal sunlight exposure require a minimum of 2.5  $\mu\text{g/day}$  (100 IU) to prevent rickets.

The physiological dependence on ultraviolet light for

normal vitamin D status is most evident when one considers that approximately 6 litres of human milk daily would be necessary to obtain the minimal amount of vitamin D needed to prevent rickets where sun exposure is inadequate. Two hours is the required minimum weekly amount of sunlight for infants if only the face is exposed, or 30 minutes if the upper and lower extremities are exposed. Breastfed infants who are exposed to less sunlight present low 25(OH)D serum concentrations (115). Because of the normal dependence on sunshine for vitamin D adequacy, it is not currently possible to provide precise estimates of vitamin D requirements.

**Level of vitamin D intake and serum 25(OH)D**

Serum 25(OH)D is considered the best indicator of vitamin D status because it reflects the combined vitamin D obtained from diet, sunlight and liver stores (123, 124). The cut-off for defining vitamin D deficiency in adults is based on the level of serum 25(OH)D below which high serum parathyroid hormone (PTH) concentrations are observed (122). However, a cut-off based on PTH has not been determined for infants. The current cut-off of 27.5 nmol/l for infants is based on serum 25(OH)D levels observed in cases of vitamin D deficiency rickets. It is also important to note that although there are significant correlations between milk and maternal serum 25(OH)D levels, no such associations are reported between milk vitamin D and infant serum 25(OH)D. This is likely a reflection of the infant's endogenous synthesis of vitamin D (112).

**Table 9. Vitamin D status of breastfed infants**

Reference	N	Country	Age and duration of supplementation	Vitamin D Supplement	Serum 25 (OH) D (ng/ml)	BMC	Growth
Roberts et al. (129)	22	USA	14 days	0	17 ± 3	Normal in all groups	Normal
	19		14 days–4 months	400 IU	22 ± 3		
Markestad et al. (130)	7	Norway	9–12 months	0	50% with less than 11 ng/ml		Dropped at 6 months from 60th to 40th percentile
Greer & Marshall (128)	24	USA (Caucasian)	0–7 days	0	Dropped at 1.5 months Normal		
	22		0–6 months	400 IU			
Chan et al. (125)	22	USA (Caucasian)	Birth	0	19 ± 2	Normal in all groups	Normal in all groups
	29		0–6 months	400 IU	23 ± 3		
Feliciano et al. (144)	255	China	3–5 days 0–6 months	100 IU 200 IU 300 IU			Normal in all groups
Fomon et al. (145)	26	USA (Caucasian)	8 days	300 IU		Normal in all groups	
	11		0–6 months	400 IU			
	13			1600 IU			
Atiq et al. (141)	38	Pakistan	< 6 months	0	34 nmol/l		
	24		> 6 months				
Specker et al. (133)	52	China (North)	3–5 days	100 IU	8 ± 13	Normal in all groups	
	52		0–6 months	200 IU	6 ± 9		
	52			400 IU	11 ± 10		

**Abbreviations:**

BF = breastfed

BMC = bone mineral content

S = supplemented with vitamin D

Breastfed infants in regions where sunlight is plentiful have adequate serum concentrations of 25(OH)D before 6 months of age (125–129, 115). In contrast, infants less than 6 months of age living in regions where sunlight exposure is minimal have serum 25(OH)D concentrations within ranges typically observed in cases of rickets (130–133) (Table 9).

### ***Other biochemical and clinical parameters associated with vitamin D deficiency***

Decreases in the serum concentrations of phosphorus and increases in PTH are early signs of vitamin D deficiency. Decreases in serum calcium are observed only in very severe cases. Zeghoud et al. (134) evaluated responses to either 500 IU/day or 1000 IU/day of supplementary vitamin D in 42 infants born with subclinical vitamin D deficiency (low serum calcium

and 25(OH)D and high PTH). Infants who received the higher dose achieved normal serum 25(OH)D and PTH concentrations in the first month with no further changes in either 25(OH)D or PTH. Infants who received the lower dose also had increased 25(OH)D concentrations and decreased PTH levels. Although PTH and 25(OH)D levels were within the normal range by the end of the first month in infants who received 500 IU/day of supplementary vitamin D, PTH and 25(OH)D levels continued to change through the third month, approaching levels in infants supplemented at the higher dose. In the control group with normal serum calcium, 25(OH)D and PTH concentrations presented only slight increases in 25(OH)D (15 nmol/l); PTH concentrations remained stable. All three groups were fed a formula containing 400 IU/l of vitamin D. Thus, the authors concluded that infants born with subclinical vitamin D deficiency can require higher vitamin D

intakes in early life than those born with more adequate stores (134).

Clinical manifestations of severe vitamin D deficiency include hypocalcaemic seizures. A study in the United Kingdom of Great Britain and Northern Ireland of 2- to 14-month-old infants born to parents of Pakistani origin presented with hypocalcaemic seizures and were found to be vitamin D deficient. The diagnosis was based on high concentrations of alkaline phosphatase and low concentrations of serum 25(OH)D, loss of metaphyseal definition and a positive response to vitamin D supplementation (117).

### ***Bone mineralization***

Severe vitamin D insufficiency results in inadequate mineralization of the skeleton. In growing infants deficient mineralization leads to rickets, a disease characterized by a widening of the ends of long bones, deformation of the rib cage (rachitic rosary), and limb deformations such as bowed legs and knocked knees. Studies of subclinical vitamin D deficiency and its effects on bone mineralization are inconsistent, and this likely reflects both the differential effects of PTH on cortical and trabecular bone and the ability of various measurements to distinguish such effects. Primary hyperthyroidism decreases cortical bone mineral density (BMD), and either it has no effect on, or it increases, trabecular BMD (135, 136). In a study of exclusively breastfed infants in the USA, 400 IU of supplemental vitamin D prevented a decline in bone mineralization at the distal radius that has been observed in infants administered a placebo (126). However, Specker et al. did not observe rickets at 6 months of age in any of 256 breastfed infants enrolled in a randomized, double-blind controlled study in China that provided either 100 IU, 200 IU or 400 IU of vitamin D per day (133). This study found supplementation with 100 IU to be sufficient to prevent rickets in breastfed infants with limited sun exposure and vitamin D stores.

#### **3.4.4 Vitamin D status and rickets**

In 1925 Elliot reported a large number of breastfed infants with rickets in poor urban areas of the USA. Rickets in breastfed infants also has been reported more recently in Greece (137), Nigeria (138), Pakistan (119), and in the USA, mainly among African American infants (139, 140). In a study conducted in Chicago, Edidin et al. (140) described the social conditions that can lead to rickets in developed countries. Minimal sun exposure due to protective clothing, or unsafe neigh-

bourhood conditions that prohibit outdoor activities, placed at risk infants of middle and lower socioeconomic groups.

However, other studies have failed to identify breastfed infants with rickets despite low 25(OH)D serum concentrations. For rickets to develop, sustained low 25(OH)D concentrations for long periods are probably necessary. In a study conducted in northern China (40 to 47°N latitude) approximately 33% of the breastfed infants presented 25(OH)D concentrations below 11 ng/ml, and this despite supplements of 2.5 to 5 µg (100 to 200 IU) of vitamin D (133). In the Republic of Korea 97% of breastfed infants born during winter and 47% born during summer were reported to be vitamin D-deficient (131). In Pakistan, 55% of breastfed infants presented with vitamin D serum concentrations below 10 ng/ml (141). In the USA Greer & Tsang (142) reported that exclusively breastfed 6-month-old infants presented with very low 25(OH)D serum concentrations, but none in any of these populations presented with rickets.

Thus, although there is abundant evidence suggesting that breastfed infants often receive less vitamin D than is required, most studies fail to find rickets in breastfed infants less than 6 months of age. However, this conclusion is tempered by studies of older infants. In 1979 Bacharach et al. reported rickets in breastfed infants older than 6 months whose mothers were vitamin D-deficient during pregnancy and lactation (139). In 1980, 9 cases of rickets were reported in Chicago in exclusively breastfed infants aged 7 to 24 months (140). None of the infants received any food from animal sources. Thirty children (median age 15.5 months) were diagnosed with rickets in North Carolina. All were African American and were breastfed for a median duration of 12.5 months with no vitamin D supplements (143).

It thus seems that infants who are exclusively or predominantly breastfed for 6 months or longer can be at an increased risk of rickets if their mothers are at risk of vitamin D deficiency, and the infants receive limited sun exposure and no vitamin D supplements.

#### **3.4.5 Vitamin D and growth in young infants**

The effects of vitamin D on growth in early infancy are best evaluated from results of a study conducted in China of breastfed infants assigned randomly to either 100, 200 or 400 IU of vitamin D per day. The different doses of vitamin D did not affect growth rates of infants who were born at the same latitude, but significant

differences were found in length gains over a 6-month period between infants living in northern or southern China. Length gains were greater in infants born in the north independent of supplement level. No seasonal differences were noted in either the south or the north. These data suggest that differences (e.g. genetic or environmental) other than vitamin D status may have influenced regional differences in length gain (144).

### 3.4.6 Vitamin D and growth in older infants

The effect of marginal vitamin D status on growth in older infants remains somewhat controversial because of inconsistent findings. On the whole, however, there appear to be few data supporting adverse effects on growth. Fomon, Younoszai & Thomas (145) reported similar growth rates from birth to 140 days among infants receiving either a formula supplemented with 400 or 1600 IU of vitamin D and breastfed infants. Breastfed infants were allowed one formula feeding daily that provided 500 IU of vitamin D/l. Breastfed infants also received a multivitamin preparation containing 1500 IU vitamin A, 200 IU vitamin D, several of the B vitamins and ferrous sulphate. Also, infants were unlikely to be born with marginal vitamin D stores. In another study in the USA of infants less than 1 year of age, no differences in weight or length were detected between breastfed infants, breastfed infants supplemented with 400 IU of vitamin D, and infants fed formula with added vitamin D. However, all mothers in the study were supplemented with vitamin D while infants were permitted one formula feeding per day during the first 4 months, after which solids were added to their diet (125).

In a series of studies conducted by Brooke et al., slow statural growth was reported in the first year of life in infants who were born to vitamin D-deficient mothers supplemented with vitamin D in the postpartum period (146–148). Greer et al. (127) reported that Caucasian infants exclusively breastfed for 6 months in Wisconsin were 2 cm shorter at 1 year than infants who received 400 IU daily of supplemental vitamin D, although the difference did not attain statistical significance. It is unclear if there was no difference in attained stature or if failure to detect a difference reflected a lack of sufficient power in the experimental design. In another study of vitamin D-deficient infants who were exclusively breastfed for a mean of 7.5 months, length-for-age percentiles dropped from 60 to 40 between 6 to 12 months of age (130). Thus, breastfed infants of women with poor vitamin D status, or infants with biochemical evidence of vitamin D deficiency, appear

to experience impaired growth unless supplemented with vitamin D. However, other nutrient deficiencies may account for growth retardation.

### 3.4.7 Summary

The vitamin D content of human milk is low and dependent on maternal vitamin D status as reflected by maternal serum 25(OH)D. Breastfed infants can maintain normal vitamin D status in the early postnatal period only when their mothers' vitamin D status is normal and/or the infants are exposed to adequate amounts of sunlight. Risk of vitamin D deficiency increases as infants' sun exposure decreases, and the ability of infants of vitamin D-replete mothers to maintain normal vitamin D status in the absence of sun exposure remains unknown. Infants born at high latitudes, or in places where sun exposure is restricted for cultural or other reasons, are at special risk; they are likely to be born with low vitamin D stores due to low maternal vitamin D status. If sunlight exposure or exogenous intakes of vitamin D remain inadequate, the risk of vitamin D deficiency rises with age as stores are depleted.

## 3.5 Vitamin B6

### 3.5.1 Introduction

Vitamin B6 functions as a coenzyme in the metabolism of protein, carbohydrate and fat. The term refers to several compounds, e.g. pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their respective phosphate forms – PLP, PNP and PMP. The major forms of vitamin B6 are PLP and PMP in animal tissues, and PN and PNP in plant tissues. Signs and symptoms of vitamin B6 deficiency include dermatitis, microcytic anaemia, seizures, depression and confusion. In infants vitamin B6 deficiency appears to adversely influence growth.

### 3.5.2 Vitamin B6 content in human milk

The vitamin B6 content of human milk varies with maternal B6 status and intake. The mean B6 concentration in human milk of women with B6 intakes below 2.5 mg/day is 0.13 mg/l (778 nmol/l). Mean B6 levels in milk of women with B6 intakes between 2.5 and 5 mg/day are substantially higher – approximately 0.24 mg/l (149). Thus, the daily B6 intakes of infants 1 to 6 months of age who consume at least 780 ml/day of human milk with a B6 concentration of 0.13 mg/l

should, as expected, meet the 0.1 mg/day estimated adequate intake (AI) for this age group (150) since the AI was based on B6 intake from human milk.

The milk concentration of vitamin B6 in populations at risk of vitamin B6 deficiency may be sub-optimal. The concentration of B6 in the milk of 70 Egyptian women consuming 1.0 mg vitamin B6/day was 0.073 mg/l, substantially lower than that reported for women in Western societies (151). Lower concentrations of milk B6 were associated with lower birth weight and altered infant behaviour. Thus, human milk's vitamin B6 content closely parallels the mother's intake of this vitamin.

Other factors, e.g. length of gestation, stage of lactation and the use of B6 supplements, influence the vitamin B6 concentration in human milk. The vitamin B6 concentrations in milk of women supplemented during lactation with 2 or 27 mg B6/day and who delivered prematurely were reported to be 0.05 mg/l and 0.22 mg/l, respectively. These levels were sustained for 28 days postpartum. Levels in milk of women who delivered at term and were supplemented at the same levels were, respectively, 0.08 mg/l and 0.38 mg/l during the first week postpartum. Vitamin B6 levels in milk rose to 0.10 mg/l and 0.50 mg/l, respectively, by 28 days postpartum (152). Similarly, in a study conducted by Udipi et al., throughout the first month postpartum vitamin B6 levels in milk of women delivering prematurely were lower than the levels of women who delivered at term (153). Also analysed was the effect on vitamin B6 milk concentrations of different levels of supplementation. Women received 0, 2.5, 10 or 20 mg PN(HCL) for 3 consecutive days. Non-supplemented mothers had the lowest vitamin B6 levels in their milk ( $0.09 \pm 0.01$  mg/l) compared to the other groups ( $0.19 \pm 0.02$  mg/l,  $0.25 \pm 0.02$  mg/l and  $0.41 \pm 0.04$  mg/l, respectively) (154).

### 3.5.3 Approaches used to estimate vitamin B6 requirements

Measurements of vitamin B6 concentrations in plasma, blood cells or urine have been used as indicators of B6 status. Functional indicators such as erythrocyte aminotransferase saturation by PLP or measurements of various tryptophan metabolites also have been considered as indicators of B6 status in depletion-repletion studies because of their responsiveness to changes in B6 intakes. Plasma PLP often has been used to assess vitamin B6 status because it reflects tissue stores, responds to changes in dietary B6 and correlates well with other B6 indices (155). However, erythrocyte PLP may be a more reliable indicator, particularly in infants (156).

### 3.5.4 Estimates of requirements

The AI for vitamin B6 is 0.1 mg/day for infants 0 to 6 months and 0.3 mg/day for infants 6 to 12 months of age (160). The AI for the younger age group is based on intakes of exclusively breastfed infants. The AI for older infants is based on extrapolations from data obtained in both the younger age group and adults.

### 3.5.5 Vitamin B6 status of breastfed infants and lactating women

Blood PLP concentrations are high in the fetus and newborn, and they decrease progressively throughout the first year of life (157). Reference ranges for erythrocyte PLP (EPLP) concentrations and erythrocyte aspartate transaminase (EAST) activities in lactating Finnish women and their infants were established by Heiskanen et al. (156). To be included in the reference, infants had to be exclusively breastfed for 6 months by women with adequate B6 status who were supplemented with 1 mg PN/day, who fed appropriate complementary foods after 6 months, and who weaned to a cow's milk-based formula at approximately 9 months. The 10th centile values for EPLP concentrations and EAST activity and activation coefficients were defined based on subsets ( $n=90$  at 2,  $n=106$  at 4,  $n=99$  at 6,  $n=39$  at 9, and  $n=100$  at 12 months postpartum) of the original sample ( $n=198$ ).

In a follow-up analysis, Heiskanen et al. (158) evaluated the B6 status of 44 infants from the original sample of 198 who met WHO's feeding recommendation (exclusive breastfeeding for 6 months and continued breastfeeding for 12 months with appropriate complementary feeding). Low vitamin B6 status – diagnosed as at least two reference values below the 10th centile cut-offs – was observed in 7 of the 44 infants between 4 and 6 months of age. Weight velocities of these infants did not differ from infants with normal B6 status, but their length velocity was significantly lower at 6 to 9 months.

The vitamin B6 status of exclusively breastfed infants was evaluated at 2 months ( $n=118$ ), 4 months ( $n=118$ ), 6 months ( $n=112$ ), 7.5 months ( $n=70$ ), 9 months ( $n=36$ ), 10 months ( $n=14$ ), 11 months ( $n=11$ ) and 12 months ( $n=7$ ) (159). During the first 4 months the vitamin B6 status of the infants was adequate and independent of maternal status. By 6 months 30% of the infants breastfed by mothers with low vitamin B6 status also had low status. By 6 months of exclusive breastfeeding, the low vitamin B6 status of mothers was reflected in the vitamin B6 status of their infants. At 6

and 7.5 months indicators of vitamin B6 status in mothers and infants were significantly correlated. Despite a daily PN supplement of 1 mg/day, maternal B6 status was inadequate in ~8% of mothers in the first 6 months and in 11% of mothers at 9 months postpartum. Prenatal vitamin B6 stores appear important for the maintenance of adequate vitamin B6 status of breastfed infants in the first 4 months of life. Human milk alone may not sustain vitamin B6 requirements beyond 6 months.

### 3.5.6 Growth of breastfed infants in relation to vitamin B6 status

In the series of studies conducted by Heiskanen et al. (156, 158, 158) EPLP concentrations at 4 months of age correlated positively with length velocity from 0 to 6 months ( $r=0.46$ ,  $p=0.006$ ), and EAST activity in the entire sample correlated with length velocity and changes in length-for-age at 9 months. Weight velocity assessed during the entire first year did not differ statistically among infants with adequate or low B6 indices ( $n=7$ ). Between 6 and 9 months of age, infants with low B6 indices experienced slower length velocities than infants with adequate B6 indices. Despite similar protein status at 4, 6 and 9 months determined by plasma total proteins, prealbumin and transferrin, all 7 infants with low B6 indices presented declines in length-for-age Z-scores.

Kang-Yoon et al. (160) evaluated the growth of infants of well-nourished women supplemented with 2 or 27 mg PL-HCl/day during the first month postpartum. A subgroup was selected from infants born to women who received a 2 mg vitamin B6 supplement. This subgroup of infants was supplemented postnatally with 0.4 mg of vitamin B6. This subgroup, and infants whose mothers were supplemented at the higher level, achieved higher weight-for-age and length-for-age Z-scores than infants of women supplemented at the lowest level despite similar values at entry.

### 3.5.7 Summary

Maternal B6 status and intake, length of gestation, stage of lactation and use of B6 supplements affect the B6 content of human milk. In well-nourished populations, human milk appears to maintain normal vitamin B6 status in most exclusively breastfed infants during the first 4 to 6 months of age; the risk of B6 inadequacy appears to increase beyond 6 months. After 6 months of exclusive breastfeeding, low vitamin B6 status in a mother was associated with low vitamin B6 status in

her infant. Compromised linear growth associated with low vitamin B6 status in infants exclusively breastfed for 6 months was reversible through appropriate complementary feeding. In populations with poor vitamin B6 nutriture, the concentration of B6 in human milk will be sub-optimal, with possible adverse effects on infant growth and neurological development.

## 3.6 Calcium

### 3.6.1 Human milk composition

Human milk contains 250–300 mg/l of calcium with no pronounced changes during lactation (33). Generally, maternal diet does not appear to influence the concentration of calcium in milk. However, recent studies from the Gambia indicated that poorly nourished women on low-calcium diets produced milk with lower-than-normal calcium levels (161), which did not increase with calcium supplementation (162).

### 3.6.2 Estimates of calcium requirements

Calcium requirements are affected substantially by genetic variability and other dietary factors (163). Pronounced calcium deficiency resulting in tetany rarely occurs in the healthy, breastfed infant and therefore is not helpful in determining requirements. Assessment of calcium status is difficult since serum levels are homeostatically regulated and therefore do not reflect body content. Inadequate calcium intake can result in lower-than-normal bone mineralization. Single-beam X-ray densitometry and, more recently, dual-energy X-ray absorptiometry (DXA) have been used to measure bone mineral content (BMC) and BMD.

Using DXA, breastfed infants have been shown to have lower BMC and BMD than formula-fed infants at 6 months (164) and 12 months (37). However, the clinical relevance of this is uncertain since the differences in bone mineralization did not persist beyond weaning (37, 164). Since bone mineralization did not differ between breastfed and formula-fed infants after weaning, retention of more calcium than that achieved by breastfed infants does not seem to benefit bone mineralization later in life.

Compared to British children, BMC at the radius in Gambian infants was slightly lower at birth, and it fell progressively during early childhood such that by 36 months it was 31% lower (165). The difference remained significant after correction for body weight, height and bone width. Although the BMC of Gambian and British women is remarkably similar, it could be



argued that the BMC of Gambian women is less than their genetic potential, as African Americans are known to have significantly higher BMC than their fairer-skinned counterparts. Weaning breastfed infants onto low-calcium diets may compromise later bone mineralization.

Balance studies in breastfed term infants indicate rates of absorption ranging from 40 to 70% (166, 167). In breastfed infants, a mean calcium intake was 327 mg/day and the retention was 80 mg/day (166). Losses amounted to 247 mg/day.

Stable-isotope studies using  $^{44}$ calcium and  $^{46}$ calcium have been used to determine calcium absorption and retention in term infants (168). Calcium absorption measured using stable isotopes averaged  $61 \pm 23\%$  (range 27–89%) in a study of 14 human milk-fed infants, aged 5 to 7 months, with a mean weight of 7.8 kg (168). Based on an assumed milk concentration of 0.25 mg/ml and 766 ml/day, an endogenous faecal excretion of 3 mg/kg per day and a urinary excretion of 3 mg/kg per day, these authors estimated from their observations that 68 mg/day of calcium were retained from human milk (168).

The accretion of body calcium during the first year of life has been estimated from changes in body weight

(140 mg/day) (169) and metacarpal morphometry (80 mg/day) (170, 171).

The calcium requirements of breastfed infants have been estimated from urinary calcium losses (3 mg/kg per day), faecal endogenous losses (3 mg/kg per day) (168), and rates of calcium accretion (Table 10). We estimated calcium accretion from BMC measurements by DXA taken at 15 days and 12 months of age (172) on the assumption that 32.2% of BMC was calcium (173). These estimated requirements were based on a small number of infants and several assumptions and thus should be confirmed by further study. Based on the estimated calcium intakes of exclusively breastfed infants, the efficiency of calcium absorption would have to be greater than 70% to cover these estimated requirements.

### 3.6.3 Summary

Calcium requirements during infancy were derived from stable isotope studies of calcium absorption and retention, and calcium accretion rates. Calcium content of human milk is fairly constant throughout lactation and is not influenced by maternal diet. Based on the estimated calcium intakes of exclusively breastfed infants, human milk meets the calcium requirements

**Table 10. Calcium requirements of breastfed infants**

Age (months)	Calcium urinary losses (mg/day)	Calcium urinary losses (mg/day)	Calcium endogenous faecal losses (mg/day)	Calcium endogenous faecal losses (mg/day)	Calcium gain (mg/day)	Calcium gain (mg/day)	Calcium gain (mg/day)	Total requirement for net calcium absorption (mg/day)	Total requirement for net calcium absorption (mg/day)	Total requirement for net calcium absorption (mg/day)
	BOYS <sup>A</sup>	GIRLS <sup>A</sup>	BOYS <sup>B</sup>	GIRLS <sup>B</sup>	BOYS <sup>C</sup>	GIRLS <sup>C</sup>	ALL	BOYS	GIRLS	ALL
1	14	13	14	13	131	109	120	158	135	147
2	17	15	17	15	131	109	120	164	140	152
3	19	17	19	17	131	109	120	169	144	156
4	21	19	21	19	131	109	120	173	147	160
5	22	21	22	21	131	109	120	176	151	163
6	24	22	24	22	131	109	120	179	153	166
7	25	23	25	23	131	109	120	181	155	168
8	26	24	26	24	131	109	120	183	157	170
9	27	25	27	25	131	109	120	184	159	172
10	27	26	27	26	131	109	120	186	160	173
11	28	26	28	26	131	109	120	187	161	174
12	29	27	29	27	131	109	120	189	163	176

<sup>a</sup> Calcium urinary losses (3 mg/kg per day) (168).

<sup>b</sup> Calcium endogenous faecal losses (3 mg/kg per day) (168).

<sup>c</sup> Calcium gain (172).

of infants during the first 6 months of life if the efficiency of absorption is maintained at ~70%, which is within reported rates (166, 167).

## 3.7 Iron

### 3.7.1 Human milk composition

The concentration of iron in human milk declines from ~0.4–0.8 mg/l in colostrum to ~0.2–0.4 mg/l in mature human milk (33). The iron content of human milk appears to be homeostatically controlled by up- and down-regulation of transferrin receptors in the mammary gland (174); consequently, it is unaffected by maternal iron status or diet.

### 3.7.2 Estimates of iron requirements

Major factors determining iron requirements during infancy are iron endowment at birth, requirements for growth and a need to replace losses. The newborn infant is well endowed with iron stores and a high concentration of haemoglobin. In the first 6 to 8 weeks of life, there is a marked decline in haemoglobin from the highest to the lowest observed during development due to the abrupt decrease in erythropoiesis in response to increased postnatal delivery of oxygen to tissues (175). In the next stage, between 2 and 4 months of age, haemoglobin concentration gradually increases. Erythropoiesis becomes more active, and there is an increase in erythroid precursors in the bone marrow and an elevation of the reticulocyte count. Between 4 and 6 months of age, there is an increased dependence on dietary iron. Dietary iron provides ~30% of the requirement for haemoglobin iron turnover, compared to 5% in adults (175). Because of the considerable iron requirement for growth and the marginal supply of iron in infant diets, iron deficiency is prevalent among infants between 6 and 12 months of age.

Iron-containing compounds in the body serve metabolic or enzymatic functions or are used for storage. Haemoglobin, myoglobin, the cytochromes and several other proteins function in transport, storage and utilization of oxygen. Iron is stored primarily as ferritin and haemosiderin. Iron is mobilized from these reserves to maintain haemoglobin and other iron-containing compounds. Body function is unlikely to be impaired as long as iron reserves are available. When iron reserves are depleted, iron deficiency will result in anaemia. Haemoglobin can be used to diagnose iron deficiency anaemia although the cut-off value for infants is debated. Serum ferritin, transferrin saturation, trans-

ferrin receptor and mean corpuscular volume can be used to assess iron deficiency.

Total body iron is relatively stable from birth to ~4 months of age, but the proportion of body iron in distinct compartments (e.g. red blood cells, myoglobin and stores) shifts dramatically as stores are depleted and demands for iron increase to meet needs imposed from 4 to 12 months of age by expanding red blood cell and myoglobin compartments. Iron requirements thus rise markedly around 4 to 6 months of age (176). These requirements are very high relative to infants' energy requirements at this age. Factorial, balance and stable isotope methods have been used to estimate infants' iron requirements. Iron needed to recover endogenous losses through the gastrointestinal tract (62 mg/year) and skin (29 mg/year) has been estimated to be approximately 91 mg/year. Iron at 1 year of life as haemoglobin (270 mg), myoglobin and enzymes (54 mg), and storage (53 mg) amounts to 109 mg above the amount present at birth (268 mg) (177). Using this factorial approach, the total iron requirement during infancy is ~200 mg/year or 0.55 mg/day. Since there is a substantial increase in erythrocyte mass and myoglobin between 4 and 12 months (176, 178), the iron requirement is thought to be higher in later than early infancy. Iron requirements are thus estimated to be 0.5 mg/day for infants from 0 to 6 months of age and 0.9 mg/day for infants 6 to 12 months of age (Table 11).

**Table 11. Iron Requirements of breastfed infants**

Age (months)	Faecal and skin losses (mg/day)	Iron gain (mg/day) All	Total iron requirement (mg/day)
1–6	0.24	0.25	0.49
7–12	0.37	0.53	0.90

Source: reference 177.

Iron intakes from human milk are summarized in Table 4. At a fractional iron absorption rate of 0.20, it is clear that breastfed infants subsidize their requirements from iron reserves in the body. Stable isotope studies using <sup>59</sup>Fe have tended to overestimate the absorption of iron from human milk because of unequal distribution of the extrinsic label with intrinsic iron in human milk. Recent studies have indicated that the absorption of iron from human milk is more likely to be lower – ~19–20% (179, 180). A balance study in exclusively breastfed term infants resulted in positive iron balances up to 4 months

of age (181). Recent results using  $^{59}\text{Fe}$  and  $^{58}\text{Fe}$  indicated a median absorption of iron from human milk of 14% at 6 months of age, 49% in non Fe-supplemented infants and 18% in Fe-supplemented infants at 9 months of age. Although iron absorption was enhanced, the iron in human milk would not be sufficient to meet estimated iron requirements (Abrams, personal communication).

Iron status – assessed by the determination of haemoglobin, red blood cell counts, transferrin, transferrin saturation, serum iron and ferritin – of exclusively breastfed infants was satisfactory up to 6 months of age in studies by Duncan et al. (182), Lönnerdal & Hernell (183), Saarinen & Siimes (184) and Simes et al. (185). Iron status was adequate in one study up to 9 to 12 months of age in exclusively breastfed infants (186). However, other studies demonstrated that breastfed infants who do not receive iron supplements are at risk of becoming iron-deficient in the second half of infancy (187, 185).

### 3.7.3 Summary

Human milk is a poor source of iron and cannot be altered by maternal iron supplementation. It is clear that the estimated iron requirements of infants cannot be met by human milk alone at any stage of infancy. The iron endowment at birth adequately provides for the iron needs of the breastfed infant in the first half of infancy. The iron available for growth and development should be adequate until iron stores are exhausted.

Factorial and balance methods have been used to estimate the iron requirements of infants. Iron requirements are estimated to be 0.5 mg/day for infants from 0 to 6 months of age and 0.9 mg/day for infants 7 to 12 months of age. Human milk's iron content, which declines from ~0.4–0.8 mg/l in colostrum to ~0.2–0.4 mg/l in mature milk, is unaffected by maternal iron status or diet. The estimated iron intakes of exclusively breastfed infants are insufficient to meet their iron requirements. At a fractional iron absorption rate of 0.20, it is clear that breastfed infants subsidize their requirements from iron body reserves. It appears that breastfed infants who do not receive additional iron from supplements or complementary foods are at risk of becoming iron-deficient in the second half of infancy.

## 3.8 Zinc

### 3.8.1 Human milk composition

The concentration of zinc in human milk declines precipitously from 4–5 mg/l in early milk, to 1–2 mg/l

at 3 months postpartum, and to ~0.5 mg/l at 6 months (33). There is considerable inter-individual variation in milk zinc concentrations; in one study the coefficient of variation was 0.25 at 2 weeks postpartum and > 0.50 at 5 to 7 months (188). Interestingly, milk zinc concentration displays channelling or tracking in individuals throughout lactation. A significant correlation ( $r=0.60$ ) was detected between the concentration of zinc in early milk at 2 weeks postpartum and mature milk at 5 to 7 months (188).

Maternal dietary zinc has not been shown to affect the zinc content of human milk, while concentrations in human milk seem resistant to zinc supplementation. Studies of lactating women receiving daily zinc supplements did not show any effect on milk zinc concentration (188, 189), nor did daily doses of 50–150 mg zinc prevent a decline in milk zinc concentration (190). A slower rate of decline, however, was observed in lactating women supplemented with 15 mg/day zinc for 9 months of lactation (191). A randomized, controlled supplementation trial by the same group of investigators failed to confirm their earlier observations (188). A supplement of 20 mg/day for 9 months did not increase mean serum or milk zinc in Finnish women (191). However, a 40-mg supplement increased maternal serum levels at 2 months and the milk level after 6 months of supplementation. A recent study in lactating Spanish women provided evidence that both dietary zinc intake and serum zinc concentrations were positively correlated with milk zinc concentrations (193). Women with low zinc intake in their third trimester of pregnancy (< 10 mg/day zinc) had lower concentrations of zinc in their milk. A comparison of milk zinc concentrations from lactating women in developing and developed countries supports the hypothesis that chronically low dietary zinc is associated with lower milk zinc concentrations (194).

### 3.8.2 Estimates of zinc requirements

Severe zinc deficiency results in acrodermatitis enteropathica, impaired immune function, diarrhoea and growth retardation. Zinc status is commonly assessed by serum zinc; however, this indicator is affected by other factors, notably infection, stress and growth rate. Serum zinc is informative for groups of healthy infants but not for assessing individuals.

There are several case reports of severe zinc deficiency in breastfed term infants receiving milk having lower-than-normal concentrations of zinc (195–200). Since maternal zinc supplementation failed to increase milk concentrations, zinc uptake or secretion by the mammary gland appeared defective in these cases.

Mean serum zinc was stable in breastfed infants from 2 to 9 months, but the number of infants in the low range (0.55mg/l) increased from 3% at birth to 30% at between 4 to 9 months (192). Serum zinc correlated with zinc intake and milk zinc concentrations. However, neither low zinc intakes nor low serum zinc levels were associated with poor growth.

In contrast, a decline in serum zinc and erythrocyte metallothionein concentration from 6 to 9 months was observed in breastfed Danish infants (201). Serum zinc at 9 months was positively correlated with weight gain between 6 and 9 months. Mean serum zinc did not change significantly between 2 and 6 months, and then fell significantly between 6 and 9 months, reaching a low mean of 8.4 µmol/l.

Median zinc balance in term predominantly breastfed infants studied at 17, 35, 57, 85 and 113 days of age has been shown to be positive (0.1 mg/kg per day); however, the range of zinc balances was high (202) and 33% of the infants were in negative balance. Stable-isotope studies using <sup>67</sup>Zn and <sup>70</sup>Zn demonstrated equilibration of the extrinsic label with intrinsic milk zinc (203). The mean fractional zinc absorption from human milk was 0.55 or 0.08 mg/kg per day in 2- to 5-month-old breastfed infants with some variation with infant age (204).

In the latter study, the infants all achieved positive zinc balance through relatively high fractional zinc absorption and conservation of endogenous zinc losses.

Since there is no pharmacological effect of zinc on growth, zinc supplementation trials of breastfed infants provide evidence as to whether zinc is limiting growth. A 3-month intervention trial was undertaken in 4- to 9-month-old breastfed infants who received either 5 mg/day zinc or a placebo (205). A significant increase in weight gain and linear growth was observed in the supplemented infants. Complementary foods and formula use were not reported. Whether the amount of zinc provided by human milk during the complementary feeding period was insufficient or whether properties of food interfered with the absorption of zinc from human milk is uncertain (205).

In another random double-blind study, exclusively breastfed infants were assigned to receive a 5 mg/day zinc-supplement or placebo from 2 to 6.5 months of age (206). Zinc supplementation did not enhance the growth of exclusively breastfed infants. This suggests either that zinc intakes and stores in these infants were sufficient to sustain growth or, alternatively, that zinc alone may not be limiting the growth of exclusively breastfed infants. Zinc, in combination with other trace

**Table 12. Zinc requirements of breastfed infants**

Age (months)	Urine and sweat losses (mg/day) BOYS <sup>a</sup>	Urine and sweat losses (mg/day) GIRLS <sup>a</sup>	Urine and sweat losses (mg/day) ALL	Endogenous faecal losses (mg/day) BOYS <sup>b</sup>	Endogenous faecal losses (mg/day) GIRLS <sup>b</sup>	Endogenous faecal losses (mg/day) ALL	Zinc gain (mg/day) BOYS <sup>c</sup>	Zinc gain (mg/day) GIRLS <sup>c</sup>	Zinc gain (mg/day) ALL	Total requirement for net zinc absorption (mg/day)
1	0.092	0.087	0.089	0.229	0.218	0.223	0.704	0.566	0.635	0.947
2	0.110	0.103	0.106	0.275	0.257	0.266	0.599	0.507	0.553	0.925
3	0.126	0.116	0.121	0.314	0.291	0.303	0.461	0.421	0.441	0.864
4	0.139	0.128	0.134	0.347	0.321	0.334	0.375	0.362	0.368	0.836
5	0.150	0.138	0.144	0.374	0.346	0.360	0.322	0.309	0.316	0.820
6	0.159	0.147	0.153	0.397	0.368	0.382	0.257	0.257	0.257	0.791
7	0.166	0.154	0.160	0.415	0.386	0.400	0.230	0.217	0.224	0.784
8	0.172	0.161	0.167	0.431	0.402	0.416	0.211	0.184	0.197	0.780
9	0.178	0.166	0.172	0.444	0.416	0.430	0.178	0.171	0.174	0.776
10	0.183	0.171	0.177	0.456	0.428	0.442	0.158	0.158	0.158	0.777
11	0.187	0.175	0.181	0.469	0.437	0.453	0.158	0.145	0.151	0.785
12	0.192	0.180	0.186	0.481	0.450	0.466	0.158	0.145	0.151	0.803

<sup>a</sup> Urinary and sweat zinc losses (20 µg/kg per day) (209).

<sup>b</sup> Endogenous faecal zinc losses (50 µg/kg per day) (204).

<sup>c</sup> Zinc gain (20 µg/g weight gain) (210, 211).

minerals present in very small amounts in human milk, might be limiting the growth of older exclusively breastfed infants.

Using stable isotope studies, the estimated mean net zinc absorption, which does not include urinary or integumental losses, was 0.26 mg/day at 2 months and 0.29 mg/day at 4 to 5 months (207). Even with very efficient absorption and conservation of endogenous losses, net zinc absorption did not meet zinc requirements at 2 months or 4 to 5 months. Mobilization of hepatic zinc bound to metallothionein may supplement the infant's needs during the first months of life, but by 4.4 months hepatic metallothionein levels fall to those found in older children (208).

Zinc requirements of infants may be estimated by the factorial method (209). Urinary and sweat zinc losses are estimated to be 20 µg/kg per day (209). Zinc required for new tissue accretion is estimated to be 20 µg/g weight gain or 30 µg/g lean tissue gain (210, 211). Endogenous faecal zinc losses are estimated to be 50 µg/kg per day (204). Total zinc requirements for net zinc absorption are summarized in Table 12. These estimated zinc requirements should be considered provisional since they were based on studies with small sample sizes and extrapolated data. Zinc requirements are higher in boys than in girls and are highest in early infancy, at the time of greatest weight gain. As growth velocity slows in later infancy, the losses in urine and sweat exceed the amount deposited in tissues.

Mean zinc intakes from human milk are summarized in Table 4. At an estimated fractional zinc absorption of 0.55 (204), net zinc absorption will fall short of actual zinc needs. Zinc intakes from human milk are subject to inter-individual variation in milk zinc concentrations. Since milk zinc concentration displays significant tracking ( $r=0.60$ ) in individuals throughout lactation (188), infants whose mothers produce low zinc concentrations will be at increased risk of zinc deficiency. Since milk intakes are driven by energy needs and not by zinc requirements, and since milk energy and zinc concentrations are not correlated, milk zinc intakes will not be determined by infant size or growth potential.

### 3.8.3 Summary

The concentration of zinc in human milk declines precipitously between early and mature milk and is basically unaffected by maternal zinc supplementation. There is some evidence that chronically low dietary zinc is associated with lower milk zinc concentrations. Zinc requirements have been estimated by the factorial method. At an estimated fractional zinc absorption of 0.55, the net zinc absorption from human milk will fall short of zinc needs, which appear to be subsidized by prenatal stores.

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Fifty-fifth World Health Assembly, May 2002,  
document A55/15.

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This review, which was prepared as part of the background documentation for a WHO expert consultation, evaluates the nutrient adequacy of exclusive breastfeeding for term infants during the first 6 months of life. Nutrient intakes provided by human milk are compared with infant nutrient requirements. To avoid circular arguments, biochemical and physiological methods, independent of human milk, are used to define these requirements.

The review focuses on human-milk nutrients, which may become growth limiting, and on nutrients for which there is a high prevalence of maternal dietary deficiency in some parts of the world; it assesses the adequacy of energy, protein, calcium, iron, zinc, and vitamins A, B6, and D. This task is confounded by the fact that the physiological needs for vitamins A and D, iron, zinc – and possibly other nutrients – are met by the combined availability of nutrients in human milk and endogenous nutrient stores.

In evaluating the nutrient adequacy of exclusive breastfeeding, infant nutrient requirements are assessed in terms of relevant functional outcomes. Nutrient adequacy is most commonly evaluated in terms of growth, but other functional outcomes, e.g. immune response and neurodevelopment, are also considered to the extent that available data permit.

This review is limited to the nutrient needs of infants. It does not evaluate functional outcomes that depend on other bioactive factors in human milk, or behaviours and practices that are inseparable from breastfeeding, nor does it consider consequences for mothers. In determining the optimal duration of exclusive breastfeeding in specific contexts, it is important that functional outcomes, e.g. infant morbidity and mortality, also are taken into consideration.

For further information please contact:

**Department of Nutrition for  
Health and Development (NHD)**

World Health Organization  
20 Avenue Appia  
1211 Geneva 27  
Switzerland  
Tel: +41 22 791 3320  
Fax: +41 22 791 4156  
email: [deonism@who.int](mailto:deonism@who.int)  
website: [www.who.int/nut](http://www.who.int/nut)

**Department of Child and Adolescent  
Health and Development (CAH)**

World Health Organization  
20 Avenue Appia  
1211 Geneva 27  
Switzerland  
Tel +41-22 791 3281  
Fax +41-22 791 4853  
email: [cah@who.int](mailto:cah@who.int)  
website: <http://www.who.int/child-adolescent-health>