Protein and Energy Requirements in Infancy and Childhood

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Foreword

The topic ‘Protein and Energy Requirements in Infancy and Childhood’ was chosen for the 58th Nestlé Nutrition Pediatric Workshop, which took place in November 2005 in Ho Chi Minh City, Viet Nam. In 1993 the 33rd Nestlé Nutrition Workshop on ‘Protein Metabolism during Infancy’, chaired by Prof. Niels Raihã, was held in South Africa. In this workshop, Prof. Raihã introduced a new concept in terms of protein requirements, proposing a reduction in the protein level of infant formulas in order to come closer to that of human milk. In 2000, the workshop entitled ‘Infant Formula: Closer to the Reference’ (Nestlé Nutrition Workshop Series, Vol. 47, supplement) chaired by Prof. Niels Raihã and Prof. Firmino Rubaltelli, included a review of more recent information showing the safety and efficacy of reducing the protein content of infant formulas to a level closer to that of human milk.

The outcome of these workshops has enabled Nestlé R&D to successfully develop a new generation of starter infant formulas with significantly improved protein quality at the level of 1.8 g/100 kcal, coming closer to that of human milk during early lactation. After successful clinical testing, these starter infant formulas are now soliciting high interest among infant health professionals. Of late, attention has also focused on whether or not it may also be desirable to optimize the protein quality and reduce the protein level of follow-up infant formulas. Moreover, other studies have suggested associations between protein nutrition in the first year of life, and the subsequent risk of obesity, diabetes and other related chronic diseases. Whilst protein requirements have received a great deal of attention, the aspect of energy requirements has never been reviewed on a systematic basis in the Nestlé Nutrition Workshop Series. Hence we believe it is timely to review the latest knowledge on the energy and protein requirements of infants and children.

We wish to thank the two chairpersons, Prof. Ekhard E. Ziegler and Prof. Jacques Rigo, recognized experts in this field, for establishing the workshop program and for bringing together the key opinion leaders in the field of protein and energy requirements.

Ekhard E. Ziegler, Professor of Pediatrics and Head of the Samuel J. Fomon Infant Nutrition Unit, at the University of Iowa, USA, is well known for his work on protein metabolism. Prof. Jacques Rigo is the Head of the Neonatal Department of the University of Liege in Belgium and a member of the ESPGHAN Committee of Nutrition.
We also thank Mrs. Montip Nagsevi and Mrs. Vipapan Panitantum and their team from Nestlé Viet Nam who provided all logistical support, enabling participants from around the globe to enjoy the superb Vietnamese hospitality.

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Abstract

Some 30 years ago, Günter Dörner stated that the concentrations of hormones, metabolites and neurotransmitters during critical periods of early development will program disease risk in human adulthood, a concept that since has received enormous scientific support and broad attention. Evidence has also accumulated showing that early nutrition programs later obesity risk. Breastfeeding reduces the odds ratio for obesity at school age by about 20%, relative to formula feeding, adjusted for biological and sociodemographic confounding variables. We propose that the protective effect of breastfeeding is explained at least in part by the induction of lower rates of infant weight gain, which may be related to differences in substrate intakes with breast milk and standard infant formulae. Protein intake per kilogram body weight is some 55–80% higher in formula-fed than in breast-fed infants. We hypothesize that high early protein intakes in excess of metabolic requirements may enhance weight gain in infancy and later obesity risk (the ‘early protein hypothesis’). The European Childhood Obesity Project is testing this hypothesis in a randomized double-blind intervention trial in more than 1,000 infants in 5 European countries. Infants that are not breast fed are randomized to formulae with higher or lower protein contents and are followed up to school age. If an effect of infant feeding habits on later obesity risk should be established, there is great potential for effective preventive intervention with a significant potential health benefit for the child and adult population.

Evidence is accumulating to show that metabolic events during critical time windows of pre- and postnatal development have marked modulating effects on health in later life, a concept often referred to as ‘programming’ or ‘metabolic programming’ [1]. It has been some three decades since Prof. Günter Dörner,
then head of the Institute of Experimental Endocrinology at the Charité Hospital, Humboldt University at Berlin, Germany, first introduced the term 'programming' into the scientific literature to describe these phenomena [2]. In a visionary article reviewing a series of clinical and experimental data, Dörner [3] concluded that the concentrations of hormones, metabolites and neurotransmitters during critical early periods of development are capable of pre-programming brain development and, up to human adulthood, functional disturbances, diseases as well as syndromes of reproduction and metabolism. Dörner also proposed an interaction between the genetic material of the individual and environmental influences during early development to determine later function in adult life, a concept that has only recently been confirmed by experimental data [1, 4–6]. The concept has gained wide popularity following epidemiological studies documenting inverse relationships between body weight at birth and at age 1 year, respectively, and the risks of hypertension, diabetes and coronary heart disease in adulthood [7].

These observations have led to the hypothesis that maternal malnutrition during pregnancy would induce both fetal growth restriction and increased later disease risk, the fetal origins of the adult disease hypothesis [7]. However, this interpretation has recently been challenged based on the observation that low birth weight is associated with catch-up growth after birth, and accelerated weight gain by itself seems to be a risk factor for later disease [8]. Cole [9] substantiated the latter concept by multiple regression analysis of blood pressure outcomes on weights at different ages. Data from cohort studies in Brazil and the Philippines relating blood pressure in adolescence to weight through childhood showed small inverse weight effects in infancy, but early weight proved to be less important than weight and weight gain during adolescence [9].

Furthermore, Tu et al. [10] raised the possibility that evidence for the fetal origins of adult disease hypothesis might be a statistical artifact known as the 'reversal paradox', due in part to inappropriate statistical adjustment for variables on the causal pathway such as early weight gain and current body size. They performed computer simulations for three hypothetical relations between birth weight and adult blood pressure. The effect of statistically adjusting for different correlations between current weight and birth weight and between current weight and adult blood pressure was examined to assess their impact on associations between birth weight and blood pressure. When there was no genuine relation between birth weight and blood pressure, adjustment for current weight created an inverse association the size of which depended on the magnitude of the positive correlations between current weight and birth weight and between current weight and blood pressure. When there was a genuine inverse relation between birth weight and blood pressure, the association was exaggerated following adjustment for current weight, whereas a positive relation between birth weight and blood pressure could be reversed after adjusting for current weight.
These questions need to be carefully elucidated, given the enormous preventive potential of optimizing early nutrition for long-term health, well-being and performance. Not only in view of the possible improvement in quality of life of individuals, but also in view of the enormous economic impact for societies, major investments in research are justified to explore the mechanisms and effects of early nutritional programming on long-term health. Today a large number of scientific investigators study these issues. In Europe many leading research groups collaborate in the international research cluster EARNEST (www.metabolic-programming.org) supported by the European Union.

Programming of Later Obesity Risk

Childhood obesity is now considered a global epidemic in view of the alarming increase in its prevalence and severity, not only in affluent but also in less privileged childhood populations worldwide [11–13]. Serious short- and long-term consequences of childhood obesity arise in terms of damage to quality of life, performance, health and life expectancy. In addition, the size of the obesity epidemic is estimated to create huge costs for society due to loss of productivity and ensuing costs for health care and social security. Faced with the size of the problem, widely available and effective medical management of children who are already obese is needed, but at present the results of available treatment concepts are far less than satisfactory, and costs are high [14]. A recent Cochrane review on interventions for treating obesity in children found that no conclusions on the effects of treatment strategies and their components can be drawn with confidence [15]. Thus, in the present situation the emphasis must be put on the development, evaluation and implementation of effective primary prevention of obesity. Several indications exist that modification of infant nutrition may offer opportunities for contributing to the prevention of later obesity risk [1].

Early Growth and Later Obesity Risk

Already in the 1950s, McCance and Widdowson showed that alterations in early growth by manipulating the feeding conditions of piglets during sensitive pre- and postnatal periods predetermined their ultimate weight in adulthood [16]. In humans high birth weight has been proposed as a risk factor for later overweight [17, 18], which could reflect both the roles of genetics and early priming by intrauterine environment. Additionally recent studies pointed to further priming of childhood overweight in the first 2 years of life by a high postnatal weight gain [19–22]. In order to assess the best anthropometric predictor from birth to 2 years for later overweight, we performed a cohort study in Bavaria, southern Germany, on 4,235 German children aged 5–6 years.
participating in the obligatory school entry health examination in 1999/2000 [23]. Overweight at school entry was assessed according to sex- and age-specific body mass index (BMI) cutoff points. Data collected during the preventive pediatric health care screening led to calculation of weight, length, BMI and the ponderal index difference between birth, 6, 12 and 24 months which were analyzed as possible predictors by receiver operating characteristic curves (ROC) and predictive values. For all parameters the highest areas under ROC were observed with a 24-month follow-up. The area under ROC decreased in the order from a weight gain of 0.76, to a BMI gain of 0.69, to a length gain of 0.58 (p < 0.001; table 1). The highest Youden Index ((Sensitivity + specificity) – 1) for weight gain from birth to 24 months (41%) was attained for a cutoff point of 9,764 g with a corresponding likelihood ratio of 2.39 and a positive predictive value of 19% despite an odds ratio of 5.7 (95% CI 4.5–7.1). Thus, a high weight gain during the first 24 months is the best

Table 1. Area under receiver operating characteristic (ROC) curves and cut points, sensitivity and specificity at highest Youden index for early anthropometric measurement prediction of overweight at school age in 4,235 children in Bavaria, Germany

<table>
<thead>
<tr>
<th>Measure</th>
<th>Area under ROC curve</th>
<th>Cut point at highest Youden index (Youden index)</th>
<th>Sensitivity at highest Youden index, %</th>
<th>Specificity at highest Youden index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 0–6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.63 (0.60–0.66)</td>
<td>5,100 g (19)</td>
<td>45 (40–50)</td>
<td>74 (73–76)</td>
</tr>
<tr>
<td>Length</td>
<td>0.51 (0.48–0.55)</td>
<td>20 cm (4)</td>
<td>21 (17–25)</td>
<td>83 (81–84)</td>
</tr>
<tr>
<td>BMI²</td>
<td>0.60 (0.57–0.63)</td>
<td>5 (15)</td>
<td>43 (38–48)</td>
<td>72 (70–73)</td>
</tr>
<tr>
<td>Ponderal index³</td>
<td>0.59 (0.53–0.60)</td>
<td>0.2 (11)</td>
<td>32 (27–37)</td>
<td>78 (76–79)</td>
</tr>
<tr>
<td>Age 0–12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.68 (0.65–0.70)</td>
<td>6,933 g (27)</td>
<td>68 (63–72)</td>
<td>59 (58–61)</td>
</tr>
<tr>
<td>Length</td>
<td>0.55 (0.52–0.58)</td>
<td>26 cm (9)</td>
<td>66 (61–71)</td>
<td>43 (42–45)</td>
</tr>
<tr>
<td>BMI²</td>
<td>0.63 (0.60–0.66)</td>
<td>4 (20)</td>
<td>66 (62–71)</td>
<td>53 (51–55)</td>
</tr>
<tr>
<td>Ponderal index³</td>
<td>0.57 (0.54–0.60)</td>
<td>−0.3 (11)</td>
<td>64 (59–69)</td>
<td>47 (45–48)</td>
</tr>
<tr>
<td>Age 0–24 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.76 (0.74–0.79)</td>
<td>9,764 g (41)</td>
<td>70 (65–75)</td>
<td>71 (69–72)</td>
</tr>
<tr>
<td>Length</td>
<td>0.58 (0.55–0.61)</td>
<td>39 cm (13)</td>
<td>45 (40–50)</td>
<td>68 (66–69)</td>
</tr>
<tr>
<td>BMI²</td>
<td>0.70 (0.67–0.72)</td>
<td>4 (31)</td>
<td>57 (52–62)</td>
<td>74 (73–75)</td>
</tr>
<tr>
<td>Ponderal index³</td>
<td>0.61 (0.58–0.64)</td>
<td>−0.5 (17)</td>
<td>44 (39–49)</td>
<td>72 (71–74)</td>
</tr>
</tbody>
</table>

Weight gain from birth to age 2 years is the best predictor of overweight at school age. Adapted from Toschke et al. [24].

Unless otherwise indicated, data are given as values with 95% confidence intervals in parentheses.

¹(Sensitivity + specificity) – 1.
²Calculated as weight in kilograms divided by the square of height in meters.
³Calculated as weight in kilograms divided by the length in meters cubed.
overall predictor of overweight at school entry compared to other anthropometric markers and time intervals.

In contrast to our results, Stettler et al. [25] recently proposed that the weight change in the first week after birth might be critical for modulating later obesity risk. They analyzed data obtained in interviews with 653 adults aged 20–32 years who had participated as infants in controlled but not randomized trials testing the feeding of infant formulae based on cows milk or soy proteins from 8 to 112 days of life. For an additional 100 g weight gain between birth and age 8 days, the odds ratio for being overweight in young adulthood (BMI > 25) increased significantly by 20%, whereas there was only a trend to an increase by 6% for 100 g weight gain between birth and age 122 days. However, the data on which the authors’ conclusions are based appear questionable because the dimension of weight gain between birth and either 8 or 122 days, respectively, is markedly different. Thus the use of one and the same absolute weight gain (100 g) as the denominator appears inappropriate. In fact, the recalculation of their data to percentage weight change indicates that a 10% change in weight gain between birth and day 8 (approx. 20.5 g) increases adult overweight risk by only 4.1%, whereas a 10% higher weight gain between birth and day 112 (approx. 309 g) leads to a 18.5% higher risk of adult overweight. Thus, also these data seem to support that it is growth rates over a long period in infancy that are predictive of later obesity.

The rate of weight gain in the first 2 years of life is influenced by the genetic factors of the individual and its mother, birth weight, metabolic influences during pregnancy, health and disease factors, such as the occurrence of infections, and not least by diet and nutrient supply.

Protective Effects of Breastfeeding against Later Obesity

It has long been known that populations of infants fed breast milk or formula differ in their growth kinetics, with formula-fed infants showing higher weight and length gains [26]. Based on a systematic review of 19 studies in affluent populations, Dewey [27] concluded that by the age of 12 months, the cumulative difference in body weight amounts to approximately 400 g in infants breast fed for 9 months and as much as 600–650 g in infants who are breast fed for 12 months. Given this very large effect of the mode of feeding on early weight gain, we attempted to study whether breastfeeding might also confer protection against later obesity risk.

In a cross-sectional survey in Bavaria, Germany, we assessed the impact of breastfeeding on the risk of obesity and the risk of being overweight in children at the time of school entry [28]. Routine data were collected on the height and weight of 134,577 children participating in the obligatory health examination at the time of school entry in Bavaria. In a sub-sample of 13,345 children, early feeding, diet, and lifestyle factors were assessed using responses to a
questionnaire completed by the parents. The data of 9,357 children aged 5 and 6 years who had German nationality were included in the final analysis. Being overweight was defined as having a BMI above the 90th percentile and obesity was defined as a BMI above the 97th percentile of all 134,577 German children examined in this year. The prevalence of obesity in children who had never been breast fed was 4.5% as compared with 2.8% in breast-fed children. A clear dose-response effect was identified for the duration of breastfeeding on the prevalence of obesity: the prevalence was 3.8% for 2 months of exclusive breastfeeding, 2.3% for 3–5 months, 1.7% for 6–12 months, and 0.8% for more than 12 months. Similar relations were found with the prevalence of being overweight. The protective effect of breastfeeding was not attributable to differences in social class or lifestyle. After adjusting for potential confounding factors, breastfeeding remained a significant protective factor against the development of obesity (odds ratio 0.75, 95% CI 0.57–0.98) and being overweight (odds ratio 0.79, 95% CI 0.68–0.93), again with a clear dose-response relationship between the duration of breastfeeding and later risk of overweight and obesity, respectively (fig. 1). We conclude that promoting prolonged breastfeeding may help decrease the prevalence of obesity.

Following our publication, a number of other investigators studied this relationship in data from various cohorts around the world. We thus performed a systematic review and meta-analysis of published epidemiological studies (cohort, case-control or cross-sectional studies) comparing early feeding mode and adjusting for potential confounding factors [29]. Electronic databases were searched and reference lists of relevant articles were checked. Calculations of pooled estimates were conducted in fixed-effects and random-effects models. Heterogeneity was tested by Q-test. Publication bias was assessed from funnel plots and by a linear regression method. Nine studies with more than 69,000 participants met the inclusion criteria. The meta-analysis showed that breastfeeding reduced the risk of obesity in childhood.

![Fig. 1. The adjusted odds ratio for overweight (■) and obesity (□) at school entry decreases with increasing duration of breastfeeding. Adapted from von Kries et al. [28].](image-url)
significantly. The adjusted odds ratio was 0.78 (95% CI 0.71–0.85) in the fixed model (fig. 2). The assumption of homogeneity of the results of the studies included could not be refuted (Q-test for heterogeneity, p > 0.3), stratified analyses showed no differences regarding different study types, age groups, definition of breastfeeding or obesity and number of confounding factors adjusted for. A dose-dependent effect of breastfeeding duration on the prevalence of obesity was reported in 4 studies. Funnel plot regression gave no indication of publication bias. Another recently published meta-analysis confirmed a protective effect of breastfeeding, but reported a smaller effect size with an odds ratio of 0.87, primarily influenced by the results of one publication from the USA with a very large sample size [30].

**Potential Causes for the Protective Effects of Breastfeeding on Later Obesity**

A number of hypotheses can be raised on the potential causes of a protective effect of breastfeeding. Even though the inverse relationship of both breastfeeding and breastfeeding duration with later obesity persists after adjustment for measurable confounding variables, residual confounding cannot be fully excluded. Since one cannot randomize healthy babies to either breast milk or formula feeding for ethical and practical reasons, undisputable
proof for a protective effect of breastfeeding can hardly be obtained. However, the consistent results of many studies and the dose-response effect between the duration of breastfeeding and the later reduction of obesity risk observed in a number of studies make an effect of breastfeeding highly likely.

Differences in feeding behavior and mother-child interactions between populations of breast- and formula-fed infants might play a role. Breast-fed infants show a different suckling pattern and a higher suckling frequency [31, 32]. Breast-fed infants seem to have a greater degree of control on meal sizes and intervals than those fed formula. Sievers et al. [33] monitored marked differences in feeding patterns, with a 20–30% higher feeding volume of formula-fed infants after 6 weeks of life as well as a smaller number of total meals and of nightly meals in bottle-fed babies at 4 months of age. Such differences may modulate later body size. Agras et al. [34] reported that early feeding patterns were predictive of BMI at 3 years of age, with high-pressure sucking measured in the laboratory at 2 and 4 weeks of age (denoting a vigorous feeding style) associated with a greater degree of adiposity in toddlers.

In contrast to infant formula, breast milk shows marked variation in its taste and smell from day to day, and even from meal to meal, depending on maternal dietary habits and other metabolic factors. Since early taste experience in infancy has been shown to favor later consumption of foods with the same taste [35], it is conceivable that breast-fed infants might be programmed to different food selection and dietary habits in later life.

Moreover, breastfeeding appears to enhance the emotional bonding of the mother to her child, mediated in part by the stimulation of maternal oxytocin release by infant suckling, and breastfeeding has been shown to lead to decreased neuroendocrine response to stressors and decreased negative mood in the mothers [36, 37]. These effects of breastfeeding might well have repercussions on the interaction between mother and child and health-related behaviors. These and further behavioral hypotheses are plausible and attractive, but are difficult to test experimentally, thus for the time being they remain somewhat speculative.

The mode of infant feeding at the breast cannot be copied by human milk substitutes, but if the protective effects of breastfeeding were related to the compositional aspects of breast milk and to the nature of substrate supply, such benefits might potentially be extended, at least in part, also to formula-fed populations by appropriate modifications to infant formula composition. Promising hypotheses can be deducted from studies evaluating physiological differences of breast- and bottle-fed infants. The higher growth rates observed in populations of formula-fed infants compared to infants fed breast milk are most likely due to differences in metabolizable substrate intakes.

Infant formulae have a higher average caloric density (kcal/100 ml) than the mean values for breast milk, and energy supplies per kilogram body weight to formula-fed infants are 10–18% higher than those to breast-fed babies between 3 and 12 months of age [38]. Even larger is the difference in
protein intake per kilogram body weight, which is 55–80% higher in formula-than in breast-fed infants (fig. 3) [39].

In rats, prenatal exposure to high protein decreased energy expenditure and increased later adiposity [40], and a high postnatal protein and nutrient supply led to higher adult body fat deposition [41] and increased adult weight by 10–40% [42]. A high protein intake in excess of metabolic requirements may enhance the secretion of insulin and insulin-like growth factor-1 (IGF-1). Indeed, infants fed formula had far greater postprandial levels of insulin on day 6 of life than infants fed cow’s milk-based formula [43]. High insulin and IGF-1 values can enhance both growth during the first 2 years of life [44, 45] as well as adipogenic activity and adipocyte differentiation [46] (fig. 4). High protein intakes may also decrease human growth hormone secretion and lipolysis.

Indeed, high protein intakes in early childhood, but not the intakes of energy, fat or carbohydrate, were significantly related to an early occurrence
of adiposity rebound and to high childhood BMI, corrected for parental BMI [47–50]. Thus, we hypothesize that a high protein intake with infant formula, in excess of metabolic requirements, may predispose to an increased obesity risk in later life (early protein hypothesis).

**Testing the Early Protein Hypothesis: The European Childhood Obesity Project**

In addition to prospective epidemiological and experimental studies, human intervention trials are needed to test this ‘early protein hypothesis’. Therefore, we have set up the European Childhood Obesity Project (www.childhood-obesity.org) funded by the European Commission's 5th Framework Research Programme to test, in a randomized double-blind intervention trial, whether variation in protein intakes during the first year of life affects growth kinetics and later obesity risk. This trial is being conducted in 5 European countries which differ substantially in their prevalence of adult obesity and also in the nutritional characteristics of the habitual diet of infants and children, in particular in protein supply with complementary feeding, i.e. Germany (project and center coordinator Prof. Berthold Koletzko, Munich), Belgium (center coordinator Prof. Philippe Goyens, Brussels), Italy (center coordinator Prof. Marcello Giovannini, Milan), Poland (center coordinator Prof. Jerzy Socha, Warsaw) and Spain (center coordinator Dr. Ricardo Closa, Tarragona). Therefore the trial offers the opportunity to combine a multicenter intervention trial on infant formulae which differ in their balance of protein and fat (Bledina, Steenvoorde, France), with an epidemiological observation study which can assess the balance of protein and fat in the overall early diet. This approach will enable us to assess the effect of variables which differ substantially within Europe, as well as allowing the intervention trial results to be analyzed within centers. The inclusion of a group of breast-fed infants in each center will also allow an epidemiological comparison of the effects of breastfeeding and formula feeding in the different countries. This approach will provide the opportunity for an external validation of the underlying hypothesis.

Growth from birth to age 2 years, a marker of later obesity risk, was chosen as the primary outcome variable. In addition, a variety of further variables are measured, including detailed data on diet, lifestyle and behavior, biochemical and endocrine markers, markers of renal function, and others (fig. 5). Randomization and data collection are performed via the internet based on uniform electronic case report forms, using specially developed information technology architecture with a central database and 12 remote data entry stations as well as dedicated software that enables secure data protection. Mechanisms for quality assurance have also been established. Data input and transfer to the central database are supervised by a contract research organization participating in the project.
The intervention trial started on October 1, 2002, and recruitment was completed on June 30, 2004. Following the study protocol and the requirements to report the first results to the EU at the end of the first funding periods, the study will not be blinded in the second half of 2006 to allow first data evaluations. However, the children participating and their families will be invited for further follow-up until 2010 as part of the EU 6th Framework Research Project, EARNEST, which investigates long-term health effects or early nutrition (www.metabolic-programming.org). Therefore, the European Childhood Obesity Project offers unique and exciting opportunities for evaluating the effects of early diet on long-term health in later life. If an effect of infant feeding habits and, in particular, of high protein intakes on long-term growth, development of later body composition and obesity risk can be established, there is great potential for effective preventive intervention by modification of the composition and use of dietary products for infants. Thus, the expected results might have a very direct application with a significant potential health benefit for the child and adult population.

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Statement on Conflict of Interest

The author declares that there is no conflict of interest according to the definitions of the International Committee of Medical Journal Editors (http://www.icmje.org/).

References

Long-Term Consequences of Early Feeding

Discussion

Dr. Cong Khanh Nguyen: What are the factors that lead to weight gain during the first week? Are they related to maternal nutrition? The second thing is you addressed the issue of nutrition before 2 years of age as being really critical, not only breastfeeding but also weaning food, the starting time and quality. Somehow it is dealing with protein and energy. So our topic is really interesting with regard to the protein and energy requirements for infancy and childhood. Again it is exactly what we are doing and understand about the requirements of protein. In early childhood protein plays a role in development, but we need to do something with protein in terms of controlling weight gain. It is really important for Viet Nam at the moment; we have to do nutritional controls and also control weight because obesity occurs even in the malnourished population.

Dr. Koletzko: Thank you, I agree this is very important for Viet Nam but also for many other countries around the world that are affected by this double burden of disease, where on the one hand you have a significant proportion of the population that is born with low birth weight under less optimal conditions and then exposed to a diet that predisposes to a high risk of obesity. As we have learned from a number of studies, it appears that if you are born with a low birth weight then at a later age you have a higher risk not only for obesity but also for the metabolic consequences of obesity. There are studies showing that at the same body mass index (BMI) populations born with a low birth weight have a higher body fat content and a much worse metabolic pattern, and a higher disease risk. This is particularly relevant for countries such as Viet Nam where you have this double burden of disease. Now the question whether the first week is relevant; I have my doubts. I have discussed my interpretation of the data published by Stettler et al., but perhaps Dr. Ziegler can also contribute something to that. I have my doubts whether the first week is really so relevant. If you think about the weight change in the first week, there are a lot of variables including water balance, and weight change after birth often is not really the substance of the body. If you look at the first 2 years I think we still have a lot of questions because the analyses we have now, not only from these two studies but also from many other studies, show that there is a relationship between early weight gain and later overweight. But we are really not sure what the critical factors involved are. I think it is a bit premature to come to direct conclusions about intervention. We need to have more data to draw these conclusions. But I agree with you absolutely that it may be a bit short sighted just to look at milk intake, and perhaps there are other factors involved such as complementary feeding. Also non-nutritional factors may be involved which could be relevant. We have seen in our own studies that smoking during early pregnancy also is
a marked risk factor for children later becoming obese, as opposed to smoking before or after pregnancy. Smoking during the first weeks and months of pregnancy is a strong independent risk factor which points out that not only are dietary and nutritional factors relevant but other factors as well.

Dr. Ziegler: I would like to make two comments regarding the Stettler study. As Dr. Koletzko pointed out, the subjects were studied as infants in Iowa. Although they had a totally different objective, we collaborated with a group in Philadelphia in a telephone follow-up study when the subjects were young adults. We asked the participants about their current weight and height. So their weights are self-reported, with all the reservations one has to have about that. The data showed that overweight status in adulthood was associated with weight gain during the first week of life and with weight gain from birth to 112 days of age, but not from 8 to 112 days of age. We have to conclude that it is really the weight change during the first week of life that predicted later obesity. Now that brings me to my second comment. There is an association between the weight change during the first week of life and later obesity. It does not imply or prove causality, and if there is no causality established, it does also imply that preventive measures aimed at altering weight change during the first week of life should not be expected to be effective, although an effect cannot be ruled out. In other words, if you were to prevent a child from gaining weight in the first week of life, it does not follow that you protect this child from being obese later in life, and i.e. because an association does not in itself establish causality. The studies that you mentioned established an association between breastfeeding in infancy and obesity in childhood, but they did not establish causality.

Dr. Koletzko: I couldn't agree more with you. There is an association with the medium size or modest size effect, and we don't really know where the causality lies and whether that gives an opportunity for preventive intervention. However, if you think about a 20 or 25% impact on later overweight and obesity on a population basis, this could be very relevant. Therefore I think it is worthwhile to look at this and investigate the potential for improving infant feeding to utilize that preventive effect. But I agree this is a research question, it is not a question that we can turn into policy at present. With respect to the comment on Stettler's study; yes, I agree birth weight is an important factor and it has been shown in many studies that birth weight is a contributing factor to later overweight risk. But I disagree with the conclusion that weight gain during the first week is a most important factor because it just results from using the same dimension, a 100-gram weight gain from day 0 to 8 and day 0 to 112. I think this is inappropriate because 100 g is a huge difference in 1 week but it is a small difference in 112 days. If you use the same proportion, i.e. percentage change in weight as I showed, then it is not the first week of life that is the most predictive time period but the total weight gain in 112 days is more relevant.

Dr. Dewey: We are all fascinated by these recent data on which age interval during the first 2 years might be most critical with regard to weight gain. I think it is important to point out that breastfed infants on the average don't have a net weight gain in the first week of life, they tend to lose a little bit and then come back up, whereas formula-fed infants tend to gain from the very beginning. So it is very intriguing to wonder whether those first 3 days or so of caloric intake, which is minimal in the breastfed infant, might be important metabolically. I think the jury is still out, and I agree with you that we need much more data. I don't know if you have the first week of life in your data set, but the question I have for the whole first 2 years is whether you looked separately at the effect of weight gain during any of those intervals for the breastfed infants in your study as compared to the formula-fed infants? I think there are questions as to whether rapid weight gain in a breastfed infant has the same consequences as it does in a formula-fed infant.
Dr. Koletzko: That is a very good question. We have included in the publication data obtained on infant feeding at school age (5–6 years). While the data are very reliable on whether the child was breastfed or not breastfed, the question on how long the child was fed exclusively breast milk is not very precise in a retrospective interview at age 6. Even less precise is the question on when complementary feeding was introduced and the kind of complementary feeding. So we felt a bit uncomfortable about putting too much trust in these details of that study. But now we have the opportunity in the European Childhood Obesity Project to obtain very detailed characteristics of these populations, also in terms of the dietary habits with 3-day records every month in the first year. It will be a much more reliable analysis, and we look forward to seeing what comes from that.

Dr. Haschke: The Nestlé Nutrition Institute recently appointed a committee under the chairmanship of Prof. Lucas to look at all published data on the association between breastfeeding and obesity. The committee pointed out that unfortunately in most meta-analyses there is a selection bias towards positive studies showing an association. However, one study which is continuously neglected is the Iowa Growth Study where more than 400 infants were followed prospectively until 8 years of age; they were either breastfed or formula-fed and all the data on the duration of breastfeeding and type of formula feeding are available. So I would like to ask you to comment on this; first what is the outcome at 8 years of age, and why is this study continuously neglected in meta-analyses?

Dr. Ziegler: The study we conducted was published in 1984 [1]. We followed up a total of 471 children, of whom about one third had been breastfed and two thirds had been formula-fed during the first 4 months of life. All infants participated in the studies in Iowa. Follow-up measurements of height, weight and blood lipids were performed within 2 months of the subjects’ 8th birthday. Weight, height and BMI were nearly identical in the formula-fed and breastfed infants; there was no difference whatsoever. The reason why this follow-up study is consistently ignored in all the meta-analyses I think has to do with the fact that our breastfed babies were permitted to receive modest amounts of other foods from an early age, so they were not all exclusively breastfed. But it shows absolutely no difference in adiposity at 8 years. I have to emphasize that we live and work in a small community which is dominated by the university, so most families are somehow affiliated with the university and that may explain why the families are more health conscious than the average family, and it may also explain why on follow-up in the Stettler study the rate of obesity and overweight was unusually low, it was much lower than in the general population. Under these circumstances, when all subjects are health conscious, one sees no association between breast-feeding and late obesity in childhood.

Dr. Koletzko: Let me try to give some comfort as to whether you include 400 infants more or less in a meta-analysis that includes 70,000 or 200,000 infants would not change the result at all. I cannot speak for the meta-analyses published by Harder and by Owen but I can speak for our own meta-analysis and we, in keeping with the concept of evidence-based medicine, defined upfront our criteria for inclusion which included a clear description of exposure, a clear description of results, overweight-obesity as odds ratios so that they could be compared, and outcome measured at the age of at least 5 years because we felt that overweight-obesity before the time of obesity rebound doesn’t really give a strong predictive result. A journal plot analysis did not give any indication of publication bias. So based on those criteria I think we came to a very clear unbiased conclusion. But I agree, this is the observation of an association, it does not allow any conclusions on cause and effect.

Dr. Butte: Two of the biggest predictors of later obesity in children are maternal and paternal BMI as well as the birth weight of the infant. If you look around the world we tend to put all this early programming into the same pot, and in some populations we
have very poor gestational weight gain and in other populations, like in United States
and Europe, we see very high gestational weight gains, and I think it is unfair to put
these in the same basket. What might be occurring to infants born above 4,000 g is very
different from infants born at less than 2,500 g. Could you comment on the effects of
both maternal and paternal BMI and gestational weight gain in your studies in Germany?

**Dr. Koletzko:** As you have probably seen we have done several studies on this issue.
In the first study published in the British Medical Journal in 1999, we didn't have data on
maternal BMI [2]. In the consecutive studies we collected such data, and what we found
is that if we adjust for maternal BMI, e.g. in the Czech study [3], then the effect size is
smaller for breastfeeding, but it is still significant in the Czech study with a 20% risk
reduction after adjusting for a variety of factors including maternal BMI. Weight gain in
pregnancy is obviously a very relevant candidate to look at, but in our studies we did not
have such data included because it is very difficult to get precise measures on that in a
retrospective analysis. We recently had a discussion with some obstetricians on the
question of whether one come up with recommendations on desirable weight gain, and I
started to appreciate how complex this issue is and how difficult it is to come to conclu-
sions. But I would agree with you yes, that is an important area to look at.

**Dr. Gomes-Pedro:** You have talked about breastfeeding decreasing overweight and
obesity at a later age. Can you comment about blood pressure?

**Dr. Koletzko:** There are some studies in the literature reporting that breastfed pop-
ulations on average have a slightly lower blood pressure, the difference is in the order
of 3 mm Hg. You may wonder whether 3 mm Hg is relevant or not; you may say 3 mm Hg
is something we can't even measure, why should that be relevant? But on a population
basis it seems to be relevant. If you look at the data from the Framingham study then a
3-mm Hg difference in average blood pressure really has a relevant effect also on the
percentage with critically high blood pressure values, and may have a marked impact
on the risk of stroke in the population. The relevant question now is whether this is
something that we can influence, and there are two insightful studies that suggest we
might. One is a study from the Netherlands [4] in which infants were given different
sodium intakes in early life, I believe it was 0.9 and 2.6 mmol sodium, total sodium
intake per day for the first 6 months. As might be expected there was a slight differ-
ence in blood pressure at the end of the 6 months; but more importantly when these
children were revisited 15 years later, there was still a difference in blood pressure of
3 mm Hg. So that would suggest that protection against very high sodium or sodium
chloride intake in the first months of life might be useful and beneficial. The second
interesting study was published by Forsyth and coworkers [5]. They revisited children
who as infants had participated in randomized trials with and without long-chain
polysaturated fatty acids in infant formula. When they looked at these children at
age 6 years, those children who had received formula with the long-chain polysatu-
rated fatty acids again had a 3-mm Hg lower blood pressure; in other words they were
closer to breastfed children. However this was a small trial, and I think it is necessary
to revisit that question in a somewhat larger, more homogeneous study concept.

**Dr. Bozo:** My question is about high protein intake during infancy in infant formula
and the hypersecretion in insulin; is it related with all kinds of amino acids or is it
related with specific amino acids present in the infant formula?

**Dr. Koletzko:** So far we don't know. If you look at the literature there is a lot of data
showing that in animals and in humans, there are certain amino acids which have a par-
ticular effect on insulin liberation. When it comes to infants first of all I would say we
don't really know whether protein intake affects the described difference in insulin
secretion between infants. We have the hypothesis but I don't think we have conclusive
data. We have to get them from the European Childhood Obesity Project obviously, but
I don't think we can make conclusions at the moment. If that is the case obviously the
next question is, is it total protein or is it the quality of protein, and maybe another
opportunity if the hypothesis proves true is not only to manipulate total protein intake but also to manipulate protein composition to try to adapt the metabolic profile. So I am excited about the question you are asking but I can’t offer a perfect answer.

**Dr. Rigo:** Most of the time in the studies they look at the weight gain. Can you speculate about the quality of the weight gain or length gain during the first part of life and the obesity rate?

**Dr. Koletzko:** In our study of 4,000 children we saw that length gain between 0 and 6, 6 and 12, 12 and 24 months, the totality had a predictive value for later overweight, but the predictive value was less than the value of weight gain. Mind you what we chose as the outcome measure was BMI at 5–6 years. You can ask now is BMI really the best indicator of health risk, and looking back it might have been better to have other measures included to describe obesity worldwide than just BMI, perhaps central obesity, waist-hip ratio or body fat content or whatever might have been more useful than just BMI. In our prospective studies now we are looking at a variety of descriptors of body size and body composition to study some of these questions because BMI in children may not be the best predictor of later health. We need to look at that more closely.

**Dr. Kah-Tzay Low:** From the epidemiological study by Barker et al. [6] on the relation of birth weight and later death from acute myocardial infarction during adulthood, it was found that those who are born small (<3.4 kg) and do not reach 10 kg by 1 year of age are at a higher risk of death from acute myocardial infarction. What are your comments on their conclusion recommending that those who are born with a low birth weight should try to catch up by the first year of life?

**Dr. Koletzko:** That is a very important question. It points out that it is not only fetal development and fetal growth that are relevant but also postnatal development. It is in contrast with the conclusion of a paper from the same group just published in the New England Journal of Medicine [7] where they propose that it would only be growth after the age of 2 years that would be predictive of later health. The difficulty of course is that some of these are studies performed retrospectively in subjects born in England between 1911 and 1930. So what has induced a high weight gain during the first year in these children is not necessarily what induces weight gain today. I would suspect that, e.g., socioeconomic status would be a very important factor influencing the choice of feeding. I suppose those who were not fed breast milk were fed cow’s milk or preparations made from cow’s milk rather than what we consider today infant formula. So I would be very careful in drawing conclusions from these observations to what we should do with infants born today.

### References

Energy Requirements of Infants and Children

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Abstract

The energy requirements of infants and children are defined as the amount of food energy needed to balance total energy expenditure (TEE) at a desirable level of physical activity, and to support optimal growth and development. New TEE data from doubly labeled water and heart rate monitoring are available to derive the energy requirements. Compared with the 1985 FAO/WHO/UNU recommendations, the 2004 FAO/WHO/UNU and 2002 IOM recommendations are ~12–20% lower during infancy. The 2004 FAO/WHO/UNU recommendations are on average 18% lower for boys and 20% lower for girls <7 years of age, and 12% lower for boys and 5% lower for girls 7–11 years of age. From 12 to 18 years of age, the requirements are 12% higher for boys and girls. The 2002 IOM recommendations are 8% lower for children <7 years of age, 2% lower for children 7–11 years of age, and 8% higher for children 12–18 years of age. Although the basic principles underlying energy requirements have not changed, the recommendations for energy intake have been decreased in infancy and early childhood, and increased in adolescence based on newly available TEE data.

Introduction

The energy requirements of infants and children are defined as the amount of food energy needed to balance total energy expenditure (TEE) at a desirable level of physical activity, and to support optimal growth and development consistent with long-term health [1, 2]. Recommendations for dietary energy intake must meet energy requirements to avoid the double burden of under- and overnutrition. Unlike recommendations for other nutrients which meet or exceed the requirements of practically all individuals in the population,
recommendations for dietary energy intake are based on the average require-
ment of the population group to avoid energy intakes that exceed require-
ments. Recommendations for energy intake and physical activity are intended to support and maintain the growth and development of well-
nourished and healthy children and adolescents.

**Components of Energy Requirements**

Energy requirements during development can be partitioned into compo-
nents of basal metabolism, thermogenesis, physical activity, and energy cost of growth. Basal metabolism is defined as that energy expended to maintain cellular and tissue processes fundamental to the organism. Energy is needed to maintain body temperature, support the minimal work of the heart and respiratory muscles, and supply energy to other tissues at rest. The basal metabolic rate (BMR) is measured under standard conditions defined as awake and supine in a fasting, relaxed state in a thermoneutral environment. The thermic effect of feeding (TEF) refers to the energy required for the ingestion and digestion of food and for the absorption, transport and utilization of nutrients. The TEF amounts to about 10% of the daily energy expen-
diture [3]. Thermoregulation can constitute an additional energy cost when exposed to temperatures below and above thermoneutrality; however, clothing and behavior usually counteract such environmental influences. Physical activity is the most variable component of energy requirements, and entails both obligatory and discretionary physical activities. The energy requirement for growth relative to maintenance is low, except for the first months of life. The energy cost of growth as a percentage of total energy requirements decreases from around 35% at 1 month to 3% at 12 months of age, and remains low until the pubertal growth spurt, at which time it increases to about 4% [1].

**Approaches for Derivation of Energy Requirements**

Energy requirements can be derived using the factorial estimates of TEE or measurements of TEE by the doubly labeled water (DLW) method or heart rate monitoring. The factorial approach is based on the time allocated to activities that are performed habitually and the energy cost of those activi-
ties. Factorial calculations are heavily dependent upon the estimation of basal metabolism, and energy expended in obligatory and discretionary activities. DLW is a stable (nonradioactive) isotope method that provides an estimate of TEE in free-living individuals [4, 5]. The DLW method entails administration of two isotopic forms of water (H$_2^{18}$O and $^3$H$_2$O) and is based on the principle
that the disappearance rate of $^2$H reflects the water turnover rate and the disappearance rate of $^{18}$O reflects both water and CO$_2$ turnover rates. The difference between the disappearance rates of $^2$H and $^{18}$O represents the rate of CO$_2$ production. The DLW method has been validated in preterm and term [6, 7]. In the heart rate method, TEE is predicted from heart rate based on the nearly linear relationship between heart rate and O$_2$ uptake during submaximal muscular work [8].

**Consequences of Deficit and Excess Energy Intake**

Deficit energy intake and negative energy balance can be acute or chronic [9]. Acute energy deficiency results in short-term negative energy balance during which there is a progressive loss of body weight. Chronic energy deficiency reflects long-term inadequate food intake during which a steady state is achieved at a suboptimal nutritional status. Energy deficit in children leads to growth retardation, loss of fat and muscle, delayed motor, cognitive and behavioral development, diminished immunocompetence, and increased morbidity and mortality [10]. Adaptations in metabolic rate and physical activity in response to chronic energy deficiency in children are difficult to assess for technical problems and mitigating geographical and social circumstances. The functional and behavioral consequences of energy deficiency are responsive to food supplementation. Food policies and nutrition programs aimed at meeting the energy requirements of children are clearly warranted, but their implementation should promote healthy diets with adequate, not excess, calories. Supplementation programs, especially in stunted populations, can contribute to obesity. Program targeting to undernourished children, growth monitoring and qualitative improvement in food provisions may be instrumental in preventing energy excess [11].

Excess energy intake and positive energy balance are promoted by readily available, energy-dense foods and sedentary lifestyles [11]. The consequences of excess energy and obesity are well described in children [12]. Obesity-related co-morbidities include type-2 diabetes, hyperlipidemia, hypertension, hyperandrogenism in girls, sleep disorders, respiratory difficulties, nonalcoholic fatty liver disease, gallbladder disease, orthopedic problems, and idiopathic intracranial hypertension. Serious psychosocial problems including poor self-esteem and depression also are common. Childhood obesity and its co-morbidities have a significant likelihood of persisting throughout adolescence and into adulthood.

Societies in transition are afflicted with the double burden of energy deficit and energy excess. As societies develop economically and adopt more Westernized food habits and sedentary lifestyles, there is a shift away from undernutrition towards overnutrition, as evidenced by the rise in the prevalence of childhood obesity worldwide in the past two decades [13, 14].
Energy Requirements of Infants

Previous recommendations for dietary energy intake of infants were based on the observed intakes of healthy infants growing normally, largely due to the lack of information on the energy expended in physical activity needed to estimate TEE. Since the 1985 FAO/WHO/UNU report [15], new scientific data have allowed recommendations to be based on TEE plus the energy needs for growth [16].

Total Energy Expenditure of Infants

Thirteen DLW studies were available on infants from the United Kingdom, United States, Netherlands, Chile and China [16]. TEE ranged from 255 to 393 kJ/kg/day (61–94 kcal/kg/day). Four studies demonstrated higher rates of TEE in formula-fed than breast-fed infants in the first year of life, in the order of 12, 7, 6 and 3% at 3, 6, 9 and 12 months of age [17–20]. A prediction equation (equation 1) for TEE was developed based on longitudinal data on 76 healthy infants studied at 3-month intervals for the first 2 years of life [18]. Although TEE was a function of age, weight and height, only weight was used in the prediction equation because of the high inter-correlations between variables (r = 0.91–0.96).

\[
\text{TEE (kJ/day)} = 0.416 + 0.371 \text{ weight (kg)} \text{ SEE} = 0.456
\]

\[
\text{TEE (kcal/day)} = 99.4 + 88.6 \text{ weight (kg)} \text{ SEE} = 109
\]

Energy Cost of Growth during Infancy

During infancy, energy is required to support the substantial changes in body weight and composition. Not only does the infant increase in size, but body fat increases from about 11% in newborns to 31% at 3–6 months [21]. The energy cost of growth consists of the energy deposited in newly synthesized tissues plus the cost of synthesis which is encompassed in the DLW-derived TEE. Energy deposition can be computed from the rates of protein and fat deposition assuming energy equivalents of protein (23.6 kJ/g or 5.65 kcal/g) and fat (38.7 kJ/g or 9.25 kcal/day). Serial measurements of body composition changes during infancy are used to compute energy deposition [21, 22]. The energy cost of growth decreases substantially during the first year of life from approximately 730 kJ/day (175 kcal/day) at 0–3 months to 250 kJ/day (60 kcal/day) at 4–6 months and 85 kJ/day (20 kcal/day) for 1–12 months of age.

Physical Activity Level of Infants

As infants grow and develop, physical activity represents an increasing, yet minor, component of TEE. The physical activity level (PAL) of infants can be
estimated from TEE, in conjunction with a measured or estimated BMR (PAL = TEE/BMR). The basal metabolism of infants has been investigated extensively and prediction equations for BMR are available. Schofield et al. [23] compiled ~300 measurements from historical data to develop predictive models based on weight and length. These equations have been evaluated in more recent investigations, and have been found to underestimate BMR at early ages by about 5–12% from 1 to 9 months of age [24, 25]. In infants, PAL increases from 1.2 at 3 months to 1.4 at 12 months of age [18, 26].

### Dietary Energy Recommendations for Infants

In the recent FAO/WHO/UNU [1] and IOM [2] reports, the average energy requirements of infants were based upon the TEE and growth rates of healthy, well-nourished infants (tables 1, 2). In the 2004 FAO/WHO/UNU report, energy requirements are presented for all infants combined, as well as separately for breast-fed and formula-fed infants [1]. In the FAO/WHO/UNU report, the median weight-for-age and monthly rates of weight gain of the WHO pooled breast-fed data set were used to calculate energy requirements [27]. In the 2002 IOM report, the 50th percentiles for weight gain published by Guo [28] were used to compute the energy cost of growth. Compared with the 1985 FAO/WHO/UNU report [15], the 2004 FAO/WHO/UNU and 2002 IOM recommendations are ~12% lower from 0 to 3 months, 17% lower from 3 to 9 months, and 20% lower from 9 to 12 months of age (figs. 1, 2).
Table 2. Energy requirements of girls during the first year of life

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Fig. 1. Energy requirements for girls 0–12 months of age.
Energy Requirements of Children and Adolescents

Because of insufficient data on the activity patterns of children under 10 years of age, previous estimations of energy requirements were based upon observed energy intakes [15]. For children over 10 years of age, the factorial approach was taken. Since then, reliable information on the TEE of children and adolescents has become available to derive the energy requirements of children and adolescents.

Total Energy Expenditure of Children and Adolescents

A substantial amount of DLW data have now accumulated across a wide range of ages and body sizes, so that the energy requirements of children and adolescents can be based on DLW measurements of TEE [2]. However, the available DLW data are not representative of the wide diversity in lifestyles from around the world. The majority of measurements were obtained from the children living in the United States or Europe where TEE is influenced by modern technology and transportation systems that tend to demand low physical effort [29]. There are, however, TEE data derived from heart rate monitoring available from a broader spectrum of populations.

In the 2004 FAO/WHO/UNU report [1], DLW and heart rate monitoring were used to predict the TEE of children and adolescents. The TEE data of
801 boys and 808 girls ages 1–18 years were compiled from Canada, Denmark, Italy, Sweden, Netherlands, Brazil, Chile, Columbia, Guatemala, and Mexico; specific prediction equations for TEE were developed for boys and girls [29].

For boys:

\[
\text{TEE (MJ/day)} = 1.298 + 0.265 \text{ weight (kg)} - 0.0011 \text{ weight}^2 (\text{kg}^2) \text{ SEE} = 0.518 \\
\text{TEE (kcal/day)} = 310.2 + 63.3 \text{ weight (kg)} - 0.263 \text{ weight}^2 (\text{kg}^2) \text{ SEE} = 124
\]

(2)

For girls:

\[
\text{TEE (MJ/day)} = 1.102 + 0.273 \text{ weight (kg)} - 0.0019 \text{ weight}^2 (\text{kg}^2) \text{ SEE} = 0.650 \\
\text{TEE (kcal/day)} = 263.4 + 65.3 \text{ weight (kg)} - 0.454 \text{ weight}^2 (\text{kg}^2) \text{ SEE} = 155
\]

(3)

In the 2002 IOM report [2], TEE was estimated from DLW measurements compiled on normal weight children with body mass indexes between the 5th to 85th percentiles. Separate TEE predictive equations were developed for normal-weight boys and girls from age, height, weight, and PAL category using nonlinear regression techniques. The PAL categories were defined as sedentary (PAL ≥ 1.0 < 1.4), low active (PAL ≥ 1.4 < 1.6), active (PAL ≥ 1.6 < 1.9), and very active (PAL ≥ 1.9 < 2.5). Specific prediction equations for TEE were developed for boys and girls.

For boys:

\[
\text{TEE} = 88.5 - (61.9 \times \text{age [years]}) + \text{PA} \times (26.7 \times \text{weight [kg]} + 903 \times \text{height [m]})
\]

(4)

where PA, the physical activity coefficient, was equal to 1.00, 1.13, 1.26 or 1.42 for the sedentary, low active, active and very active categories.

For girls:

\[
\text{TEE} = 135.3 - (30.8 \times \text{age [years]}) + \text{PA} \times (10.0 \times \text{weight [kg]} + 934 \times \text{height [m]})
\]

(5)

where PA, the physical activity coefficient, was equal to 1.00, 1.16, 1.31 or 1.56 for the sedentary, low active, active and very active categories.

**Energy Cost of Growth during Childhood and Adolescence**

During adolescence, gender differences in body size and composition are accentuated [30]. Adolescence in boys is characterized by rapid acquisition of fat-free mass (FFM) and a modest increase in fat mass in early puberty, followed by a decline. Adolescence in girls is characterized by a modest increase in FFM and a continual accumulation of fat mass. Despite these maturational changes, the energy cost of growth is minor relative to maintenance and physical activity.

In the 2004 FAO/WHO/UNU report [1], the energy cost of growth was based on mean rates of weight gain calculated from the WHO weight-for-age
standards [31]. The composition of weight gained was assumed to be 10% fat with energy content of 38.7 kJ/g (9.25 kcal/g), 20% protein with an energy content of 23.6 kJ/g (5.65 kcal/day) and 70% water, carbohydrate and minerals with negligible energy content. In the 2002 IOM report [2], the energy cost of growth was computed based on rates of weight gain of children enrolled in the FELS Longitudinal Study [32] and rates of protein and fat deposition for children and adolescents [33]. The energy cost of tissue deposition was approximately 85 kJ/day (20 kcal/day), increasing to 125 kJ/day (30 kcal/day) at peak growth velocity.

**Physical Activity Level of Children and Adolescents**

A minimum of 60 min/day of moderate-intensity physical activity is recommended for children [1, 2], although there is no direct experimental or epidemiological evidence on the minimal or optimal frequency, duration or intensity of exercise that promotes the health and well-being of children [34]. Regular physical activity is often associated with decreased body fat in both genders and, sometimes, increased FFM, at least in males [35, 36]. Physical activity is associated with greater skeletal mineralization, bone density, and bone mass [37].

Energy requirements must be adjusted in accordance with habitual physical activity. Torun [38] compiled 42 studies on the activity patterns of 6,400 children living in urban, rural, industrialized and developing settings from around the world. Compared with children in industrialized societies, children in developing rural areas expended more energy in domestic and productive work, and less energy in low energy activities such as attending school. The TEE of rural boys and girls was 10, 15 and 25% higher at 5–9, 10–14 and 15–19 years of age, respectively, than their urban counterparts.

As part of the compilation of TEE values described above, PAL values were estimated by using measured or predicted BMR [29]. The Schofield equations for BMR [23] were used to predict the PAL for children and adolescents, if not provided in the original publication. The average PAL (1.7) from these studies reflects a moderate level of activity. To estimate the energy requirements of children with different levels of habitual physical activity, a 15% allowance was subtracted or added to the average PAL to estimate light (PAL = 1.5) and vigorous (PAL = 2.0) levels of activity in the 2004 FAO/WHO/UNU report (figs. 3, 4). In the 2002 IOM report, energy requirements are computed for sedentary, low active, active and very active levels of physical activity.

**Dietary Energy Recommendations for Children and Adolescents**

A marked variability in energy requirements exists for boys and girls because of variations in growth rate and physical activity (tables 3, 4). In the
Fig. 3. Energy requirements of girls at three levels of habitual physical activity.

Fig. 4. Energy requirement of boys at three levels of habitual physical activity.
2004 FAO/WHO/UNU report [1], the average energy requirements for a moderately active child were derived using the above TEE equations (equations 2 and 3) and the energy cost of growth for the WHO reference values for median weight [31]. Compared with the 1985 FAO/WHO/UNU recommendations [15], the present recommendations are on average 18% lower for boys and 20% lower for girls under 7 years of age, and 12% lower for boys and 5% lower for girls 7–11 years of age. From 12 to 18 years, the requirements are 12% higher for boys and girls (fig. 5).

In the 2002 IOM report [2], energy requirements for an active child were based on TEE (equations 4 and 5) plus an average energy deposition of 20–25 kcal/day. The IOM recommendations are 8% lower for children under 7 years of age, 2% lower for children 7–11 years of age, and 8% higher for children 12–18 years of age than the 1985 FAO/WHO/UNU recommendations [15] (fig. 6).

Although the basic principles underlying the energy requirements of infants and children have not changed, recommendations for energy intake have been

### Table 3. Energy requirements of boys 0–18 years of age

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1FAO/WHO/UNU energy requirements computed for moderate levels of physical activity.

2IOM energy requirements computed for active level of physical activity.
Table 4. Energy requirements of girls 0–18 years of age

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\(^1\)FAO/WHO/UNU energy requirements computed for moderate levels of physical activity.

\(^2\)IOM Energy requirements computed for active level of physical activity.

Table 4. Energy requirements of girls 0–18 years of age

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\(^1\)FAO/WHO/UNU energy requirements computed for moderate levels of physical activity.

\(^2\)IOM Energy requirements computed for active level of physical activity.

Fig. 5. Energy requirements for girls 1–18 years of age.
decreased in infancy and early childhood, and increased in adolescence based on newly available TEE data. Even though energy requirements are also presented for varying levels of physical activity, moderately active lifestyles are strongly encouraged to maintain fitness and health and to reduce the risk of developing obesity and its co-morbidities.

**Fig. 6.** Energy requirements for boys 1–18 years of age.

decreased in infancy and early childhood, and increased in adolescence based on newly available TEE data. Even though energy requirements are also presented for varying levels of physical activity, moderately active lifestyles are strongly encouraged to maintain fitness and health and to reduce the risk of developing obesity and its co-morbidities.

**References**

Energy Requirements of Infants and Children


Discussion

Dr. Koletzko: One should encourage people to use such refined methodology to look further into better defining nutrient requirements where we still rely very much on estimates. I think Dr. Pencharz will probably give us another example on where this could lead to. I am also impressed by your adaptation of the estimates of energy requirements by 10–20% which have a real impact in infancy. If we consider that high energy intakes and high growth rates in this period of life might have long-term consequences on overweight and obesity at later ages. The Codex Alimentarius is presently discussing a revision of the global infant formula standard and, based on much of what you have presented, considered altering the range of energy density in formula. However, I have a concern when I see your slide showing that the energy requirement recommendations are intended to be prescriptive for populations. Here I have real difficulty in understanding because while this may be relevant for subjects who are fed on one and the same formulation, infant formula or tube feeding in sick children, I am not sure how you can prescribe energy intake to populations. So I am not sure where the real impact of that would be beyond infancy.

Dr. Butte: In many ways we implement these requirements on a population basis, on a group basis and on an individual basis in the United States. One thing that comes to mind, for instance, is how we feed children in school. They start somewhere and by law in the United States we are required to actually feed a certain percentage of the energy requirement at lunch and at breakfast. So there are ways in which these requirements get translated into policy and into programs. So in that sense I think we are contributing to the public health of individuals. Certainly I can’t dictate what an individual child is going to eat under those circumstances, but at the group level we are influencing what the schools for instance will get. When you think of infant feeding programs and preschool feeding programs, there has to be an estimate where to start, and I think to the best of our knowledge we should start where we think the requirement is. Dr. Uauy has presented very nice data where policy in Chile has really influenced what happening to preschool children. Those programs were initiated many years ago to combat malnutrition; but malnutrition has now decreased in those countries and overweight is being seen, he has had to fight hard to get some of those policies changed and they are actually fitting in a very large national program in preschoolers. So in that sense I think we can effect the health of infants and children through our programming. I certainly appreciate your first comment that we can take very sophisticated techniques to better our knowledge of what the requirements really are. One of the things that was really very distressing for me is when we met with the FAO and tried to get the global data for doubly labeled water studies, and found there were no data for the developing world. That is really a pity, and it coincides with when
we were trying to use the technique, $^{18}\text{O}$ became very scarce; so just as the study was gaining momentum our supply was cutoff. But fortunately $^{18}\text{O}$ is again available, and it is a wonderful opportunity at the Nestlé Institute to go into these transitional societies and capture the energy expenditure of children. There is a lot of discussion; has physical activity in children really changed? We don’t have good historical data in Westernized populations, but here is an opportunity to study traditional societies, societies in transition, as well as developed societies, and see what the range of physical activities of children is. Here in Viet Nam the children ride bicycles; they can’t get a motorcycle before 18 years of age, so they still ride bicycles. It would be fascinating to really see what the energy expenditure of these children is.

*Dr. van Goudevoer:* You showed us a lot of graphs and equations, and one of them attracted my attention. It showed that the energy expenditure of formula-fed infants is higher than that of breastfed infants. Is that due to malabsorption problems for formulas; is it due to other qualities of protein or energy, or is it due to differences in growth?

*Dr. Butte:* We don’t know why the breastfed infants have lower energy expenditure than formula-fed infants. It doesn’t have anything to do with the absorption. We are really talking about total energy expenditure which then impacts their requirements. We know that theoretically because they are eating less, the thermic effect of feeding would contribute proportionally less; we know that if they are growing less, the energy cost of growth will be different; we know the composition of the tissues being laid down also differs between breastfed and formula-fed infants. So all these although they seem quite minor they do add up and contribute to that difference in energy expenditure we see. We started out measuring sleeping metabolic rates in our earlier studies, and found a difference in the sleeping metabolic rate as well as total energy expenditure. So there is a component of basal as well as a component of physical activity. We know that there are potential chemicals in breast milk that may have a different effect on energy expenditure, a depressive effect. But no one has actually proven that any of these are the source of that difference.

*Dr. Pencharz:* The one thing I wanted to ask you about, in you childhood data you chose, at least for the IOM, to have a body mass index between the 5th and 85th percentiles, but surely normal distribution should be between the 15th and 85th percentiles. There are recent data that actually say that the 15th percentile is a better cutoff for undernutrition than the 5th percentile. What difference would it make on your overall data, the choice of the 5th rather than 15th?

*Dr. Butte:* I would say very little because if we go back and eliminate all the children that were less than 5th percentile, the actual doubly labeled water that is available is very minor in that group. So that extra 10% from the 5th to the 15th percentile would make very little difference. We took the 5th percentile for a pediatric convention defining undernutrition, not on the distribution of the population.

*Dr. Dewey:* I want to follow up on the question about the difference between breastfed and formula-fed infants in energy expenditure. My understanding from your data is that there wasn’t a difference in the physical activity component of the total energy expenditure between breastfed and formula-fed infants. So it is just the basal metabolic rate and the thermic effect of food that differ. I think that is important because it is not that they are less active. The question I have has to do with the difference between the IOM and FAO requirements for preschool children. You showed lower estimates for the FAO than for the IOM. Can you explain why that is and what the implications are?

*Dr. Butte:* It is really based on different databases. The FAO has a combination of those doubly labeled water studies and also the heart rate monitoring studies which were taken from around the world, and that is why it is based on different data. The actual fitting of the curves was slightly different; a different approach was taken to the
development of the curves. The implications will just have to stand the test of time, and in that one period from about 5 to 10 years of age there is a difference.

**Dr. Rigo:** I am concerned about the energy intake evaluation, especially for the newborn infant during the first week of life and also for the preterm infant. In fact energy intake is evaluated according to the feeding volume and the energy content, but the energy content is calculated by the metabolizable energy content using the up-water factor, and is the up-water factor adapted for the newborn infant? In fact when we analyze metabolic balance studies we see that the energy available differs and probably improves according to postnatal age, and there is some difference between breastfed and formula-fed infants. So what is your opinion about that?

**Dr. Butte:** We don’t have as much information as we would like on the term infant. The committees have met and looked for more data, but they simply don’t exist for the term breastfed infant. There are a lot of data on formula-balanced studies and there are now many studies in the preterm infant, but there is just very little on the term breastfed infant to address that question. We can avoid that whole debate now because total energy expenditure is measured directly and then metabolizable energy. So in the older studies you start with the gross energy intake doing bomb calorimetry on the milk source and you have to assume or measure metabolizable energy. But that is not an issue in these requirements or in these recommendations. But I agree there are differences in the availability of energy from human milk vs. the formula sources.

**Dr. Cong Khanh Nguyen:** Your presentation on the new FAO/WHO/UNU recommendation for energy expenditure is very interesting, and I am concerned about the implication of this new recommendation compared to the 1985 recommendation for energy expenditure, especially for children. Interestingly for children under 10 years it is lower than the previous recommendation, but from 10 years onwards it is higher than the previous recommendation. In Viet Nam we are in a period of developing recommendations for dietary allowance, and we are also looking at physical growth activity programs. So this has very important implications for us. There are three graphs, one from FAO/WHO/UNU in 1985, the second one in 2002 and the third one from the IOM. Is the thermic effect of food, the 10% of energy expenditure, the same? For example the pattern of food consumption, food intake, may have different energy expenditures. Is 10% something standard for different food patterns? Perhaps we need to do the same kind of study in Viet Nam because the doubly labeled water method is quite standard. Do you think that Viet Nam should do this kind of study because of different patterns of food intake? We need to do more and look at the physical activity levels in different populations.

**Dr. Butte:** I think the most important thing is to measure the total energy expenditure and the physical activity component in your population of children. The thermic effect of feeding is going to be around 10% and there will be some variation depending on the composition of the diet, and possibly an effect on the pattern of intake as well. More importantly we know that the carbohydrate, protein and fat will affect the thermic effect of feeding, but not greatly. Where there is going to be the most variability is in knowing what the basal metabolic rate is and the physical activity of the children. So I encourage you to focus on getting a handle on the total energy expenditure of your children so that you don’t under- or over-feed them.

**Dr. Haschke:** I have a practical question. Infant formulas and in particular follow-up formulas are now produced and used assuming that an energy intake of 100 kcal/kg body weight/day is adequate. According to what you have just shown, after 4 months of age the energy requirement is consistently around 80 kcal/kg/day. So if there is a practical consequence, these new requirements should be translated into adequate recommendations for formula-fed infants, resulting in a change in the packaging labels and feeding recommendations. So what is your recommendation? Should manufacturers now produce formulas with lower energy density for infants after 4 months of age,
and also probably with a lower protein concentration? What would be the practical consequences? The breastfed infant can self-select what it wants to consume, whereas the formula-fed infant relies on premixed formulas. What would be the consequences?

Dr. Butte: Up to now the formula companies have tried to simulate the energy content of human milk and it is fairly close on average. Of course there are variations within a feed or between feeds, but the formula is set close to the energy content of the human milk. There are several trials going on where companies are altering the protein and energy ratio in formulas and to see what impact that has on weight gain. What we know from Fomon and Nelson [1] is that probably in that early phase of infancy the children who receive a feeding at a lower energy density level just consume more. Also the studies show that, after a certain point, regulation is not as tight. We simply don't know what the impact on weight gain will be at various stages of infancy if the protein-energy ratio in the formula is changed. As I said there are trials going on and we will have the answer to that. Also as far as the recommendation on the label: yes, they should be changed to reflect more what we think the requirements of infants are. In the studies where we have carefully measured the formula intake of formula-fed infants, it is less than 100–120 kcal/kg/day. So I feel very comfortable in changing what is on the labels, on how we suggest mothers feed their infants.

Dr. Haschke: It seems that we have also overestimated the energy density of breast milk. It is 58–60 rather than 67 kcal/l as shown by more recent publications. This would also have an impact on the energy density of follow-up formulas.

Dr. Butte: I think it depends on what studies you look at. We measured the 24-hour volumes of human milk for years and our bomb calorimetry gave us averages of about 0.67–0.68. Depending on the fraction you want to take for metabolizable energy, you do get down to 62–64 kcal. I don't think it has changed; if we look at the studies that were done very carefully to get 24-hour expressions of human milk, we have seen lower energy content all along. It is very difficult to get a representative sample of human milk, and so when it is taken without paying attention to the time of day or what part of the feed, the answers can be different.

Dr. Sauve: You made your assumption on active children for the IOM and then moderate activity for the FAO and WHO. We know in North America in fact that very few children even do minor activity. I am worried that when people look at these numbers they think that these are the requirements for all children. In fact you use them for healthy active children, whereas very few children in North America are active.

Dr. Butte: The requirements have the flexibility of determining the physical activity levels of your group. For instance, if you know that your children don't get their 60 min/day and you are feeding them for some program, then you could potentially use the lower activity level. If you plug into the pyramid used in the USA you actually set what the physical activity of that individual child is or the group of children you are concerned with. I think the strong recommendation for physical activity of at least 60 min is what we would like to promote. But slowly we are seeing policy changing, and more physical education and recesses are being re-instituted in schools. Both committees have recognized that there are differences and they can be customized to your needs.

Dr. Dewey: I would like to come back to the issue of compensation for the energy density of the feeding. The earlier studies that Dr. Fomon and colleagues did were wonderful and really helped us to understand that issue. But I think it is important to remember that, if I recall correctly, the babies were fed by nurses and they were fed in a responsive way, in other words it was after the baby signaled fullness that they would stop in those trials. What I think we don't know is the degree to which babies are reasonably good at compensating when fed by parents.

Dr. Ziegler: You are referring to our studies with formulas with decreased caloric density. These were all free-living infants at home, all feeding was done by the parents.
Dr. Dewey: Were they given instructions then?

Dr. Ziegler: No, they were not even told that there was a different caloric density. We just gave them the formula and told them to feed it to the babies.

Dr. Dewey: Alright, but I do want to point out that perhaps we do need more studies on how parents of different socioeconomic levels feed formulas and the degree to which babies do or do not compensate for different energy levels.

Dr. Butte: Equally important in this whole area of early infancy is that there has been a lot of emphasis on the first 6 months of life, but from 6 to 24 months we really don’t have the data. Complementary feeding is critical in that area as well.

Dr. Ziegler: I have a question on the hypothetic assumption that it is possible to feed formula-fed babies the caloric intakes that the new recommendations show which are largely based on energy expenditures of breastfed babies.

Dr. Butte: In the IOM we included both, breastfed and formula-fed infants.

Dr. Ziegler: On the assumption that it is possible to make formula-fed babies have energy intakes matching the recommendations, what do you think would happen?

Dr. Butte: There are two things going on here. We talked a lot about total weight gain but we often have to think about the composition of that weight gain. If the protein-energy (PE) ratio is not changed, you still might see a higher accretion of fat-free mass in the formula-fed infants, as we found in our studies at Houston. If both the total energy consumed and the PE ratio are changed to simulate human milk, you might find a difference both in the weight gain and the composition of the tissue being deposited. I know it has been quite controversial, but our studies showed that we had higher rates of fat deposition in our breastfed infants during the period of exclusive breastfeeding. The differences decrease as we approach 12 and 24 months, but in the early period of exclusively breastfeeding we clearly saw higher rates of fat deposition, and that was by diverse techniques. Both components, the total amount of energy delivered as well as the PE ratio, must be addressed. Here we are focusing on protein and energy, but we often notice differences in the other nutrients in human milk vs. formula. In the formula we have much higher levels of the other minerals, and all this may be contributing to the differences we see in growth between the breastfed and formula-fed infants.

Reference

Protein Requirements of Infants and Children

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Abstract

During the last 35 years there have been various published assessments of human protein needs, including those of infants and children. Most recently, the Institute of Medicine of the US National Academies has published its report on Dietary Reference Intakes (DRI) for Macronutrients, and WHO/FAO/UNU have convened a new Expert Consultation, which is due to be published soon.

Although there have been a number of published studies on children’s protein requirements determined by the nitrogen balance technique, the results of these studies in themselves are insufficient to derive requirement values for all ages. Instead, a meta-analysis of the data from a range of studies in children has been used to derive values for the requirement for maintenance (i.e. no growth), and for the efficiency of utilization of dietary protein for growth. These values were then combined with age-specific rates of protein deposition to calculate the average requirement at any age. New values for protein deposition in children have been derived from studies of potassium-40 accumulation in infants and children, and these have been employed in new calculations of the average protein requirement. By combining values for the maintenance requirement, derived from adults, with the requirement for growth, estimated by analysis of the data for total-body potassium at different ages, the age-specific average protein requirement was determined. The safe level of intake is the amount of dietary protein that will provide the needs of almost all of the specified age group, and this is usually taken as the average requirement plus 2 times the standard deviation (SD) of the requirement. The SD of the children’s requirement was determined by combining the SD for maintenance (from the adult data) with the SD for protein deposition, derived by combining data for rates of growth of body mass and data for the whole body potassium-40 content at different ages.

The resulting values for the average protein requirement range from 1.12 g/kg/day at age 6 months to 0.74 g/kg/day at 10 years, with a small decline towards the adult value thereafter. The corresponding values for the safe level are 1.43 g/kg/day at 6 months and 0.91 g/kg/day at 10 years.
Introduction

The proteins of the body are in a dynamic state, involving continuous degradation to free amino acids, and re-synthesis of new protein molecules, the process known as ‘protein turnover’. At the same time, free amino acids are continually being degraded and oxidized to carbon dioxide and nitrogenous end products, such as urea and ammonia. These losses of amino acids must therefore be replaced by consumption of dietary protein. Moreover, in children there must be an additional intake of protein from the diet to furnish new tissue growth. The ‘requirement’ for dietary protein is therefore the sum of these two separate needs for ‘maintenance’ and ‘growth’.

Over the last few decades, various committees have been convened to determine the values for the requirement for dietary protein of children and adults, notably the FAO/WHO/UNU report on ‘Energy and Protein Requirements’ in 1985 [1], the recently published ‘Dietary Reference Intakes’ report of the US National Academies [2], and the pending WHO/FAO/UNU report on ‘Protein and Amino Acid Requirements in Human Nutrition’ [3]. In addition, the requirements of infants and children were analyzed in detail by Dewey et al. [4].

Definitions

The protein requirement for an individual is defined as the minimum intake of high quality dietary protein that will provide the needs for maintenance at an appropriate body composition, and will permit growth at the normal rate for age, assuming energy balance and normal physical activity. However, the requirement is expressed in two different ways. The first is the ‘average requirement’, which is simply the mean value for the requirement of the population under study. The second is the ‘safe level’ [1, 3] or ‘recommended dietary allowance’ (DRI) [2], which is the amount of dietary protein that will provide for the requirements of almost all (97.5%) of the population. This is higher than the average requirement by two times (to be more precise, 1.96 times) the standard deviation (SD) of the mean requirement of the population under study. It is therefore important when considering the ‘requirement’ to make a clear distinction between these two.

The use of these two values is often misinterpreted. The average requirement is the amount of protein that will satisfy the needs of approximately 50% of the population. Thus, for a population that has an average intake equal to the average requirement, half of the individuals will be receiving less than their requirement. The safe level is set at a value higher than the average requirement so that only a very small proportion of the population will have inadequate intakes when consuming that amount. However, since the intake also has a distribution, one cannot assume that if the average intake is equal to the safe level, then there will be no prevalence of inadequacy. If the average and distribution of
both intake and requirement are normally distributed and their means and SDs known, then it is possible to calculate the prevalence of inadequacy [1, 3, 4].

**Measurement of the Protein Requirement**

The procedure known as ‘nitrogen balance’ has been, and still is, the basis of all the procedures used to estimate the protein requirement. Nitrogen balance is the difference between the dietary intake of nitrogen and the total losses of nitrogen (including urine, feces and miscellaneous losses such as sweat, skin and hair losses). As almost all the nitrogen in the body is in the form of protein, the nitrogen balance represents the protein balance, with protein equal to 6.25 times nitrogen.

To measure the protein requirement of an individual, it is necessary to measure the nitrogen balance at a series of different intakes above and below the estimated requirement value. For the adult, the requirement is then the amount of dietary protein at which the intake equals the total losses, as the healthy adult is not gaining or losing body protein. For the child, the interpretation is somewhat more complex due to growth, which is accounted for as follows.

The total literature on children’s protein requirements includes a number of investigations, from various parts of the world, of nitrogen balance in infants and children, in each of whom a series of different protein intakes was studied [5–13]. For each individual a straight line relating the nitrogen balance to the protein (as nitrogen) intake was plotted and expressed mathematically as the intercept (on the intake axis) and the gradient. The intercept is the amount of nitrogen intake that is required to balance the nitrogen losses (i.e. maintenance), and the gradient of the line is the efficiency of the utilization of dietary nitrogen. In reality, and as illustrated in figure 1, the scatter of points is usually large, so that calculation of the requirement for an individual from their own data would be subject to considerable error. Hence, in practice the data from all of the published studies in which nitrogen balance was determined in children at various levels of nitrogen intake have been utilized. Ten studies of nitrogen balance in children have been published, and analysis has been performed to determine the average values for the parameters of the line [2–4]. These are conveniently expressed as the value for maintenance (i.e. when nitrogen balance is zero and there is no growth), and the gradient which expresses the efficiency of utilization of dietary protein for growth. The requirement can then be calculated for any target rate of growth, as maintenance + (rate of protein deposition ÷ efficiency).

The analysis included 10 studies with measurements on 53 individual subjects, varying in age between 9 months and 14 years, over a range of dietary protein intakes. In addition, there were 4 more studies that only determined the basal loss of nitrogen, i.e. the nitrogen excretion when a protein-free diet is given. When all studies were included in the analysis, the value for maintenance
was 110 mg nitrogen/kg/day (0.687 g protein/kg/day) and the slope was 0.58. However, when those studies that included only animal protein (milk and egg) in the diets were used in the analysis, the maintenance value was 93 mg nitrogen/kg/day (0.58 g protein/kg/day) and the slope was 0.66.

In order to use the data from the analysis of nitrogen balance vs. nitrogen intake for determining the protein requirements of infants or children, it is also necessary to know the rate of protein deposition (i.e. growth) at each age. The following therefore describes the methods that have been used to determine age-specific rates of growth and protein deposition.

**Fig. 1.** Simulated nitrogen balance data.

Rates of Protein Deposition in Infants and Children

In the analysis of the protein requirement of infants by Dewey et al. [4], the rate of growth of body mass, adjusted for the percentage of deposition as fat from the data of Fomon et al. [7], was employed to estimate the growth of protein mass. The potential errors in this assessment have been discussed in detail, as well as their impact on the recommendations. More recently, there have been more direct determinations of the rate of gain of protein mass in infants and children by measurements of total body potassium, which can be used to calculate total body protein content [14]. Butte et al. [14] assessed total body potassium in infants longitudinally from 2 weeks to 2 years of age, whereas Ellis et al. [15] made cross-sectional measurements in children from 4 to 18 years. These studies provide the most comprehensive data set so far of protein growth in children, and have been used by the DRI [2] and WHO/FAO [3] committees in their determinations of children's protein requirement. However, because the data for the two age ranges were collected differently (cross-sectional for ages 0–2 vs. longitudinal for ages 4–18), and no measurements were made between ages 4–8.
2 and 4 years, the calculation is not straightforward. The DRI committee took the mean values for total body protein at each age over the full age range, 2 weeks to 18 years, and used nonlinear regression to derive a fourth-order polynomial function for age vs. protein content. This equation was then differentiated, enabling the gradient of the curve, which is the growth rate, to be calculated for any chosen age. This approach, although theoretically sound, has several drawbacks. The first is that it did not take advantage of the longitudinal nature of the data on each individual infant during the first 2 years, and second, it was not possible to derive the SD of growth (needed to calculate the recommended daily allowance/safe level) from the curves. A single value of 43%, derived from the data of Butte et al. [14], was employed. Subsequently, the WHO/FAO Committee employed more sophisticated curve-fitting techniques to derive the growth rates. In particular, the SDs of growth rates were calculated by the following procedure: (1) for the first 2 years, each infant’s data were fitted to a polynomial function, so that growth rates at any age in the range could be calculated for each infant, and the mean value and the SD were determined; (2) for the ages 4–18 years the mean values for body protein were fitted to a polynomial function to obtain the mean rate of growth at any age; (3) a separate function was used to bridge the gap in experimental data between 2 and 4 years, and (4) because the cross-sectional data between 4 and 18 years did not allow calculation of the between individual SD for the rate of protein growth, values from a separate longitudinal database on growth of body weight of children [16] were used to calculate the between individual SD of the body growth rate at each age. This was then adjusted for the proportion of body weight that is protein, using the total body potassium data. By this process, it was possible to calculate the rate of growth plus its SD at each age. A subset of these values is shown in table 1.

### Table 1. Protein deposition for infants and children

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Protein deposition, g protein/kg/day</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>0.5$^c$</td>
<td>0.266</td>
</tr>
<tr>
<td>1.0$^c$</td>
<td>0.168</td>
</tr>
<tr>
<td>1.5$^c$</td>
<td>0.108</td>
</tr>
<tr>
<td>2.0</td>
<td>0.076</td>
</tr>
<tr>
<td>3.0</td>
<td>0.044</td>
</tr>
<tr>
<td>4–5</td>
<td>0.024</td>
</tr>
<tr>
<td>6–10</td>
<td>0.047</td>
</tr>
<tr>
<td>11–15</td>
<td>0.031</td>
</tr>
<tr>
<td>16–18</td>
<td>0.009</td>
</tr>
</tbody>
</table>

$^a$Derived from Butte et al. [15] and Ellis et al. [16] (see text).

$^b$See text.

$^c$Before age 3, data for males and females were pooled.
In table 1, the selected age ranges are chosen because they represent phases of faster or slower growth. Growth is very rapid at 6 months of age, but declines quickly until the end of the 5th year. There is then an acceleration in growth in both genders between 6 and 10 years, declining somewhat between 11 and 15 years. By 16 years, growth has almost ceased in females, but continues at a relatively slow rate for a further 3 years in males.

**Determination of Protein Requirements for Infants and Children**

**Infants**
Various groups have concluded that for infants up to age 6 months, breast milk is the best source of nutrition [2–4]. Thus, the recommended intakes have been modeled on the intake of milk by healthy breastfed infants [2]. Dewey et al. [4] compared the intake of milk with the requirement calculated by the factorial method, on the basis that the calculated requirement should not be greater than the intake. However, this comparison is complicated by the fact that human milk contains about 25% of nitrogen that is not in protein. Some of this non-protein nitrogen (NPN) is in the form of free amino acids, which would be utilized as if it were protein, but a substantial part of the NPN consists of urea and other compounds that might not be utilized as well as protein. It is therefore of interest to examine how the requirement for infants predicted by the factorial approach compares with the actual intake of milk protein and NPN.

For the factorial calculation, the values for maintenance and efficiency of utilization of dietary protein are those derived from the analysis of nitrogen balance in children described earlier. In particular, it is most appropriate to use the values that are derived from children consuming only animal protein, mostly from milk and eggs. For these children the value for maintenance was 0.58 g protein/kg/day and the slope (efficiency) was 0.66. Table 2 shows the details of the factorial calculation for the 6-month-old infant.

With the above derivation it is also possible to calculate the prevalence of inadequacy by relating the mean values and the SDs for requirement and intake using the unit normal distribution [1, 3]. This indicates a prevalence of inadequacy in the above example of about 15%.

It can be seen from the calculation that the predicted safe level of intake is intermediate between the intake of milk protein and the intake of milk nitrogen, suggesting that unless some infants are receiving less than their requirements, some of the NPN must be utilized. Alternatively, there is the possibility that the efficiency of utilization of dietary protein employed in the calculation is too low for infants at this age, giving rise to an apparent requirement that is too high. In practice it may be better to produce a requirement value that is on the high side (i.e. conservative) as the alternative, to have a value that is too low, might place some infants at risk of receiving too little protein.
The procedure for calculating the average requirement and safe level for children follows the same procedure as that for infants, with the exception that different values have been used for the maintenance and efficiency of utilization. As these children will no longer be relying on breast milk, it is assumed that they will be taking a mixed diet from normal food. Therefore, in selecting the appropriate values for maintenance and the efficiency of utilization...
from the analysis of nitrogen balance studies in children described above, values for all children, including those taking mixed diets, have been used. This is appropriate, as children older than 6 months will progressively be consuming diets with a wide range of constituents of both animal and plant origin. However, in view of the close similarity of the value for maintenance in children (0.68) with the value derived from a much larger data set in adults (0.66), the adult value was also used for maintenance in children.

**Conclusions**

The protein requirement of the 6-month-old infant is 75% higher than in the adult due to the dominance of the requirement for growth. However, growth slows rapidly over the first 2 years of life, at which time the protein requirement is less than 20% higher than that of the adult. The new values for children are about 25% higher than those derived 20 years ago by FAO/WHO/UNU [1], but for teenagers the difference is much less apparent. The differences are mostly the result of more sophisticated statistical analysis of the data, as well as the new techniques of body composition measurement (total body potassium), which have enabled the growth of protein mass in children and its variability to be evaluated more accurately.

**References**

Protein Requirements of Infants and Children

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Discussion

Dr. Pencharz: Something I would like everyone to appreciate is that the late Peter Reeds worked on this a great deal, and a lot of the work Dr. Garlick has presented, and has worked very hard on, was worked on first by Peter Reeds as well as Nancy Butte. The one thing that is also a new and which you didn't emphasize was the work by Butte and Ellis. I mean the maintenance components are really reworking of existing data, but the growth data are much more sophisticated from Beila and Butte.

Dr. Koletzko: You discussed the question of maintenance requirements and the standard deviation (SD) and pointed out that there might be some limitations in the statistical approach you used, but basically you are confident with the assumption that maintenance requirement and SD for adults can be used for infants and children as well. I wonder whether we should take into account that there might be differences. One factor that could probably contribute is the intestinal physiology. I am not sure how large the contribution of intestinal cell turnover and shedding is to the total maintenance requirement. I would assume that there might well be a difference between
infants and young children and adults if you consider intestinal cell mass to total body size, if you think about cell turnover in the intestine, and also if you consider that it is almost intrinsic to young age, to preschool age and toddler age to have enteric infection. It is a sort of physiological phenomenon to have frequent intestinal infection which of course will enhance the intestinal cell turnover even more, particularly if you think about less privileged populations. So my question simply is, do we need to put a caveat in that assumption? I was noting that you assume 75% non-protein nitrogen utilization in 3- to 4-month-old infants. My question here is how good are the data that we can base this on, both for breastfed and formula-fed infants? In formula-fed infants we usually assume much less than 50% non-protein nitrogen utilization. Can you comment on that, on how good databases are?

**Dr. Garlick:** Firstly the non-protein nitrogen utilization is a chemical point, as to how much of the milk nitrogen is not protein. We assumed about 75% just as an example to see how much of that would have to be used by making that assumption. Obviously it is going to be somewhat variable, but those are the figures in the literature, the approximate percentage. We are not trying to claim this is the requirement for infants of that age. That wasn’t the reason for the exercise. The reason was to see if by making these assumptions, do we get a requirement which is consistent with their intake, which is obviously what is needed. If it had been inconsistent we would have had to alter the calculation in some way to try and make it consistent. So we are not trying to claim this is the requirement for children of that age, it is merely a check on the numbers we are getting.

**Dr. Koletzko:** Are the maintenance requirement and SD for adults appropriate for infants and young children, specifically considering differences in intestinal physiology?

**Dr. Garlick:** It is only a small assumption really because the data that came from children for the maintenance was 0.68. We chose 0.66 because simply they are so close that they are not statistically significantly different. So we used 0.66 simply because the adult data have a much larger number of subjects. Statistically I don’t think it is a bad assumption to make.

**Dr. Dewey:** You mentioned that the studies used were on children between 9 months and 14 years of age, and my recollection is that there were very few studies of children under 12 months, perhaps one or two. So I think that question is still very important. We just don’t have enough nitrogen balance data for children under 12 months, especially healthy children living in a normal environment. Many of the earlier studies were from malnourished recovering infants who were confined to their beds and not active. So it is questionable whether to use those data. There were some earlier studies by Dr. Fomon and his colleagues on nitrogen balance in healthy young infants under 6 months of age that showed a quite low basal nitrogen requirement. Were you able to use that information in trying to assess this? The reason I bring it up is that in the 1995 calculations that we did with the factorial approach, we used that information and came up with lower levels of requirements than you have shown here for infants, which meant that the utilization of non-protein nitrogen did not have to be anywhere nearly as high as 75%, it was more in the order of 30% or so as I recall.

**Dr. Garlick:** There is an almost complete absence of really good nitrogen balance data in small infants. I looked at Fomon’s data, tried to plot graphs and curves from them and get some idea of the reliability of the numbers, and they were so variable that we decided not to use them. The other reason was if we had been using these data for older children then we would have had to include these data obviously, but we weren’t producing a requirement for children below 6 months of age, it was the real thing. Neither the IOM nor WHO are dealing with children of that age group who they say should be breastfed. So in other words the recommendations we are producing are only for children 6 months and older.
**Dr. Hilmanto:** Can you give a reason or equation why total body potassium can be used to calculate the total body protein? If we look at the last table, the safe level is about 1.2 times the average requirement, but in the introduction you mentioned that the safe level is about 2 times the average.

**Dr. Garlick:** On the second point, if I said it was above 2 times I was wrong; the safe level is the average requirement plus 2 times the SD, which usually comes to about 25% higher than the average requirement. About the total body potassium in relation to protein, there is a relationship between the potassium content of lean tissue and the protein content of lean tissue that enables the calculation. I think it will be better if Dr. Butte answers that question in more detail because they are her data.

**Dr. Butte:** There is a constant relationship between the potassium to nitrogen ratio, and it does change with growth and the actual value that we used was 2.15. This is the same value that was used in the reference infant that Dr. Fomon and Dr. Ziegler had taken to look at body composition of infants. At the time we applied it, I did search the animal literature trying to see how confident we are of that ratio. It does change with growth and there are values that are different during pregnancy in adult animals and such, but I really could not come up with anything better than what Dr. Fomon and Dr. Ziegler used many years ago. So that is the value we used.

**Dr. Ziegler:** Most of the protein in the body is intracellular, and almost all of the potassium is intracellular, and therefore the ratio of potassium to protein is quite constant. That is the basis for estimating the protein content by determining total body potassium.

**Dr. Do Van Dung:** You gave us a new approach in the calculation of nutritional requirements. Because I work mostly in the methodology of nutrition I would like to raise some questions on your methodology and especially your assumption. First I would like to challenge your assumption on the regression of the nitrogen composition on nitrogen intake because we think that if we have a low nitrogen intake the deposition efficiency will be increased and if you have more nitrogen intake you will waste a lot of nitrogen. Therefore I wonder why you use the linear regression model. The second question concerns the graph from the study by Ellis et al. [1] on body composition and I would like to know why there are some negative values in that chart? The last question, you said that the distribution of the nitrogen requirement is not normal and I wonder why you used a rather complicated formula and you admit that it is not particularly correct. Why don’t you use the percentile instead of using the mean value plus 2 times the SD. You said that the SD of the estimated average requirement is equal to the square of SD for maintenance plus the square of SD for growth. Is the formula correct if the two variables are independent, that means when the maintenance and growth are independent?

**Dr. Garlick:** As I said previously, the data are limited. We don’t have sufficient data to be able to do any perfect calculations. We just have to make do with what we have. You can see from the actual distribution of data that it isn’t normal. We satisfy ourselves that by making it into log normal then at least we can calculate the reasonably correct safe level, and I think that is a sound way of doing it. So statistically I don’t think we really have a problem with that, we are just finding a different way of calculating the 97.5 or 98th percentile, that is all. The assumption that the maintenance for the children is the same as the average requirement for the adult, the average numbers are almost the same, so I don’t think there is any problem there.

**Dr. Do Van Dung:** Why do you use linear regression?

**Dr. Garlick:** That is the way the data are. You cannot statistically show any difference from linearity. We have no basis to assume any other relationship. If the data suggested a curve then we would have to use the curve, but it doesn’t. The data show it to be pretty linear. So statistically one always starts with the simplest model and proves it wrong and before moving to a more complex one. In this case we could not prove that the simple linear model was not valid.
Dr. van Goudevoer: Protein deposition is basically based upon the availability of the most limiting amino acid. I think that the quality of milk proteins around the world is rather standard, but whenever you go up into higher ages the intake of proteins differs and also the quality of the proteins that you eat is different. So are you justifying making recommendations on a whole protein intake level, whereas the quality of that protein might differ quite substantially?

Dr. Garlick: The assumption throughout the calculation, which I should have stated, was that it is high quality protein. This is the requirement for high quality protein. If we are dealing with populations who typically have diets that consist of lower quality protein, that has to be allowed for in the calculation.

Dr. Rigo: As you show there is a gap in the data of protein accretion between 2 and 4 years. So you make the assumption that we can calculate the protein accretion according to the data that we obtain before and after with an extrapolation of the polynomial data. If we look at your data you suggest that protein accretion during this period between 2 and 4 years of age was lower than before and after. Do you think that your extrapolation was correct or not? If we look at the curve of growth velocity between birth and adolescence, it does not show this decrease between 2 and 4 years of life.

Dr. Garlick: I didn’t emphasize this because it gets too complicated to explain. But the actual body weight of children was used in the interpretation of those data, which were not from Dr. Butte or Ellis. Separate standard curves for growth were used to construct that bridging function. So I think it does take account of the actual growth.

Dr. Rigo: How do you explain that the protein accretion was lower at that period of time?

Dr. Garlick: I am showing rates of protein accretion, so they slow down slightly and then speed up again. That would not be seen unless you plot the actual rates of growth, the gradients of the growth curve.

Dr. Axelsson: I know it is a very hard work to make recommendations. We have many recommendations. What are the consequences of the new recommendations? Is it possible to follow them because when intake is calculated, even if milk and egg proteins are considered, it is very difficult. What are the practical consequences of the new recommendations to make diets consistent to this?

Dr. Garlick: They are slightly lower for children than the previous recommendations.

Dr. Axelsson: But still we have problems to follow them; even the recommendations we have today are difficult to follow in practice. If you calculate the intake of a child or an infant and then look at the intake and the requirement, there is a big difference. The intake is much higher than the requirements.

Dr. Garlick: The reason for that is that we are in a society which has ample food. For many areas of the world that would not be the case. The children will be eating a much lower protein content in relation to energy, and they are getting less than the requirements. The total intake is usually determined by energy intake.

Dr. Yates: One of the reasons, although the protein might be higher, is that there are other nutrients that a food-based diet would be providing and that might not be met even where there are high levels of protein consumed. If you are talking about a formula that is one thing, if you are talking about food-based diets it would be different.

Reference

Growth and nutrition during infancy are being viewed with renewed interest because of the possibility that they may be linked to cardiovascular and metabolic health in later life. Of particular interest are differences between breast- and formula-fed infants with regard to nutrient intake and growth because breastfeeding has been shown to be associated with a reduced risk of obesity in later life. During the first 6–8 weeks of life there is little difference in growth (gain in weight and length) between breast- and formula-fed infants. However, from about 2 months of age to the end of the first year of life formula-fed infants gain weight and length more rapidly than breast-fed infants. There are no consistent differences in adiposity during the first 4–5 months of life, but during the later part of the first year of life the preponderance of the evidence suggests that breast-fed infants are leaner than formula-fed infants. Formula-fed infants at 4–5 months of age show higher plasma levels of insulin-like growth factor-1 (IGF-1), insulin and certain amino acids than breast-fed infants. Whereas the protein intake of breast-fed infants decreases with age and closely matches the requirements for protein during the early months of life, the protein intake of formula-fed infants exceeds requirements after the first 1–2 months of life. The data are consistent with the hypothesis that differences in protein intake are mainly responsible for differences in growth between breast- and formula-fed infants. Differences in energy intake probably are responsible for differences in adiposity observed in older infants.

Introduction

In recent years growth and nutrition during infancy have been the focus of renewed and broad interest because of their association with health outcomes later in life. The seminal study by von Kries et al. [1] exemplifies
recent studies that show breastfeeding during infancy to be associated with a reduced risk of obesity in childhood. It has been appreciated for some time that breast-fed infants grow less rapidly than formula-fed infants. Also, it has been known or suspected for a long time that breastfeeding, besides its well-known health benefits in the short-term, confers protection against certain diseases in adult life. For example, subjects who were breast-fed as infants have lower plasma lipid concentrations and are therefore at a lower risk of cardiovascular disease than subjects who were fed formula during infancy [2–4]. A number of subsequent reports [5] have confirmed the initial observation by von Kries et al. [1] that breastfeeding is associated with less risk of obesity in childhood. These observations have generated considerable interest in view of the worldwide increase in overweight and obesity.

It is likely that breastfeeding in infancy and improved later health are manifestations of one and the same genetic or other causative factor. But it is also possible that breastfeeding per se is causally related to improved health in later life. If that is the case, it is pertinent to ask what it is that may confer to the breast-fed infant protection against disease in later life. Although it would seem implausible to expect a single factor, such as a single component of breast milk, to be responsible for protection against a range of conditions later in life, such a possibility is actually not entirely implausible. In premature infants it has been shown that a single factor (i.e., low protein intake during a relatively short period in infancy) is associated with lower blood pressure during adolescence [6] as well as reduced insulin resistance [7]. It is important to note that in the studies by Singhal et al. [6, 7] those subjects who enjoyed relative protection as young adults showed slower growth as infants than those with less protection. In other words, slow growth during infancy is a marker of health advantages later in life. It appears possible that the rate of growth in infancy is what determines individuals’ later cardiovascular health. Indeed, Singhal and Lucas [8] have proposed in their ‘growth acceleration hypothesis’ that it is a high rate of growth during infancy that causes increased later cardiovascular risk. A high rate of growth has, incidentally, also been shown by the same group to be associated with better neurocognitive outcome [9].

It is also possible that cardiovascular health in later life, rather than being associated with the rate of growth during infancy, is determined by the level of protein intake that produces the differences in growth in the first place. We shall show that high protein intakes during infancy elicit metabolic and hormonal responses that could explain not only the observed differences in the rate of growth but could also program individuals with regard to lifelong cardiovascular health. It is against this backdrop that the difference in growth between breast- and formula-fed term infants has become of renewed interest. A close examination of the growth differences, together with other relevant information, may not only shed light on the cause(s) of differential growth but also provide hypotheses regarding the association between infant feeding and later health.
Growth from Birth to 4 Months

From the perspective of comparing the growth of breast- and formula-fed infants, the early months of life are of particular interest. This is so because during that period breast- and formula-feeding exist for the most part in 'pure' form, i.e. without the confounding presence of complementary foods and, in the case of breast-fed infants, of supplemental formula. Also, because growth is most rapid during the early months of life, the effect of factor(s) causing differences in growth is likely to be most marked during that period.

Differences in growth between breast- and formula-fed infants are evident already during the first week of life. Breast-fed infants typically have not quite regained birth weight by 8 days of age [10], whereas formula-fed infants by 8 days of age exceed birth weight on average by 50–100 g [11]. This difference is largely explained by the fact that breast-fed infants receive only small amounts of colostrum during the first 2 days of life, a time during which formula-fed infants already have free access to formula. The difference in early weight change is potentially significant in view of the findings by Stettler et al. [12] who reported that, among formula-fed infants, the risk of overweight and obesity in early adulthood was increased in proportion to weight changes during the first week of life.

Dewey [13] presented a comprehensive review of data concerning the growth of breast- and formula-fed infants published since 1980. The review was appropriately limited to studies in which data on breast- and formula-fed infants were collected simultaneously using identical methods. Of 19 studies, 5 reported data separately for the first 3–4 months of life. Of these, 2 showed significantly greater weight gain among formula-fed infants, with 1 study [14] showing also greater length gain. Two studies showed no difference in weight gain between breast- and formula-fed infants and 1 study showed actually higher weight gain in breast-fed than in formula-fed infants.

The report by Nelson et al. [14] concerned by far the largest cohort (419 breast-fed infants and 720 formula-fed infants). Infants were studied using identical methods between 1965 and 1987. Formula-fed infants received a variety of milk- and soy-based formulas. Until 1978 infants were permitted to receive limited amounts of solid foods and, in the case of breast-fed infants, of supplemental formula, and the majority of infants did actually consume these foods, although generally only in modest amounts. After 1978 infants received no foods other than breast milk or formula through 112 days of age.

The data of Nelson et al. [14] show very convincingly that during the first 6 weeks of life, although the small difference in weight that is established during the first week of life persists, gain in weight and length are almost identical in breast- and formula-fed infants. Data on gain in weight and length between 8 and 112 days of age reported by Nelson et al. [14] are summarized in table 1 on a sex-specific basis. It is evident that between 8 and 42 days of
age, differences in gain between breast- and formula-fed infants are very small and not statistically significant. In contrast, for the age interval 42–112 days, and also for the entire period from 8 to 112 days of age, differences in gain in weight and length are larger and statistically significant.

To answer the question of whether consumption of solid foods and, in the case of breast-fed infants, supplemental formula, may have had effects on growth during the early months of life, Nelson et al. [14] compared data for infants observed before 1978 when infants were permitted to receive limited amounts of other foods and formula, with data for infants observed after 1978 when no other foods were permitted until 4 months of age. There were no significant differences in any of the growth parameters before vs. after 1978, and growth differed between breast- and formula-fed infants before as well as after 1978. This shows that consumption of modest amounts of other foods does not affect the growth of infants and does not blunt the growth difference between breast- and formula-fed infants.

It is important that the differences in growth reported by Nelson et al. [14] concern not only weight gain but also length gain. It is well established that the energy intakes of breast-fed infants are substantially less than those of formula-fed infants [15, 16]. A difference in energy intake alone in the presence of an ample protein intake would be expected to lead to a modest difference in weight gain, reflecting a difference in gain of fat mass, but it would not be expected to lead to a difference in length gain since it would not have an effect on gain in fat-free body mass. The fact that there is a difference in length gain between breast- and formula-fed infants strongly suggests that the intake of protein is limiting gain in fat-free mass in breast-fed infants.

**Table 1.** Gain in weight and length according to gender and type of feeding

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>breast-fed (n = 230)</td>
<td>formula-fed (n = 380)</td>
</tr>
<tr>
<td>Weight, g/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–42 days</td>
<td>38.9 ± 9.7</td>
<td>39.8 ± 7.7</td>
</tr>
<tr>
<td>42–112 days</td>
<td>25.4 ± 6.2*</td>
<td>28.5 ± 6.4</td>
</tr>
<tr>
<td>8–112 days</td>
<td>29.8 ± 5.8*</td>
<td>32.2 ± 5.6</td>
</tr>
<tr>
<td>Length, mm/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–42 days</td>
<td>1.29 ± 0.22</td>
<td>1.33 ± 0.22</td>
</tr>
<tr>
<td>42–112 days</td>
<td>0.96 ± 0.17*</td>
<td>1.03 ± 0.12</td>
</tr>
<tr>
<td>8–112 days</td>
<td>1.07 ± 0.12*</td>
<td>1.13 ± 0.11</td>
</tr>
</tbody>
</table>

Modified from Nelson et al. [14] with permission.

*Significantly (p < 0.001) lower than corresponding value for formula-fed infants.
It is well established that insulin-like growth factor-1 (IGF-1) plasma concentrations reflect the intake of dietary protein [17, 18]. In a study comparing growth of infants fed formulas with different protein concentrations [19], determinations of plasma concentrations of IGF-1 were obtained [20]. One formula contained protein at a concentration of 2.39 g/100 kcal and the other at 1.90 g/100 kcal. Similar determinations were made in a group of exclusively breast-fed infants participating in another study (unpublished). The results summarized in table 2 show that plasma IGF-1 concentrations were similar in the 2 groups of formula-fed infants and in the breast-fed infants at 1 month of age. However, by 4 months of age, the IGF-1 concentrations of breast-fed infants and infants fed the lower protein formula had declined significantly, whereas they remained unchanged in infants fed the higher protein formula. At 4 months of age, the IGF-1 levels of breast-fed infants were significantly lower than the levels in either formula-fed group, and the IGF-1 levels in the lower protein group were significantly lower than those in the higher protein group.

The level of dietary protein also influences plasma insulin levels, probably mediated through the effect of the protein level on plasma concentrations of certain amino acids. Dewey et al. [21] reported that at 5 months of age breast-fed infants had lower plasma concentrations of insulin and of insulin-releasing amino acids than formula-fed infants (table 2).

### Table 2. Concentrations of IGF-1, insulin and insulin-releasing amino acids (IRAA) in breast-fed and formula-fed infants

<table>
<thead>
<tr>
<th>Age</th>
<th>1 month IGF-1, μg/l</th>
<th>4 months IGF-1, μg/l</th>
<th>5 months IGF-1, μg/l</th>
<th>insulin, pmol/l</th>
<th>IRAA, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast-fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>74.9 ± 33.3 (n = 56)</td>
<td>32.0 ± 18.7* (n = 35)</td>
<td>42.6 ± 25.8 (n = 52)</td>
<td>0.967 ± 0.178 (n = 52)</td>
<td></td>
</tr>
<tr>
<td>Formula-fed</td>
<td>79.3 ± 34.0 (n = 35)</td>
<td>58.9 ± 37.8* (n = 41)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula-fed</td>
<td>77.5 ± 31.0 (n = 21)</td>
<td>80.7 ± 37.8 (n = 27)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IGF-1 data for formula-fed infants are from Steenhout et al. [20] and those for breast-fed infants are unpublished data. The insulin and IRAA data are from Dewey et al. [21]. Values in same column with different superscripts differ significantly: p < 0.01.

*Value significantly lower than corresponding value at 1 month: p < 0.01.
The protein requirements of infants decline relatively rapidly during the first few months of life [22] (fig. 1). Although the protein intakes of breast-fed infants match the requirements relatively closely, figure 1 suggests that, contrary to the first 2 months, beginning at about 2 months of age protein intakes may be marginally low relative to the requirements. The age at which protein intake begins to be marginal coincides with the age at which growth of breast-fed infants begins to be less than that of formula-fed infants. This is consistent with the hypothesis that protein intake begins to limit the growth of breast-fed infants beginning at 2 months of age.

Contrary to the variable protein content of breast milk, the protein content of formulas is constant. Their protein content is such that it meets the protein needs of infants at all times, including the first 2 months of life when protein needs are highest. Therefore, the protein intakes of formula-fed infants exceed the protein needs by an increasing margin beginning at 2 months of age. This relative excess over requirements is reflected in the plasma levels of IGF-1, insulin and amino acids of formula-fed infants at 4–5 months of age (table 2). These hormonal responses to protein intakes are consistent with the hypothesis that differences in protein intake explain differences in growth between breast- and formula-fed infants.

**Growth from 4 to 12 Months**

After 4 months of age the majority of breast-fed infants begin to receive complementary foods and many receive supplemental formula. Among the
studies reviewed by Dewey [12], 12 reported measurements beyond 6 months of age. Ten of these studies reported that breast-fed infants gained significantly less weight at least during some portions of the first year of life. In 4 of the 8 studies reporting length data, breast-fed infants also showed significantly slower gain in length than formula-fed infants. Thus, a clear predominance of studies shows that breast- and formula-fed infants differ in growth during the later parts of the first year of life.

As part of 2 unpublished studies concerning the iron nutritional status of breast-fed infants, weight and length were measured during the first year of life. Although many of the breast-fed infants also received supplemental formula after 4 months of age, a sizable number of infants did not receive supplemental formula in accordance with parental choice. The data therefore offer the opportunity to examine the effect of supplemental formula on growth. Infants in the iron study were exclusively breast-fed for the first 4 months of life. They were permitted to receive cereal and other complementary foods from 4 months of age and most infants did receive complementary foods. Regardless of whether they received complementary foods, infants were classified as ‘breast-fed’ if they did not receive formula at 9 months of age, as ‘breast + formula-fed’ if they received formula at 9 months of age, and as ‘formula-fed’ if they were no longer being breast-fed at 9 months of age. The data are summarized in table 3 which, for reference purposes, includes data for predominantly formula-fed infants [23] and for infants observed in the Euro-Growth study who were breast-fed according to WHO recommendations [24].

At 4 months of age there were no differences in weight. At 9 months of age, female infants who were breast-fed with no supplemental formula were

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**Table 3.** Weight of breast-fed infants at 4, 9 and 12 months (mean ± SD)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Iowa</th>
<th>Euro-Growthb</th>
<th>Fels [23]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>breast-feda</td>
<td>breast+ formula-feda</td>
<td>formula-feda</td>
</tr>
<tr>
<td>Females n = 33</td>
<td>n = 16</td>
<td>n = 15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6,232 ± 595</td>
<td>6,267 ± 550</td>
<td>6,310 ± 369</td>
</tr>
<tr>
<td>9</td>
<td>8,168 ± 797a</td>
<td>8,527 ± 872</td>
<td>8,758 ± 637b</td>
</tr>
<tr>
<td>12</td>
<td>8,968 ± 873</td>
<td>9,238 ± 893</td>
<td>9,471 ± 714</td>
</tr>
<tr>
<td>Males n = 26</td>
<td>n = 19</td>
<td>n = 22</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6,898 ± 739</td>
<td>6,737 ± 758</td>
<td>6,668 ± 560</td>
</tr>
<tr>
<td>9</td>
<td>8,937 ± 628</td>
<td>8,883 ± 869</td>
<td>9,165 ± 837</td>
</tr>
<tr>
<td>12</td>
<td>9,704 ± 686</td>
<td>9,707 ± 957</td>
<td>9,925 ± 964</td>
</tr>
</tbody>
</table>

Values in same row with different letters differ significantly (p < 0.05); analysis of combined genders by ANOVA shows differences between breast- and formula-fed infants to be significant at 9 (p = 0.014) and 12 months (p < 0.05).

aFeeding at 9 months of age.

bInfants breast-fed according to WHO recommendations [23].
significantly ($p = 0.018$) lighter than infants fed formula. Breast-fed infants who received supplemental formula (‘breast + formula’) weighed substantially more than breast-fed infants receiving no formula, but the difference was not statistically significant. Breast-fed infants receiving supplemental formula were similar in weight to the Euro-Growth reference [24] and to formula-fed infants [23]. By 12 months of age, the difference between breast- and formula-fed infants no longer was statistically significant ($p = 0.0612$), although substantial differences persisted. Similar differences were observed among male infants, but the differences were smaller than in female infants and were not statistically significant. However, for the combined (females and males) data, the differences between breast- and formula-fed infants were significant at 9 months ($p = 0.014$) and remained significant ($p < 0.05$) at 12 months.

**Body Composition**

Dewey [12] found that of 9 studies that reported measures of adiposity, 5 found that adiposity was less in breast-fed infants, 3 reported no difference and 1 found that breast-fed infants had higher adiposity than formula-fed infants. The latter study [25] concerned exclusively breast-fed infants who had restricted length gain in addition to increased adiposity. In 2 of the studies that found differences in adiposity, the differences persisted to 18–24 months of age. The 3 studies that found no difference in adiposity were limited to the first 4–6 months. This agrees with our own findings [26] in a group of 18 infants who had total body water determinations at 42 days and again at 84 days of age. Gain of fat-free mass between 42 and 84 days was significantly greater in formula-fed than in breast-fed infants but gain in fat mass was not, regardless of whether fat mass was expressed as grams per day or as percentage body mass. Thus it appears that during the first 4–6 months of life breast-fed infants do not differ in adiposity from formula-fed infants. In contrast, after 6 months of age there is considerable evidence that breast-fed infants are leaner than formula-fed infants.

**References**

Growth of Breast-Fed and Formula-Fed Infants


Discussion

Dr. Lönnerdal: I wonder how you can call the low blood urea nitrogen (BUN) levels in the formula-fed infants unsafe when they were similar to those of breastfed infants?
Dr. Ziegler: The term ‘safe’ in the title of our paper did not apply to the BUN. It applied to the fact that these babies seemed to consume an excessive amount of calories on the low protein formula.

Dr. Lönnerdal: I just question the validity of using BUN as an indicator for adequacy of protein intake.

Dr. Ziegler: The BUN is an indicator of current protein intake and that is true in all infants. The BUN can be influenced by other factors such as the hydration state, but given other things being equal, the BUN is a reflection of protein intake.

Dr. Dewey: I am a bit puzzled by your conclusion. From what I understand you did not find a significant difference in growth in the first study, in which you had the lower protein, and in the second one you also did not find a significant difference in length gain. We did a study in Honduras of babies who were exclusively breastfed to either 6 or 4 months of age and then given complementary foods that were high in protein and had high quality (egg) protein as well. These foods were fortified with iron and they were nutritionally adequate so the infants’ growth wasn’t being limited by any other nutrients. Those complementary foods had no significant effect on growth. So if the growth of exclusively breastfed babies was being limited by protein I would have expected to see a difference, and you didn’t see one in your experimental studies either. My conclusion is that giving them more protein does not affect their growth at that age. We also did a double-blind randomized controlled trial with formulas that had either standard levels or lower levels of protein. We reported it in the abstract that you cited for the insulin levels. We used a two-stage lower protein formula, as close as we could get to breast milk. We did not find significant differences in growth between the two groups, even though we did find differences in plasma amino acids and in BUN. So I differ in my conclusion, which is that protein is not a limiting nutrient for breastfed infants at that age.

Dr. Ziegler: We ask the same question and, looking at different data, we come to different conclusions. To come back to the study with the very low BUN, I think that is a question of statistical power. If we had more than 16 subjects, the difference in growth would almost surely have reached statistical significance. It was just a small group of babies that we studied. The data that I showed you concerning breastfed babies from 4 to 9 months were with formula supplements vs. without. I interpret that to be the effect of protein so I think that is consistent with my hypothesis. In Guatemala and Honduras you fed foods other than formula, maybe therein is the difference.

Dr. Dewey: There is fairly consistent evidence that milk products are in some way growth-enhancing or accelerating. This has been observed in populations in developing countries and in other studies where they have compared different sources of protein. Milk sources seem to have a growth-promoting effect, and we see this consistently in all the studies of infants. When they are supplemented with formula they do grow more rapidly. I certainly agree with you on that. I don't think it is protein, however; I think it is something else. I don't know what it is but definitely milk has a growth-promoting effect.

Dr. Koletzko: Following on from Dr. Dewey’s thought I would just like to point your attention to a wonderful study that Michaelson et al. have done in Copenhagen. They compared the effect of milk protein and meat protein and found that milk protein had a significant effect on enhancing IGF-1 whereas meat protein did not, underlining the concept that Dr. Dewey has just proposed, that it is something other than protein in milk that might be relevant. I would just like to raise a question about the choice of words that you used, regardless of the underlying data you and Dr. Dewey just discussed on how to interpret the data. If you chose to use the wording ‘protein intake limits the growth of breastfed infants’, imagine that there is a reporter from the Saigon
Times in this room, tomorrow the headline of the Saigon Times might be ‘breastfed infants need protein supplement from birth because breastfeeding limits growth’. I have some concern whether the your data should not be interpreted a bit more cautiously in order to avoid such a conclusion. I think with the evidence we have we really cannot say that a higher growth rate would be of benefit in the long term.

Dr. Ziegler: I appreciate your comments, but I am speaking here not to the press I am speaking to colleagues who are scientifically trained and this is a scientific discussion. I would not speak to the press and say that breastfed babies are protein-deficient. And as I said earlier this is my interpretation of the same data that we all see and I interpret it to indicate that breastfed babies grow slightly slower because their protein intake is such that they can’t grow faster. And I am not attaching any value to it. It may be that a slower growth is beneficial in the long run, just as it is in many animal species; when you restrict food intake they live longer and have other benefits.

Dr. Hernell: Just alluding to what Dr. Koletzko mentioned about the growth-promoting effect of milk; are there any data similar to what you have shown that are based on soy formulas? Do you see the same difference with soy formula as with milk-based formula?

Dr. Ziegler: The growth data I showed about formula-fed babies, about one third of the infants were fed soy-based formulas and two thirds were fed milk-based formulas, and we have never seen a difference in growth between soy formula-fed and milk formula-fed babies. We have analyzed the data very extensively and especially because the study that was mentioned by Stettler et al. [1] was based on our cohort and we carefully looked at soy vs. milk because the objective of the follow-up study was to compare those who were fed soy formula with those who were fed milk formula as infants. As you know, we found some differences in the young adult women, but we found absolutely no difference in growth, and that is why I don’t think this so-called growth-promoting effect of milk is something specific to milk.

Dr. Roggero: You said that IGF-1 is low when the protein intake is low [2]. Do you think that IGF-1 could be a good prediction marker of adiposity or only of protein intakes?

Dr. Ziegler: As I said IGF-1 reflects protein intake. I don’t think it has anything to do with later adiposity or energy intake. There was no difference between boys and girls. Yes, I think IGF-1 is a marker of protein intake.

Dr. Lönnerdal: I just want to follow up the discussion about the potential growth-promoting effect of milk proteins. Perhaps what you just said is showing that, because soy formulas have an about 20–40% higher protein content. Therefore, if you had the same growth with 20–40% higher protein, there would most likely be a growth-enhancing effect of milk protein.

Dr. Ziegler: What you said is true, soy formulas all contain more protein than milk-based formulas. But my concept of infant growth is that the infant has a certain growth potential, and you either realize it or you don’t realize it. I don’t believe that you can accelerate growth beyond the genetic potential. So whether the formula has a little bit more or less protein, as long as it meets the requirements the baby grows at its predestined rate.

Dr. Rigo: I want to come back to the discussion that we had this morning regarding the long-term effect on obesity. Your data differ from what was shown this morning which was exclusively related to the formula-fed group. You showed that there is a decrease in body weight during the first 8 days of life in the human milk group and you have an increase in body weight in the formula-fed group. So the causality is could be related only to the fact that they were fed human milk and formula, and not only to the weight gain during the first week of life.
Dr. Ziegler: What you are saying is that whatever later health benefits we see could be strictly due to some property of breast milk and have nothing to do with growth; that is entirely possible.

Dr. Turck: Did you observe differences in head circumference between breastfed and formula-fed infants at any age?

Dr. Ziegler: We did not measure head circumference. We know that length and head circumference growth are very tightly correlated. Head circumference growth can be affected in severe malnutrition, which we did not study.

Dr. Zhuoqin Jiang: How do you control the preterm babies weight gain if the body weight is increasing by less than 15 g/day? Should we use a standard fortifier or concentrated fortifier?

Dr. Ziegler: You are asking about premature infants, if the weight gain is less than 15 g/day; that is a very low weight gain. Regarding the use of a fortifier, I think all premature infants who receive mother’s milk should receive a fortifier regardless of what their initial weight gain is, and they should receive it from early on. So it is given from the beginning, not only when the baby is not growing.

Dr. Fan Yang: You mentioned that there is a difference in the plasma IGF-1 of formula-fed and breastfed babies, and we know breast milk contains IGF-1. Regarding the IGF-1 content of breast milk, does it also contribute to the difference in growth of breastfed and formula-fed babies?

Dr. Ziegler: I don’t think that the IGF-1 that is present in breast milk is absorbed intact, it is almost surely digested. So I think the IGF-1 in breast milk may have some effect on the gastrointestinal tract but no systemic effect because it is digested down to its peptides and amino acids.

Dr. Do Van Dung: I appreciated your lecture very much and I understand that you want to imply that formula feeding is better than breastfeeding, although there were differences in the measurement of the height, weight and length. To return to height and length differences between the formula-fed and breastfed infants, I wonder whether you took into consideration the role of confounding factors such as nutrition programming, the difference in height and weight, and the different anthropometric measurements of the infant’s parents? We suspect that confounders played a role because you showed that breastfed infants had a lower birth weight than the formula-fed infants.

Dr. Ziegler: I simply pointed that out because I have never noticed before that both male and female breastfed babies are a little bit lower in birth weight. I have no idea what this means. I did not say that I prefer formula feeding over breastfeeding because the babies grow faster. I simply said that formula-fed babies grow a little faster than breastfed babies, and I don’t know whether it is good or bad. I am simply stating this as a fact.

Dr. Thu Nhan Nguyen: What about immunology? For instance we have compared the immunology in breastfeeding and formula feeding and the results show that the breastfed infants have fewer diseases but formula-fed infants often become respiratory insufficient, have gastroenteritis and diarrhea. In our cities people are using formula more than breastfeeding, and the babies vomiting more after the formula. What about the immunology of formula compared to breast milk?

Dr. Ziegler: We did not measure immunologic components, but it is of course very well documented that breastfed babies have much more advanced immunologic responses because breast milk really complements the newborn infant’s immunity. My topic was to review growth. The reason why growth is currently of interest is because it seems that late health effects may be related to early growth, but there is absolutely no question that the immunology of the breastfed infants and many other things are much better and preferable. As you pointed out, there is absolutely no
question that babies should be breastfed and should only be fed formula when breast-
feeding is not possible for some reason.

Dr. Dewey: I just wanted to respond to the question about head circumference
that was asked before. In the studies that we have done we matched on birth weight.
The breastfed babies indeed grew less rapidly in weight by a substantial margin, a lit-
tle bit less rapidly in length by 12 months, but there was absolutely no difference at all
in head circumference. The same result has been found in a few other studies that
have also measured head circumference.

References

1 Stettler N, Stallings VA, Troxel AB, et al: Weight gain in the first week of life and overweight
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Body Composition during the First Year of Life

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Abstract
Knowledge of changes in body composition is of great potential benefit to the understanding of the nutritional needs and functional outcome of nutritional management for both healthy and sick infants. This review evaluates the different methods presently available to evaluate whole-body composition analysis based on different models, i.e. 2, 3, or more compartments. Analysis of the various approaches related to age, body weight and body length suggests that the major differences observed between the techniques could be preferentially related to differences in the population and that gender is one of the major determinants of whole-body composition during the first year of life. Among the techniques dual-energy X-ray absorptiometry (DEXA) and, more recently, air displacement plethysmography appear to be major techniques that have been evaluated in infants. Determination of weight gain composition is one of the major keys to the evaluation of the nutritional requirements, whereas the dynamic aspect during the first years of life could play a fundamental role in the nutritional programming of adult morbidity. Recent data suggest that protein intake and the protein:energy ratio are the main determinants of weight gain composition in preterm infants. Nevertheless, these data need to be confirmed in larger cohorts evaluated during the first year of life. To that, DEXA appears to be a useful technique to obtain sequential analysis of weight gain composition over a longer period of time and in a less invasive fashion.

Introduction
Infancy is the period of most rapid postnatal growth and is accompanied by major changes in body composition. Knowledge of these changes in body composition in healthy infants is of great potential benefit to the understanding of the nutritional needs and functional outcome of nutritional management for both healthy and sick infants [1]. Recently much interest has been focused on the relationship between early nutrition and the future health of
humans. Poor growth during early life as well as a large weight gain during infancy have been associated with disorders up to adulthood. Accurate assessment of body composition during infancy enables the determination of weight gain composition and provides key information for evaluating nutritional requirements, the efficacy of diet and medical interventions, and the influence of chronic disease [2]. Therefore, studies on how the nutritional situation interacts with the growth process and body and weight gain composition during early life in humans are very important.

### Evaluation of Body Composition

#### Direct Measurements of Body Composition
Most of our knowledge on the body composition of preterm infants is derived from body carcass analyses of stillborn preterm infants. The first values were reported in 1877, and thus far 169 infants have been analyzed [3]. However, not all analyzed fetuses can be considered for reference material because gestational age, and time and cause of death were either not reported or may not have been accurately obtained. Nevertheless, more recent studies using various technologies have confirmed the chemical analysis data and validated the interest in the evaluation of intrauterine reference values of whole body composition and in the determination of the postnatal nutritional requirements for preterm infants. With the exception of the composition of a 4-year-old male who died of tuberculous meningitis, no data on whole body chemical analyses are available from birth until adulthood and our knowledge during the first year of life is derived from indirect methods [4, 5].

#### Indirect Measurements of Body Composition
In infants, the only practical means of measuring body composition is by noninvasive and indirect methods. Several methods have been developed to indirectly measure body composition in vivo (table 1). Unfortunately, these methods have limited application in infants, i.e. they are relatively invasive, may involve significant radiation exposure, and/or need active cooperation of the subject. Whole body composition analysis may be based on different models, i.e. 2, 3, or more compartments. The basic 2-compartment model, which assumes that body mass is composed of adipose and non-adipose issue, i.e. fat mass (FM) and fat-free mass (FFM) or lean body mass (LBM), is the widely used. The total body fat (TBF) compartment is the most variable one and most sensitive to changes in nutritional status so most emphasis has been laid on its measurement [5]. The three-compartment model adds a value for skeletal or bone mass, whereas in the multi-component model, body composition is obtained by integrating data from various techniques (whole body density, total body water (TBW), bone mineral content (BMC) and anthropometry) [6, 7].
Among the various techniques, anthropometric measurements, total body electrical conductivity (TOBEC), bioelectrical impedance analysis (BIA), tracer dilution using stable isotopes (deuterium oxide: $\text{D}_2\text{O}$), dual-energy X-ray absorptiometry (DEXA) and, more recently, air displacement plethysmography (ADP) are major techniques that have been evaluated in infants.

Skin-fold thickness is a simple way of measuring FM. This approach makes two assumptions: i.e. the thickness of the subcutaneous adipose tissue reflects a constant proportion of the TBF and the sites selected for measurement represent the average thickness of the subcutaneous adipose tissue. Soft tissue composition, derived from anthropometric measurements, shows good correlation with several other techniques for in vivo body composition measurement, including isotope dilution, TOBEC, and DEXA [1]. However, the rapidly changing distribution of fat accretion in young infants makes it difficult to generate a consistent equation for predicting TBF [5, 8]. In addition, body composition data from anthropometric measurements have a poor predictive value for individual measurements of body composition [1].

**FM Evaluation Derived from LBM Determination**

FM can also be estimated indirectly by determining LBM using methods such as TBW [9], TOBEC [10] and BIA [11]. TBW may be measured by isotope dilution using deuterium ($\text{D}_2\text{O}$) or oxygen 18 ($\text{H}_2\text{O}^{18}$). Assuming that the ratio of TBW to LBM is constant [12], LBM may then be estimated. However, TBW varies with gestation and changes rapidly in the first few weeks of life, limiting application in the neonate, particularly the preterm infant. In addition, the water content of LBM in infants is higher than that generally considered for adults and decreases progressively during the first 2 years of life [7].

The principle underlying TOBEC is that lean tissue is more electrically conductive than fat, and the greater the LBM the greater the electromagnetic disturbance when a weak homogeneous electromagnetic field is applied. With this technique normative data close to older reference values have been

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**Table 1.** Indirect in vivo techniques for measuring body composition in infants

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reported in term infants between birth and 1 year of age [13]. However, this method is also sensitive to the hydration status factor of FFM. The error of estimation is inversely related to FM and therefore is relatively inappropriate for infants weighing <2,800 g [10].

BIA has the advantage that it can be used across a broad age spectrum and in a variety of settings. It appears to be a simple, cheap and easily available technique requiring little cooperation of infants. It has been widely used for measuring TBW and extracellular volume in preterm and term infants. Various algorithms have been proposed in order to improve the accuracy of TBW and extracellular volume estimations in infants [8, 11, 14]. Unfortunately, data are influenced by the hydration coefficient of FFM, whereas the precision and accuracy are still being questioned [15].

Densitometry is considered to be among the most accurate indirect body composition methods. Body composition assessment by densitometry involves measurement of the density of the whole body. Body density (body mass divided by body volume) is then used in a two-compartment model to calculate the percentage of fat, FM, and FFM. Body mass is easily measured using an accurate weighing device. Body volume is a more difficult measurement and is commonly determined either by hydrodensitometry performed in water using Archimedes’ principle or ADP performed in air using gas laws. Because hydrodensitometry requires subjects to be totally submerged during a test, compliance and safety issues prevent the implementation of this technique in the infant population [16].

ADP has been successfully used to measure the body composition of children and adults, and recently a new ADP system has been developed (the PEA POD Infant Body Composition System, Life Measurement, Inc.; fig. 1). Using body mass and volume measurements, the ADP system automatically calculates the percent body fat by using a classic densitometric approach and age- and sex-specific FFM density values obtained from a multi-compartmental study [7]. The ADP system has recently been evaluated in infants, and the reliability and accuracy results of these studies indicate that ADP is easily used by operators, comfortable for infants, reliable, and accurate compared to the deuterium ($^{2}$H$_2$O) dilution technique taken as reference. Future studies are needed to determine ADP reference values compared to other reference methods and to further evaluate weight gain composition during infancy [17].

Advances in imaging techniques have allowed more direct in vivo measurement of body composition. These methods include computed axial tomography, nuclear magnetic resonance imaging (MRI) and ultrasonography [18]. Computed tomography scanning requires significant radiation exposure in contrast to MRI and ultrasonography. However, all three methods share the problem of extrapolating cross-sectional slices from a part of the body to whole body composition slices. There are also very little data on infants using these techniques. However, data in infants during the first months of life using MRI and isotope dilution have recently been reported [2, 19].
In contrast, DEXA requires minimum radiation exposure (<0.3 mrem) and performs whole body rather than slice measurements. Given the short scanning time (6–10 min) this method is becoming the most widely used method for the in vivo measurement of whole body composition in humans. Determinations are performed without sedation, but the naked infant is swaddled in a paper blanket to minimize the movement artifact. During a DEXA determination an X-ray source generates two different energy levels and, depending upon the differential absorption of the two photon emissions, body composition (lean mass, FM, bone mineral density) is determined. In addition, total bone area is also determined and BMC can be calculated. However, the accuracy and precision of the determinations may be affected by the type of DEXA instrument [1], scan mode [20] and software programs used [21].

Using the QDR 2000 (Hologic Inc., Waltham, Mass., USA) equipped with an infant table pad and the infant whole body software V5.65P, we have performed validation studies in piglets and clinical studies in preterm and term infants. In validation studies on piglets we observed that DEXA was accurate and precise in measuring lean mass and BMC but not FM [22, 23]. However,
further evaluation indicated that FM measurements, which were overestimated, were highly improved using a correction factor [22, 23]. Using this technique and correction factor, we have obtained reference values of body composition in ‘normal’ preterm and term infants (fig. 2). Unfortunately, DEXA accuracy and precision are device- and software-dependant and up to now an optimal instrument specially designed for infants is not widely available. Thus, in a comparative study, we found that the use of the more recent QDR 4500A (Hologic Inc.) appears to underestimate FM compared to the QDR 2000 (unpublished data).

**Body Composition during the First Year of Life**

In 1982 Fomon et al. [24] published their classical body composition model that provided age- and gender-specific data for TBF, FFW, TBW, and the degree of hydration in FFW. The latter is of particular importance, as TBF is often calculated as the difference between body weight and FFW, obtained using direct or indirect estimates of TBW and a value for the degree of hydration in FFW.
However, the Fomon model, which provided data from birth to 10 years of age, was presented as preliminary and crude because it was based on quite a limited data set. Since that time, several studies have evaluated whole body composition during infancy using different techniques. TOBEC reference values were provided by de Bruin et al. [13]. DEXA reference values were determined by Koo et al. [25] as well by our group [23]. More recently Olhager et al. [2] evaluated MRI data during the first 4 months of life, and a longitudinal study of human body composition during the first 2 years of life was reported by Butte et al. [7] using a multi-compartmental system.

As shown in figure 3, there is good agreement between the various approaches. In addition, analysis of the data related to age, body weight and body length suggest that the major differences observed between the techniques could be related to differences in the population evaluated in the study. Nevertheless, these studies suggest that gender is one of the major determinants of whole body composition during the first year of life and that separate data references need to be provided.

Evaluating body composition in infants and toddlers using various techniques, Butte et al. [26] suggested that methods are not interchangeable for group or individual estimations. The magnitude of method difference is a function of age which makes it difficult for systematic biases.

Considering that the sequential evaluation of body composition is of interest for monitoring and evaluating growth patterns, efficacy of diet and medical interventions, progression of chronic disease, and recovery from malnutrition, DEXA may be considered as one of the gold standards. It is a precise, safe, noninvasive, easy to perform and widely available method providing accurate information not only on FM and FFM but also on LBM, BMC and bone mineral density in infants and toddlers. However, the accuracy of the instrument and software used remain to be validated.

**Weight Gain Composition during the First Year of Life**

Evaluation of weight gain composition is of major importance in the assessment of the nutritional requirements of preterm and term infants. Up to now, duplicate analyses of 3-day metabolic and energy balances have been performed to estimate energy expenditure in association with nitrogen and energy retention in order to estimate FM deposition, LBM gain and protein accretion in preterm and term infants according to the feeding regimen. In addition, measurement of calcium and phosphorus retention enabled estimation of the adequacy of mineral deposition compared to reference values. Those data were considered as representative of growth quality over a larger period.

Analysis of the data presently available for preterm infants made it possible to evaluate the main determinants of weight gain, nitrogen retention and FM deposition [27]. Protein intake and the protein energy ratio are the main
determinants of weight gain. Protein intake is the only determinant of LBM gain in contrast to FM gain that is positively related to energy intake and negatively to protein energy ratio. Thus, protein and energy needs are reciprocally limiting. If there is a surfeit, one affecting the ability of the infant to assimilate

Fig. 3. Evolution of fat mass (FM) and fat-free mass (FFM) related to age, weight and length. From De Bruin et al. [13], Olhager et al. [2], Rigo et al. [5], Fomon and Nelson [4] and Butte et al. [7].
the other, and if energy intake is insufficient, protein is used as an energy source and nitrogen balance becomes less positive. Increasing the caloric intake will spare protein loss and improve nitrogen retention, but with limited protein intake, protein retention reaches a plateau and the energy excess is used only for fat deposition. Nevertheless, when protein supply is in the range of the protein requirement, the effect of the energy increase on protein retention appears to be minimal. Therefore, with a view to increasing the LBM accretion and limiting the FM deposition in the premature infant, an increase in the protein energy ratio is mandatory.

Unfortunately energy and metabolic balances, requiring the use of a metabolic bed, infant relative contention and a reduction in nursing, are relatively limited. Therefore, we recently suggested that DEXA might also be used to analyze weight gain composition over a longer period of time and in a less invasive fashion [28–30].

DEXA body composition was measured at the beginning of the study and then 3 weeks later. Lean, fat and bone mass gain was determined by subtracting the second from the first determination. In preterm infants (birth weight <$1,750 g) fed either fortified human milk or preterm infant formula, the weight gain composition evaluated between a mean of 34 and 37 weeks of post-conceptional age was in the range of the data obtained previously using energy and nutrient balance (fig. 4) [30]. Formula-fed infants showed a
greater weight gain, FM deposition, BMC gain and bone area increase compared with the fortified human milk group. In contrast, calcium retention estimated from bone mineral gain was greater than that determined with nutrient balance techniques. The reasons for the latter observation are not entirely clear but might be explained by the low threshold level of bone mineral detection necessary to estimate bone mass in preterm infants [5].

In term infants, the growth pattern of breast-fed infants is known to deviate from that of formula-fed infants. Mainly after 3 months of life, breast-fed infants grow more slowly than formula-fed infants even if a relative catch-up growth tends to occur during the 2nd year of life. Evaluating body composition, Butte et al. [31] recently suggested that FFM was lower in breast-fed infants compared to formula-fed infants at 3, 6 and 9 months of age. In addition, a gender effect was also reported. Similarly, weight gain composition was also estimated in term infants breast-fed and formula-fed during the first 2 months of life.

The use of DEXA allows evaluation of the weight gain composition in term infants. From birth to 2 months of age, we compared body composition in a limited number of breast-fed (n = 16) and formula-fed (n = 47) infants. From birth up to 2 months, weight gain (31.3 ± 6.9 vs. 35.8 ± 7.4 g/day; p = 0.04) and FFM gain (19.7 ± 3.9 vs. 22.7 ± 4.5 g/day) were significantly lower in breast-fed than formula-fed infants. The percent FM increased in the 2 groups from 15.1 to 23.8% in breast-fed infants and from 14.8 to 24.0% in formula-fed infants. During the study period, weight gain corresponded to about 75% of the birth weight and was similar in the 2 groups. FM deposition accounted for 36.3 and 35.8% of the weight gain in breast-fed and formula-fed infants, respectively [29].

During these studies [29, 30], it was also possible to determine the minimal detectable changes in weight gain composition according to time in preterm and term infants in relation to the size of the population. In 3 weeks, between 1,500 and 2,200 g body weight at the time of discharge, the minimal detectable differences in body weight gain were 2.3 g for weight, 2.1 g for LBM, 1.2 g for FM, and 76 mg/kg for BMC between the 2 groups of 20 very low birth weight infants. Considering the relatively low FM content at the time of discharge, this sensitivity (±30%) appears to be better than could be obtained by other indirect methods in preterm infants [30].

In comparison, in term infants from birth to 2 months of age, the minimal detectable differences in body weight gain were 1.9 g for weight, 0.8 g for LBM, 1.5 g for FM and 50 mg/kg for BMC between the 2 groups of 15 infants. In our study, the highest differences were for weight gain (0.7 g/kg/day; p = 0.37) and FM gain (0.5 g/kg/day; p = 0.39). These values, corresponding to 27 and 33% of the minimal significant differences, should be reevaluated in a larger cohort of infants [29].

In conclusion, determination of whole body and weight gain composition is one of the major keys to the evaluation of the nutritional requirements of preterm and term infants, whereas the dynamic aspect of body composition
during the first years of life plays a fundamental role in the nutritional programming of adult morbidity. In preterm and term infants, the only practical means of measuring body composition is by noninvasive and indirect methods. Of the various techniques presently available, anthropology and impedanceometry have not yet been appropriately validated in young infants. TOBEC and stable isotope tracer dilution techniques appear more appropriate but difficult to adapt for widespread use in infants. In contrast, although in investigation, ADP seems easy to use, comfortable, reliable, and accurate in infants, but further investigations are needed to establish normative values and to validate weight gain determination. DXA, a widely available technique using the three-compartment model, is now considered by some as the reference technique for determining body and weight gain composition. The technical procedure for DXA scan acquisition and analysis is quite simple. Radiation exposure to the infant is minimal. DXA techniques have been validated for measuring body composition in infants. Unfortunately, commercial DXA instruments have major differences, and software especially adapted to preterm and term infants is not always available.

References


Discussion

Dr. Dewey: In Dr. Butte’s study the babies were exclusively breastfed during the first 4 months, but after that the infants may or may not have continued to be breastfed and I think by 1 year of age less than half of them were still being breastfed and they were mainly were being supplemented with formula. So when we try to draw conclusions about whether breastfeeding affects fatness beyond 6 months for example, I think we have to be very careful about how we define what we mean by breastfed. When we look at babies who are breastfed for the whole first year of life it is fairly consistently shown that there are differences, at least in skinfold thickness which is lower in breastfed children. But again it is those who are breastfed for a whole year and not getting infant formula. The question I have has to do with methods for measuring body composition. I was involved in a validation study of air displacement plethysmography using an instrument called the PEA POD. The PEA POD is nice because it is relatively insensitive to the babies moving around a little bit or urinating or crying, i.e. you still get a good
measurement. But I am not sure about dual-energy X-ray absorptiometry (DEXA), and I wonder if you could tell us how much it is affected by movement or crying or other activities of the babies? What do you have to do to use it under these circumstances?

**Dr. Rigo:** It is difficult to compare breastfed infants and formula-fed infants because breastfed is not completely a homogenous group. In our study we limited the data on the first 2 months of life in order to obtain exclusively breastfed and formula-fed infants. In my presentation, I stress the point that not only body composition, but also weight gain composition are very important referring to the studies evaluating the influence of protein-energy ratio on weight gain composition.

Techniques evaluating body composition are also promising for weight gain evaluation. Techniques need to be useful, practical, and not time-consuming if we want to evaluate relatively large cohorts with sequential evaluations. The problem of DEXA is that presently, the technique is still device and software dependent. DEXA is relatively sensitive to movements but in small babies it is possible to wrap the baby very carefully in a paper sheet to induce quietness and immobilization. The present technique is relatively fast, and it is so possible to obtain data without any movement. In addition, movements are recorded during the examination enabled the exclusion of the database or a second evaluation. I have never used the PEA POD, but I consider that it is a promising technique.

**Dr. Roggero:** Regarding exclusive breastfeeding, the UNICEF definition of exclusive breastfeeding is related to a baby who is only breastfed. The exclusively breastfed infant does not receive any other sort of food or water. I have another comment on a new air displacement system (PEA POD). I routinely determine the body composition in neonatal intensive care unit babies using this technique and usually we have no difficulties performing this measurement. Generally the babies don’t cry because the place where they are measured is comfortable. The temperature inside the box is adequate for them. At birth, term newborns have a mean fat mass of 6% and at 15 days of life it is 12%. Preterm infants, at term-adjusted age, have a fat mass of close to 16%. This is significantly different to that found in term neonates.

**Dr. Butte:** With the PEA POD you can combine a dilution technique with the density. So when you are uncertain of the hydration concentration the deuterium dilution is quite easy to use as well. Dr. Ellis is in the process of validating the PEA POD in both preterm and term infants and hopefully the results will be out soon. I really appreciate all the emphasis put on body composition. I think that is what we have to do and not simply look at weight gain. I was just trying to emerge the two talks of Dr. Ziegler and yourself, and it is time that we look beyond just weight gain. When we are thinking about the growth potential of the infant we have to think not only of linear growth but also ponderal growth. I wanted to ask Dr. Ziegler if he really was referring to obtaining the growth potential in terms of linear growth or in terms of ponderal growth. As we know very well from animal studies, we can manipulate the composition of ponderal growth dramatically, and I don’t think infants are any different. What we are doing with formulas, trying to look at the optimal PDE ratio and all the other nutrients and macronutrients that are present, is influencing that ponderal growth and the composition of it. Dr. Dewey and I have compared our studies over the years because they were very parallel studies. They are quite similar, but from 6 to 12 months we have some very significant differences. In our studies we didn’t control what the mothers fed and many of our mothers continued to breastfeed up to 12 months. The medium breastfeeding rate was up to 12 months, but many of the mothers were working and used formula when they were at work. That is a difference, and so we have a definition of what the predominantly breastfed infant is but we have not defined what those complementary foods are. The big debate is not about differences in breastfeeding but about differences in how the children were complementarily fed. Again we have not spent a lot of time to see what the optimal way is to feed infants from 6 to 24 months.
**Dr. Lafeber:** I would like to ask you a question about gender differences in the first year regarding body composition. It is not so much related to the breastfed and bottle-fed differences, but let us concentrate on the differences between preterm, post-discharge preterm and term formula. For instance if we look at the results of older studies on body composition using the post-discharge formula, we see differences in the body composition of boys and girls when post-discharge formula is given, and post-discharge formula seems to make boys a bit fatter and to have less influence on the girls [1, 2]. Can you speculate on the mechanism underlying the changes in body composition? You suggest very much that it would be a relation not with energy but with protein. I can understand that, but how do you explain differences between boys and girls? Is that just the physiology of the changes?

**Dr. Rigo:** There is a large difference in body composition between boys and girls. Analyzing now the weight gain in preterm infants from 1.5 g to 2.5 kg, gender appears as one of the major determinant of weight gain composition. Girls seem programmed to make more fat, but also more bone mineral deposition than boys. It is relatively the same if you investigate term and post-discharge formulas.

Nevertheless, there is some discrepancies in the published data. Using enriched post-discharge formulas. Cooke et al. [3, 4] reported a positive effect on growth rate in boys but not in girls suggesting that an increase in protein energy ratio has some influence in boys. In contrast, Lucas et al. [2], evaluating weight gain and enriched formula in intrauterine growth retarded infants, found a significant difference in girls but not boys. So it was an inverse relationship to gender between the two studies, and it is very difficult to interpret the two studies. Are the results directly related to the diet composition or to a difference in programming between boys and girls, and preterm and IUGR term infants.

**Dr. Turck:** My question is related to the model. What should be the model if we look carefully at the body composition of a formula-fed infant, should it be the breastfed infant? If you study the body composition on a clinical standpoint of a formula-fed infant, what would you do if let us say the fat content of the infant is higher than the breastfed model? Would you decrease the volume intake or would you change the formula?

**Dr. Rigo:** Your question is first about the reference; what is the body composition references that we need to take into account for formula fed infants. Presently the reference is still the breastfed infant, at least during the first 6 months of life but probably also during the second part of the first year of life. Nevertheless, it is important to accumulate data and particularly to investigate all the factors that can influence body composition during the first year of life such as exclusive breast feeding and weaning diet.

The second part of your question is related to the influence of diet content on body and weight gain composition. Is it possible to correct the differences between breastfed and formula-fed infants, by manipulations of the formula content? Up to now, limited results suggest that protein energy ratio could be major factor influencing body and weight gain composition. But we need additional investigations, and the E.U. childhood obesity project could partially answer to that question.

**References**

Dietary Reference Intakes: Concepts and Approaches Underlying Protein and Energy Requirements

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Abstract

Nutrient reference values provide guidance for maintaining and enhancing health via standard setting and development of nutritionally improved products to decrease the risk of disease. Since 1941, the Food and Nutrition Board (FNB) of the National Academy of Sciences in the United States has developed and periodically revised recommendations for nutrients; the last (10th) edition of the Recommended Dietary Allowances (RDA) was released in 1989. In 1994 the FNB initiated an expanded approach to develop dietary reference intakes (DRI), quantitative nutrient intakes that include concepts of chronic disease risk and multiple reference values more specifically suited to various applications. In concert with Canadian scientists, 10 DRI reports have been completed since 1997 and are available for review at www.nap.edu. The DRI reports explicitly review possible functional endpoints considered in determining the adequacy of each nutrient, and differentiate between the statistical basis for assessing the adequacy of intakes for individuals and groups and providing recommended intakes, as well as levels of nutrient intake that should not be exceeded. Recommended intakes for infants are based on average volumes of intake by healthy, full-term, exclusively breast-fed infants and nutrient analysis of human milk; the recommended intake also includes nutrients contributed by complementary foods consumed during the second 6-month period of life.

Introduction

Nutrient reference values provide guidance to maintain and enhance health via standard setting and development of nutritionally improved products to decrease the risk of disease. Quantitative reference values for nutrients used in food and nutrition planning have been available since the early 1900s when recommended intakes for protein were included in early United States
Department of Agriculture bulletins [1]. At the beginning of World War II, the Food and Nutrition Board (FNB) of the National Academy of Sciences established a set of nutrient standards in 1941: the Recommended Dietary Allowances (RDAs) [2]. Over the following five decades, these have been periodically revised and by 1989, served as tools to assess the adequacy of diets, as goals for intake, and as the basis for food and nutrition programs and policies in the US and other countries.

By the 1980s, research in nutrition and health had expanded beyond initial concerns focused on deficiency diseases to looking at chronic disease relationships. Such research broadened the definition of nutrient ‘deficiency’ and supported the release of two companion reports by the FNB in 1989, the 10th edition of the Recommended Dietary Allowances [3] and a new report evaluating the role of nutrients and diet on chronic disease [4].

The definition of RDA has remained markedly constant over many revisions: the levels of intake of essential nutrients that were considered, in the judgment of the FNB and on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons for nutrients known to be required [3]. Past concerns about nutrient toxicity, of consuming too much of a nutrient, were typically not significant, with major exceptions being products such as infant formulas, which might well be the sole source of nutrients for an important period of growth and development, or substances such as cod liver oil.

In 1994 the FNB proposed expanding the approach of its previous work in establishing RDAs to include concepts of disease risk beyond traditional deficiency signs and symptoms [5]. Following the approach used by Great Britain in 1991 [6] and based on the diverse situations in which RDAs were increasingly being used as the reference values (table 1), it also became increasingly important to provide multiple reference values more specifically suited to the application and which could be scientifically supported [5]. This expansion of the RDAs to provide additional reference values, termed dietary reference intakes (DRIs), has resulted in a series of 10 DRI reports since 1997. This has been a joint activity with Canadian scientists, supported by US governmental agencies, Health Canada, private foundations, and industry.

**Conceptual Basis of Dietary Reference Intakes**

Brief descriptions of each category of reference intakes included in the DRI reports are provided in table 2, along with their typical uses. Initially, only three categories of DRIs for each nutrient were planned: an estimated average requirement (EAR), an RDA, and a tolerable upper intake level (UL). Early on, however, it became apparent that for nutrients with little dose-response data, a reference value would still be needed as a recommended level of intake, established on a different basis than the RDA (fig. 1). This
surrogate recommended intake, the adequate intake (AI), was not called an RDA to explicitly show that it was less conclusive and that more judgment was involved in its determination.

**Model for Establishing Recommended Intakes**

The DRI reports explicitly review the usefulness and limitations of all possible functional endpoints considered in determining adequacy, justifying those selected in establishing the requirement for the nutrient. Typically animal data are not used. While attention is paid to observed intakes in healthy populations, recommended intakes are based on epidemiological observations, human balance study data, depletion/repletion studies, and accepted surrogate markers or biochemical indicators of adequacy, when functional outcomes, such as decreased risk of chronic disease, are not available.

The basis of both the RDA and the use of the EAR in the assessment of adequacy of group intakes requires establishing a dose response (fig. 1). A normal or symmetrical distribution of requirements in a group of individuals with similar age and gender is also needed [7] (fig. 2). The departure from past derivations of most nutrient recommendations (with the exception of protein) is that in order to have an RDA as the recommended intake for an individual, there must be data available to establish an EAR. The EAR is defined as the best estimate of the average (actually, median) requirement for a group of similar individuals. Thus, half of individuals in the subgroup will have their needs met

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**Table 1.** Past uses of recommended dietary allowances

<table>
<thead>
<tr>
<th>Examples</th>
<th>Basis of planning guides for eating patterns to achieve recommended nutrient intakes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>e.g., MyPyramid (USDA.gov); Food Guide to Healthy Eating (Health Canada)</td>
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<tr>
<td></td>
<td>Basis for planning meals for groups</td>
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<td></td>
<td>e.g., nursing homes, correctional facilities</td>
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<td></td>
<td>Reference point for evaluating adequacy of the dietary intake of population subgroups</td>
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<tr>
<td></td>
<td>e.g., WIC participants vs. non-participants</td>
</tr>
<tr>
<td></td>
<td>Component of food and nutrition education programs</td>
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<tr>
<td></td>
<td>e.g., Five-A-Day Program (US National Cancer Institute/NIH)</td>
</tr>
<tr>
<td></td>
<td>Basis for nutrient intake goals for individuals</td>
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<tr>
<td></td>
<td>e.g., Daily Values in Nutrition Facts Panel and Dietary Supplement Facts Panel in</td>
</tr>
<tr>
<td></td>
<td>the US (adopted via regulations established by the US Food and Drug Administration,</td>
</tr>
<tr>
<td></td>
<td>Maximum nutrient levels for fortification/dietary supplement formulation</td>
</tr>
<tr>
<td></td>
<td>e.g., proposed by country representatives in the revision of the Nutrient Reference</td>
</tr>
<tr>
<td></td>
<td>Values (NRV) of the Codex Committee on Nutrition and Foods for Special Dietary Uses</td>
</tr>
</tbody>
</table>

A comprehensive list of uses of dietary reference standards is included in the DRI report on assessing adequacy [7] and on planning [16].
Table 2. Dietary reference intakes: definitions and uses

<table>
<thead>
<tr>
<th>Category of dietary reference intake (DRI)</th>
<th>Use to assess dietary adequacy¹/ excess</th>
<th>plan diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate intake² (AI) = the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate – used when an RDA cannot be determined</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>Recommended dietary allowance (RDA) = the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97–98%) healthy individuals in a particular life stage and gender group</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>RDA = EAR + 2 × CV&lt;sub&gt;EAR&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceptable macronutrient distribution range (AMDR) = the range of intakes for an energy yielding macronutrient associated with reduced risk of chronic disease while providing adequate intakes of essential nutrients; given as a percent of energy intake</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>Reference intakes for individuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerable upper intake level (UL) = the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase</td>
<td>XX</td>
<td></td>
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<tr>
<td>Reference intakes for groups</td>
<td></td>
<td></td>
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<tr>
<td>Estimated average requirement³ (EAR) = the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group</td>
<td>XX</td>
<td></td>
</tr>
<tr>
<td>Estimated energy requirement (EER) = the EER is defined as the average dietary energy intake that is predicted to maintain energy balance in a healthy adult of a defined age, gender, weight, height, and level of physical activity consistent with good health. In children and pregnant and lactating women, the EER is taken to include the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health</td>
<td>XX</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. (continued)

<table>
<thead>
<tr>
<th>Category of dietary reference intake (DRI)</th>
<th>Use to</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMDR: use to estimate the proportion of the population that falls outside the range to assess adherence to recommendations and determine concern level about adverse consequences</td>
<td>assess dietary adequacy(^1)/ plan excess diets</td>
</tr>
</tbody>
</table>

\(^1\)Evaluating an individual's nutritional status requires data on biochemical, clinical, and anthropometric measures.

\(^2\)The AI for infants is based on the average intake of the nutrient from human milk for infants at the midpoint of the age range, and the corresponding average composition of the nutrient from analyses of human milk obtained during the same stage of lactation. It should be used as a guide for infants, but actual intake and needs may vary depending on growth rate, etc.

\(^3\)Requires statistically valid approximation of distribution of usual intakes.

Source: The Institute of Medicine, Food and Nutrition Board [7, 11, 16].

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**Fig. 1.** Conceptual model of dietary reference intakes (DRIs). The DRI model relating nutrient risk of inadequacy and excess includes four DRI categories. The estimated average requirement (EAR) can be used to assess adequacy of population intakes if assumptions are met and is the basis for the recommended dietary allowance (RDA). The adequate intake (AI) lies somewhere in the area depicted in the diagram, in that it is not directly related to the EAR, but may be an observed intake that appears adequate for all in the population. Its relationship to the EAR and thus the RDA is not known as it is only provided when it is not possible to determine an EAR from the available data. The goal in setting the tolerable upper intake level (UL) is that it is as high as possible without increasing the potential for adverse effects due to excess intake. It may actually be less than indicated in the conceptual model, if a great deal of uncertainty exists in the available data used to set the UL.
At the EAR, half will not. In order to estimate a recommended intake that will provide almost all healthy individuals in the group with enough to meet their needs, the EAR is increased by two standard deviations to obtain the RDA for the nutrient (fig. 2).

**Fig. 2.** Model for dietary reference values. The theoretical model for establishing the estimated average requirement (EAR) and the recommended dietary allowance (RDA). The model assumes that the distribution of requirements is normal (or symmetrical); that members of the group are of similar age, gender, and size; and that each individual's requirement for the nutrient is independent of that individual's intake (not true for energy). Within the dietary reference intake (DRI) conceptual model, the RDA is derived from the EAR + 2 standard deviations of the EAR; thus the RDA should meet the needs of almost all (97.5%) of the individuals in the population. In this diagram, two members of the group, ab and ay, each from a similar subpopulation and age, have different requirements. While consumption of the EAR would provide an adequate intake of the nutrient for individual ab, it would be inadequate for individual ay, who needs more than the EAR. Note that the EAR could be different (either greater or less) if a different indicator of adequacy was chosen, resulting in a higher or lower RDA.

At the EAR, half will not. In order to estimate a recommended intake that will provide almost all healthy individuals in the group with enough to meet their needs, the EAR is increased by two standard deviations of requirements to obtain the RDA for the nutrient (fig. 2).

**Adequate for What?**

A key question is the determination of the criterion or criteria of adequacy. Which functional outcome, surrogate marker for disease, or biochemical or

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1The definition of the EAR is that of a median, while use of the word 'average' indicates a mean. The decision to call this DRI 'EAR' was made to follow precedent set by the UK [6], and because if the distribution of requirements is symmetrical, then the mean and median are the same.

2For some nutrients whose requirements are not symmetrically distributed such as iron, other statistical methods are used to establish both an average requirement (EAR) and RDA (amount that would be adequate for 97.5% of individuals in the group).
physiological indicator best reflects adequacy for a nutrient is not a trivial matter. The DRI reports outline the possible candidates, and describe in detail choices made for establishing adequacy, explicitly recognizing that if other criteria were chosen, the average requirement and the related RDA might well be significantly different. It is at this step, choosing the indicator, that geographic and policy-based decisions may direct different choices.

**When Dose-Response Data Are Not Available**

For well-studied nutrients, response data from individuals fed varying levels of intake are available to develop EARs and, from that RDAs. For many nutrients, however, available human data may only be at markedly deficient levels of intake or at amounts known to be more than adequate, with no information on intermediate levels needed to construct a dose response. For this situation, or where there are conflicting data regarding the appropriate criterion, or where responses are not uniform, an additional reference value, the AI, is established as a recommended intake for individuals [8].

**Determination of the Coefficient of Variation of Requirements**

While the average requirement is important in order to establish the EAR and thus the RDA, so too is the variation in requirements. In order to determine a reference value that will meet the needs of almost all in the population, the distribution of requirements must be estimated. In most cases where there were adequate data points to establish an EAR, there was not enough information to estimate the standard deviation (SD_{EAR}) of the average requirement for the group; errors in estimating the distribution of requirements were considered sizable enough to not attempt to estimate it [8]. If cases where data on the variability of requirements for a nutrient was insufficient to calculate an SD_{EAR}, then the coefficient of variation (CV) for the EAR is assumed to be 10% [8].

The use of 10% as a default CV comes from variation in measured basal metabolism of similar individuals estimated to be 10% [9], and on the distribution of protein requirements estimated to be 12.5% [9]. Seventeen nutrients have EARs; for six of them, the CV applied differs from the default CV of 10%: 15% for copper, molybdenum, niacin, and carbohydrate [10–12], and 20% for iodine and vitamin A [12]. For iron and protein [11, 12], where non-normal distributions of requirements were identified, statistical modeling was used to establish the EAR and the RDA.

**Establishing the Tolerable Upper Intake Level**

With the growing availability of nutrients at high levels in the diet compared to amounts obtained from typical natural food-based sources, the need to ascertain a level of intake that would not pose an increased risk of adverse effects due to overconsumption was needed. The traditional model for risk
assessment was adapted for nutrients, giving rise to the fourth category of DRIs, the UL (table 2).

Establishing upper levels of nutrient intake that do not increase the risk of adverse effects is often hampered by the following: (1) dose-response data at high levels of nutrient intake are usually lacking, thus requiring animal data review; (2) there are few chronic human or animal studies available in the literature, as those published typically determine the toxicity of acute levels of intake; (3) few surveillance studies are available in which high intakes are estimated to establish a ‘no observed adverse effect level’ (NOAEL); (4) intakes in the few studies available usually only include supplement intake rather than total intake, thus not capturing intakes from foods, which may also be fortified, and (5) estimates of bioavailability often differ due to the nutrient source.

*Steps in Risk Assessment*

Risk is defined as the probability of an adverse effect occurring at some specified level of exposure. The risk assessment model is described in each of the DRI reports on nutrients, and is comprised of four steps, following traditional toxicological risk assessment methodology: (1) hazard identification to find adverse effects resulting from ingestion of a nutrient on a chronic basis; (2) dose-response assessment, using quantitative data relating the chosen critical adverse effect to nutrient intake to identify a NOAEL, a LOAEL (lowest observed adverse effect level), and an uncertainty factor, which varies based on the strength of the data and seriousness of the adverse effect; (3) exposure assessment, which estimates the percent of population subgroups exposed at intakes above the UL, and (4) risk characterization, which determines the proportion of each subgroup in the population who may be at risk for the critical adverse effect associated with the UL. Since the UL is established taking into account sensitive individuals in the population, it is not expected that all or even many of those whose usual or chronic intake exceeds the UL will demonstrate the adverse effect. The term ‘tolerable’, however, was chosen to point out that since no health benefits of intakes by the general population above the RDA or AI have been adequately documented, the ULs should not be considered recommended intakes or desirable levels to attain.

*Extrapolation*

Given that the data on adverse effects of overconsumption of nutrients are minimal in most cases, age and gender groups are combined in establishing ULs. Adult values based on available data are extrapolated to children, elderly, or during pregnancy and lactation, based on body size or based on differences in route of ingestion, absorption, distribution, metabolism, or excretion. For vitamin E, boron, molybdenum, nickel, and vanadium, the ULs are based on animal studies, and thus the uncertainty factor applied to each takes into account extrapolating animal data to humans [12, 13].
Application of the Model of Nutrient Risk Assessment to Macronutrients

As part of the DRI process, the risk assessment model was applied to all nutrients reviewed, including macronutrients. Much of the evidence reviewed regarding the intake of fat and carbohydrate relates to their long-term effects on chronic disease [11].

For fat and other dietary lipid components, a NOAEL could not be established as, at the lowest levels of intake, there appeared to be continued benefit in further decreasing intake to decrease the risk of cardiovascular disease. Thus, rather than provide a UL for a level at which there would be an expected and quantifiable risk, the recommendation is to decrease intake of these dietary fats as low as possible while consuming a nutritionally adequate diet [11].

In the case of added sugars which were also evaluated, no UL was established as there was ‘no clear and consistent association between increased intake of added sugars and body mass index’ [11], although there was a trend toward dietary inadequacy with higher intakes of added sugars. In place of a UL, the following statement was provided: ‘A maximal intake of 25% of energy from added sugars is suggested... based on ensuring sufficient intakes of essential micronutrients that are for the most part present in relatively low amounts in food and beverages that are major sources of added sugars in North American diets’ [11].

Distribution of Energy-Yielding Macronutrients

The dual roles that fat, carbohydrate, and protein play in normal growth and in metabolism are recognized in the DRI provision of both recommended intakes (either AIs or RDAs) based on their independent roles in health as well as their use as a source of energy. The acceptable macronutrient distribution range (AMDR) was created to describe this second role: as a percentage of total energy consumed to provide guidance to individuals and to assess population group intakes (table 2).

Chronic intake above the upper end of the range can be viewed as potentially putting an individual at risk of overconsumption, in part because it could result in under-consumption of another macronutrient resulting in an increased risk of it being inadequate for its specific role in metabolism. The lower level of the range can be considered as the minimum amount required. The lower level of the protein range approximates the RDA; intake beyond the upper end of the range may result in under-consumption of fat or carbohydrate [11].

Approaches to Recommended Intakes for Infants

The AI was initially created for infants, where it would be unethical to intentionally feed infants varying levels of intake known to be deficient and evaluate
changes in body weight, growth, or other nutritional status indicators over time. Also of concern was the tendency seen with past reference standards for inaccurate comparisons to be made between recommended intakes and human milk composition. Human milk was frequently cited as inadequate for a number of nutrients. To eliminate this misinterpretation, AIs based on the nutrient content of human milk are provided for infants for nutrients for which there is no evidence indicating that breast-fed infants should have supplementary amounts above that provided in human milk [8].

The AIs for infants are calculated by estimating an average volume of intake by healthy, full-term, exclusively breast-fed infants midway through the first 6 months or the second 6 months of life. The amount of a nutrient in that volume is estimated from analysis of human milk samples taken during the same time period of infant feeding (2–6 or 7–12 months of lactation). During the second 6 months of life, the estimated contribution of complementary foods is added to the average amount of the nutrient provided by human milk. The values should be used in context; the DRI reports point out that there will be variation in both the amount consumed and its composition during normal infancy, and thus the computed values represent average values, noting that it is expected that infants will consume increased volumes of human milk as they grow.

While separate recommendations for infants fed formula are not made, if specific issues relative to bioavailability or the source of a nutrient are relevant to developing an optimal formula, the issue is discussed and data provided for modifying the recommended intake. Because of the reliance on human milk composition, the AI is less than previous recommended intakes for infants in the US [3] or Canada [14].

Of the nutrients reviewed as part of the DRI process, two nutrients were considered inadequate in the diets of infants beyond their 6th month of life when fed exclusively human milk: iron and zinc [12]. Using a factorial method to estimate average requirements that includes amounts for growth, EARs were developed for these two nutrients for infants 7–12 months of age, resulting in the only RDAs provided for this age group.

Because of organ immaturity, no ULs were established for infants, with the statement that infants should obtain nutrients from food (including human milk) or formula only.

**Approaches to Values for Children**

**Age Groups**

Since the age groups used in recent US RDAs [3] differed from those used in the Canadian Recommended Nutrient Intakes [14], a number of experts were initially queried on appropriate age cutoffs. New data on the onset of menarche indicated a need to revise female grouping. The new age categories now take into account the age at which young children enter institutional
feeding settings (pre-kindergarten) in Canada and the United States, potentially affecting energy requirements, as well as the change in onset of adolescent growth spurts [8].

*Extrapolations due to Data Gaps*

In spite of the significant progress in research related to human nutritional needs, data were not usually available with multiple levels of nutrient intake to directly determine nutrient requirements for each gender and life-stage group, including children. Thus available data from other subgroups were extrapolated to develop EARs, Alrs, and ULs for each subgroup (each DRI report discusses extrapolation methodologies employed for nutrients included in the report). Nutrients involved in energy metabolism were extrapolated on the basis of metabolic body weight (B.Wt.\(^{3/4}\)). Extrapolation based directly on body weight was used for nutrients involved in bone maintenance and growth. The size of compartment or tissue weight was used as the basis for extrapolation for nutrients primarily distributed in the water space or other specific tissues.

*Energy and Physical Activity*

The DRIs include estimated energy expenditure at four levels of physical activity. Previous energy allowances were based on estimated time spent in various activities in addition to measured or predicted basal metabolic rates [3]. Rather than provide an RDA (a level of energy intake that would be adequate for almost all the population), an estimated energy requirement (EER) is predicted from regression equations derived from doubly labeled water data based on gender, age, weight, height, and four levels of physical activity (sedentary, low active, active, very active) [11].

The DRI process also recommended levels of activity to both decrease the risk of chronic disease and maintain body weight below a body mass index of 25 [11]. The level of total activity recommended is \( > 1.6 \) physical activity level (PAL, the ratio of total energy expenditure to basal energy expenditure), and is the equivalent of 60 min of moderate intensity activity on a daily basis above sedentary levels (moderate activity equivalent to walking at 4.8–6.4 km/h).

*Conclusions*

While an important part of the DRI process and conceptual framework is to incorporate the growing body of evidence on the role of nutrients and food components in decreasing the risk of chronic as well as deficiency diseases in order to maintain and enhance the quality of life through diet-related
benefits, there has been less evidence available to associate intakes of nutrients in childhood or infancy with long-term outcomes [15]. Future efforts to incorporate concepts of risk factor reduction in quantifying nutrient requirements and recommendations will continue to increase, focusing on nutrient intake during early life and its impact on future health.

Acknowledgements

To date, the DRI process has involved over 200 scientists in Canada and the United States over the last decade or more, funded by US and Canadian federal agencies, non-profit foundations, and corporate sponsors. The Institute of Medicine and the chairs, members, consultants, and staff of the Food and Nutrition Board, all of whom worked on aspects of this continuing project over the last decade, have been essential to its development. In particular, the contributions of the late Vernon R. Young, first Chair of the DRI Committee, who, through the grace of his wit and intellect, gave his full measure to the activity as he strove to imbed scientific validity in the process, must not be forgotten.

References

Discussion

Dr. Butte: I have been involved in evaluating the diets of toddlers from the second year of life. I was personally surprised that some of the nutrient intakes, when we compared them against the recommended daily allowances (RDAs) and the upper limits, were approaching the upper limits, specifically zinc, niacin and vitamin A. So when the upper limit committee was meeting, was one of their criteria to look at where they set the upper limit and then examine dietary intake from various age groups? We all acknowledge that it is not ideal to extrapolate from adults to toddlers, but that is all we have. I was just wondering if those committees did check to see if these were consistent with what observed intakes are?

Dr. Yates: Yes and no. They did do a check to see where the nutrient was coming from, and if they found it was coming from a heavily fortified food source then it made them less comfortable; although in fact it was acceptable, recognizing that what they were establishing was a no observed effect level (NOAEL). In the case of zinc the adverse effect was determined to be a decrease in copper absorption. The issue was are these individuals who are getting higher levels of zinc, primarily from breakfast cereal, also getting a lot of copper because it was being added to the breakfast cereals, and this is not the case. As there was documented copper deficiency resulting from the overconsumption of zinc (I believe it was in infants) their feeling was that by setting the upper limit as they did for the 1- to 3-year-old group, that was where the problem lay. In the US companies were fortifying their cereal products with the adult RDA which was probably an inappropriate level. Now the upper level is not the same as an average requirement. The agencies involved in regulating how much can be added to various food products asked for a safe level, but we didn’t want to use the term ‘safe’. So in a way it is a level at which one feels comfortable, that even going over it for a while is no problem. So it is much more of an average intake but it is on the other side of the slope of the curve. If you look at the data, the issue about where zinc is coming from, whether people have been using it over the last 5 or 10 years or companies have been putting it in a product, is a little different than if it has been part of the natural diet over the last 50 years and there is no apparent adverse effect. So I think part of it is derivation. But I think it is getting at “where are those nutrients coming from?” and it could be the extrapolation method.

Dr. Cong Khanh Nguyen: I would like to ask two questions. Regarding the dietary reference intakes, should they be based on the local bioavailability of food? The problem of food bioavailability is quite considerable in a critical period like pregnancy and lactation. In Viet Nam we did some studies on food contamination, particularly in fish because in some areas fish is contaminated with heavy metal. What is the upper limit for fish consumption in children? When we look at the level recommended by the Ministry of Public of Health the total intake is still lower than what is recommended as a toxicology issue. Do you think that we can recommend that reference intake from a food safety aspect?

Dr. Yates: The first question was about pregnancy and lactation with regard to bioavailability. The assumption is that you could determine requirements, and there
probably is very little difference (in requirements) depending on what geographic area you are in or what your food supply is. When you want to set the recommended allowance you are going to have to take into account bioavailability and, taking iron as an example and depending on the iron source, there is a different average requirement if the primary iron source is animal or vegetable. So yes, certainly you have to take bioavailability into account for the average requirement. That is why we say these RDAs are really for Canada and the US, given our food supply. How well they would apply to other areas, I don’t know. I assume that other countries are doing their own RDAs based on their food supply and the typical bioavailability of nutrients that they are interested in. Your other question was how do to deal with food such as fish which may have a lot of mercury contamination; the big question here is ‘are you below what you think is the allowable exposure to something like mercury, which is really a concern over time?’ You have to decide if fish is a really important staple for maintaining an individual in a state of health, and if there is no alternative to providing fish, then you will likely cause more problems than the potential mercury contamination. Actually there is a report from the National Academy of Sciences about 2 years ago looking at dioxin in the food supply and what should be the public health message. Because of it being in fat and certainly fish, what should they tell pregnant women? They essentially said if you follow our typical dietary guidelines which are low fat diets you will be decreasing your exposure significantly, and that is probably the best public health message. But it is a problem, certainly in the US, where people are really interested in n-3 fatty acid intake and increasing it by fish consumption, but at the same time they try to avoid types of fish that have heavy metal contamination. It is a public health problem that nobody has solved yet.

Dr. Koletzko: I would like to come back to the question from Dr. Butte as to how do you extrapolate values for children, particularly for young children. I think it is quite important which method to use because the younger the child, the more different the results are. In 2003 the Scientific Committee on Food of the European Commission critically looked back at what it has been doing and pointed out that the approach it used for extrapolating upper levels or safe levels has really severe limitations. They pointed out that if you extrapolate the upper level values for children from adult values on body weight or body surface basis, in a number of cases you come up with upper levels that are lower than the known nutrient requirements. So clearly there are some severe limitations. If one looks around the world at reference nutrient intake values it is quite remarkable how big the differences in values are for the same nutrients for the children observed. I think that this can partly be explained by the lack of scientific knowledge and data, but also I think it has to do with the very different concepts, approaches and definitions that different people use. In that regard I was wondering why the Dietary Reference Intake Committee chose as a default value a metabolic body mass with the exponent of 0.75? Looking at the recent literature there is still a hot controversy regarding the right exponent, and some people are favouring the exponent of 0.66. As you know the younger the child, the bigger the difference. If I compare extrapolations based on body surface area and body weight area then at 10 years it is only a difference of 25%, and in 1 year it is a difference of 60%; in the infant between newborn age and half a year there is a difference of 100%. So this is really an important question. The second question I have is my interpretation is that the US DRI recommendations for upper levels in children were based on body weight, except for sodium.

Dr. Yates: I am sorry, I didn’t differentiate that. But you are right, the upper levels except for the B vitamins.

Dr. Koletzko: And sodium, based on body weight and not on metabolic body weight. So the reference nutrient intake and upper levels for the same nutrient were
calculated on a different basis. I am wondering why this choice was made, because again for young children this would lead to a proportionally much lower upper level.

Dr. Yates: This is a 10-year process, and what you start out with changes as you go along; certainly there is new science and there are new people and approaches. There were quite difficult scientific discussions to get toxicologists away from uncertainty factors of 10 and 100, depending on whether there were animal data, species extrapolation, and so on. Thinking in uncertainty factors of less than 5 was a major accomplishment. The big reason that the Food and Nutrition Board wanted to look at the upper level model and try to use risk assessment was that in 1992 the Environmental Protection Agency in the United States came out with a report saying that the reference dose for zinc for children was 5 mg, I think, and at the time the RDA from 1989 for zinc for children was 7 or 8 mg. But again the Environmental Protection Agency used the traditional toxicology model. So when we started we were fairly careful to try to wrench away uncertainty factors to find something that seemed reasonable. But I guess one of the major issues is how do you extrapolate for children. I don’t think that has been solved, and I think it does make a difference whether you are looking at adequacy or upper levels. As you probably know the upper levels are not nearly as specific in terms of groupings. So there is an adult upper level and then there is a child upper level and then potentially there is pregnancy and lactation, but they are not broken up by age. They are just very general numbers at which there is assumed to be no risk of consuming that level. Beyond that there is a potential for risk but there is not a definable risk. So it is not a well-cut criterion on and I think it is because it is probably 20 years behind where we are with recommended reference intakes, and we just have to get the science to catch up.

Dr. Cong Khanh Nguyen: In Viet Nam we have a program for iron fortification in food. We did efficacy studies and published 2 studies; one in the American Journal of Clinical Nutrition [1] and the other in the Journal of Nutrition [2]. However, in implementing the program we used upper limits because excess iron is quite dangerous. But it is not easy as in the same family the women and men share the same food. So upper limit here means for men or for women. Is it better for men or for women?

Dr. Yates: The data were so limited; trying to come up with the NOAEL or the lowest observed adverse effect level (LOAEL), there might be a database of 30 subjects or cases, often mixed with both men and women. So the data are too sparse to say that for women it is 1,000 and for men it is 1,200 for iodine. This is one of the reasons that the DRI reports are so large because they list all the studies that are available, and why there are so many studies to use. So you look at all the data and for the situation establish a NOAEL or LOAEL, and that would be one way of establishing whether you are giving too much fortification.

Dr. Rigo: When we look at your conceptual model there is a relatively large gap between adequate intake and the upper level. How do you interpret this difference?

Dr. Yates: The adequate intake is an intake that is a recommended intake to consume, and in most cases it is based on the average intake of a population that does not appear to be inadequate for that nutrient. So it is definitely going to be more than most people need. Now your question is why is the adequate level so far from the upper level?

Dr. Rigo: No, it is not why it is so far because it is calculated as such in the model. But I think that when we look at the upper level and the intake in the population, we don’t know exactly if a large part of the population receives more than the adequate intake, and it is difficult to study the consequences of this relatively high level.

Dr. Yates: The only way you are going to be able to study the consequence of a high level of intake is obviously to have some examples or studies of specifically how much is too much.

Dr. Rigo: Take protein intake during infancy as an example. The upper level is difficult to define and we have the adequate intake level; so what is the difference in the long-term effect if we look at the higher level?
**Dr. Yates:** With proteins we didn’t establish upper levels because we could not come up with any real adverse effects. We have this range of caloric intake, and 30 or 35% is pretty high, but it is not set on the same basis. We don’t have an upper level for arsenic even though we know that arsenic is deadly; but there is no study of long exposure to chronic doses and then evaluation of adverse effects, and that is really what you have to have in order to establish an upper level.

**Dr. Giovannini:** When you spoke about carbohydrate, why didn’t you speak about the low or high glycemic index or about rapid or slow absorption? With the intake of carbohydrate it is very important to consider the glycemic index. It depends not only of the quality but also on the way something is cooked. I think a very important problem in nutrition is quality control. In every country it is different, but quality control is of central importance for nutrition.

**Dr. Yates:** David Jenkins was a member of the committee and he is certainly interested in the glycemic index. There is a carbohydrate recommendation related to total carbohydrate. The consensus of the committee was that the glycemic index was potentially very useful for diabetic individuals or people with pre-diabetes, but in terms of the normal healthy population there wasn’t enough information with a strong scientific basis to indicate that on a long-term basis glycemic index would maintain health. The other thing is this came out in 2002, but was finally printed in 2005. So we had the information available in 2002, but there are new data since then so that must be put into context too. Things have changed, so what is available now may well show that there is a need to look more closely at glycemic index and its role in health.

**Dr. Giovannini:** It may be useful to know the glycemic index because the most popular cereals sold in the world have the highest glycemic index, and every morning children receive milk and cereals. What Jenkins published is much higher in sucrose. For this reason it is very important to have nutritional education. In every part of the world it is important to know what kind of carbohydrate is being consumed because people think about eating fibers and do not realize that they are eating food with a high glycemic index.

**Dr. Yates:** Another interesting thing here is that at the request of the Food and Drug Administration in the US we came up with a definition of dietary fiber. Essentially fiber is endogenous in food. If it is an isolated fiber in terms of a chemical modification, then it has to have a demonstrated role in health to try and point people to the fact that it is the fiber in food and probably other things that are going to be of health benefit, rather than what one might put into a food from an external source.

**References**

Intestinal Amino Acid Metabolism in Neonates

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Abstract

The portal-drained viscera (stomach, intestine, pancreas and spleen) have a much higher rate of both energy expenditure and protein synthesis than can be estimated on the basis of their weight. A high utilization rate of dietary nutrients by the portal-drained viscera might result in a low systemic availability which determines whole-body growth. From studies in our multiple catheterized piglet model, we conclude that more than half of the dietary protein intake is utilized within the portal-drained viscera and that amino acids are a major fuel source for the visceral organs. Specific stable isotope studies reveal that there are large differences in the utilization rate amongst the different amino acids. The majority of the results obtained from the piglet studies can be extrapolated to the human (preterm) infant. First-pass, splanchnic uptake of lysine and threonine differ substantially, while non-essential amino acids are oxidized to a great extend in the human gut. Overall, these studies indicate that gut amino acid metabolism has a great impact on systemic availability and hence growth in the neonate.

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Introduction

The intestine is best known for its instrumental role in the digestion and absorption of nutrients. Less known, but important as well, is its immune function. Every fifth cell in the jejunum is a lymphocyte and, moreover, almost all
IgA and most of IgG is produced by the intestine. In addition to producing defensins, mucins and glutathione for example, the intestine shows well-developed nonspecific immunity. In conjunction with its immune function, the intestine shows hormone function as well. Hormones such as glucagon-like peptide II, peptide YY, and gastric inhibitory polypeptide are all produced in the intestine. And finally, large amounts of neuronal tissue are found in the gut: every cubic millimeter is estimated to contain 2 m of axons.

Considering all these functions, it is not surprising that the gut shows high energy expenditure and a high protein synthesis rate in order to keep up intestinal metabolism. The fractional synthetic rate of proteins in the gut is higher than in any other tissue in the body (fig. 1) [1–4]. In this review we will discuss several studies in piglets and preterm infants specifically aimed to gain insight into the intermediate amino acid metabolism of the gut.

The piglet is the animal model of choice for this kind of study, seeing that the development of the pig intestine greatly resembles that of a human infant’s intestine [5, 6]. The studies were performed in the first month of life, the period in which gut metabolism has its greatest impact on total body metabolism. Gut growth in mammals is most rapid during the first month as shown in figure 2.

**Piglet Studies**

We used a multiply catheterized piglet model, with catheters placed in the carotid artery, jugular vein, portal vein, stomach, and duodenum, and a flow probe placed around the portal vein. The catheters were placed during surgery.
in formula-fed piglets on the 21st day of life. After full recovery from surgery, as indicated by a similar weight gain rate as prior to surgery, metabolic studies were performed in the fully conscious, enterally fed piglets [7]. This model allowed us to measure the utilization rates of enteral and systemic substrates by the portal-drained viscera (stomach, spleen, pancreas, intestine).

**Intestinal Energy Expenditure**

We measured the energy expenditure of the portal-drained viscera of neonatal piglets by means of sodium bicarbonate labeled with $^{13}$C [8]. It appeared to be almost three times as high as could be expected on the basis of its weight. While some 12% of total body energy expenditure occurred within the portal-drained viscera, the weight of the portal-drained viscera accounted for only 4% of total body weight. This high energy expenditure reflects the rapid growth rate and high metabolic rate of these organs (especially the intestine).

**Intestinal Sources of Energy**

As early as 25 years ago, Windmueller and Spaeth [9] already examined amino acid utilization rates in isolated perfused intestinal loops in rats. From these in vivo experiments, they concluded that several substrates may serve as energy sources. Glutamate and glutamine, and aspartate and glucose were the major fuel sources found. We repeated these experiments in fully conscious piglets, using the model described above [10]. We found amino acids to be the major source of energy, with glutamate being the single most used amino acid. Almost all (90%) enterally administered glutamate was utilized in the first pass, of which 47% was used for oxidative purposes. Not only non-essential
amino acids were used, but also leucine (an essential branched chain amino acid) was oxidized by the portal-drained viscera, slightly more than 10% of the intake. This is a crucial finding in that essential amino acids cannot be synthesized de novo, indicating that oxidation of such essential amino acids means an irreversible loss. Also a substantial part (31%) of whole-body lysine (another essential amino acid) oxidation occurred in the intestine, although lysine oxidation contributed little as an energy source [8]. But again, it meant an irreversible loss of lysine which is one of the most limiting amino acids in the diet. Table 1 shows the contributions of different substrates to total energy expenditure. At least half of the energy generated within the intestines is derived from amino acid oxidation under normal feeding conditions. Interestingly, when we reduced protein intake to a maintenance level, visceral amino acid oxidation was substantially suppressed. This was only partially compensated for by an increase in glucose oxidation, indicating that other substrates such as fatty acids might become more important.

**Intestinal Amino Acid Utilization and Systemic Availability**

The higher the intestinal utilization rate, the lower the systemic availability of dietary amino acids. This indicates that the intestinal utilization rate of amino acids determines whole body growth. We found that in piglets the utilization rate of essential amino acids was approximately 65% of the dietary intake during the first few hours following feeding [8]. Thus the systemic availability of essential amino acids was only 35%. For some amino acids like threonine, the systemic availability was only 16% of the intake (table 2) [11]. In a subsequent study, therefore, we examined whether the amino acids that were utilized within the intestine would perhaps become systemically available the next day [12]. It appeared that 26% of the amino acids that were utilized within the intestine were released in the portal vein during the hours following the feeding period and thus became systemically available. So a substantial part of dietary intake was again released into the systemic circulation.

<table>
<thead>
<tr>
<th></th>
<th>Normal protein intake</th>
<th>Low protein intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>11 ± 3</td>
<td>–</td>
</tr>
<tr>
<td>Lysine</td>
<td>2 ± 0</td>
<td>–</td>
</tr>
<tr>
<td>Threonine</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1 ± 0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glutamate</td>
<td>32 ± 15</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Glucose</td>
<td>39 ± 10</td>
<td>52 ± 30</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>13</td>
<td>36</td>
</tr>
</tbody>
</table>


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van Goudoever et al.
on the day after feeding. This can be attributed to either amino acid release by proteolysis of constitutive proteins in the intestinal wall or amino acids derived from secreted glycoproteins that are degraded and reabsorbed from the intestinal lumen. Such secreted (glyco-)proteins can be mucins because, for instance, Muc-2 is rich in threonine, one of the most abundantly utilized amino acids by the intestine [13].

Apart from oxidation, the metabolic fate of utilized amino acids in the intestine can be protein synthesis. For instance, enteral glutamate is preferentially used for glutathione synthesis [14]. We have recently measured the metabolic fate of methionine in the intestine of piglets. Interestingly, hardly any dietary methionine was utilized in the first pass, but the gastrointestinal tissues consumed 20% of the arterially derived methionine which represented a significant site of transmethylation and transsulfuration [Riedijk et al., unpubl. data]. Threonine is also utilized from the arterial site, but in equimolar amounts as from the luminal site [11].

The combined findings are consistent with the intestine being a major consumer of amino acids, inasmuch as it uses the equivalent of approximately half of the dietary amino acid intake. These amino acids can be derived from the luminal site of the intestine or from the systemic site. The utilization grades of the various amino acids differ markedly. Some might be utilized almost completely (glutamate, threonine) whereas, for instance, less than half of the intake of lysine is utilized. Oxidation is an important metabolic fate, but a substantial part of the utilized amino acids is used for protein synthesis.

### Human Preterm Studies

For obvious reasons, we cannot determine portal-drained viscera metabolism in human neonates. It is not feasible to obtain portal blood samples and to

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**Table 2.** The net systemic availability of essential dietary amino acids as a percentage of enteral intake during the first 6 h following continuous feeding (mean ± SEM; n = 9 piglets)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Intake μmol/(kg/h)</th>
<th>Systemic availability μmol/(kg/h)</th>
<th>Systemic availability (% of intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>934</td>
<td>152 ± 36</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Valine</td>
<td>765</td>
<td>315 ± 31</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>780</td>
<td>218 ± 18</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Leucine</td>
<td>748</td>
<td>350 ± 33</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>254</td>
<td>94 ± 9</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Lysine</td>
<td>518</td>
<td>277 ± 23</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>Total essential amino acids</td>
<td>3,999</td>
<td>1,406 ± 101</td>
<td>35 ± 3</td>
</tr>
</tbody>
</table>
quantify portal blood flow in infants. But the use of dual stable isotopically labeled tracers, administered enterally and systemically, allows us to quantify the first-pass uptake of specific substrates. This is a reflection of the direct utilization rate of substrates by the duodenum, small intestine and liver, assuming that digestion and absorption are complete. The findings from several studies suggest that this is an appropriate assumption: hardly any enterally administered tracer (<1%) can be found in the stools [15], approximately 98% of milk proteins are digested [16] and intact proteins are rapidly and almost completely digested and absorbed before the terminal ileum in infants [17].

We used a labeled sodium bicarbonate infusion prior to a labeled substrate infusion in order to measure the oxidation rates of different substrates. In several studies we have shown that we can sample expiratory air directly from the tracheal tube whenever the infants were mechanically ventilated or from a gastric tube inserted 1.5 cm into the nose when the infants were breathing spontaneously [18]. The tracer can be administered enterally, which makes this kind of study minimally invasive [19].

*First-Pass Splanchnic Amino Acid Utilization and Systemic Availability during Full Enteral Feeding in the Human Preterm Neonate*

Although the pig is considered an appropriate model for the human neonate, we wanted to confirm the previously described results in the human neonate. So we determined first-pass utilization rates of lysine and threonine in fully enterally fed preterm infants. We opted for lysine as it is the first limiting amino acid in the diet of preterm infants, and for threonine as it is used by the gut at the highest rate among essential amino acids in piglets. Our results confirmed most of the results obtained in the piglets. Approximately one fifth (18 ± 7%) of dietary lysine was utilized in the first pass, versus 70% of dietary threonine [20; van der Schoor et al., unpubl. data]. In 2 earlier studies approximately 50% of both leucine and glutamine were found to be utilized in the first pass [21, 22]. We recently finished a study revealing that 72 ± 10% of glutamate was utilized in the first pass [Van der Lugt et al., unpubl. results]. All these results together show that there is a large variability between the different amino acids in splanchnic extraction. The results are summarized in table 3.

*Effect of Reduced Enteral Protein Intake on Systemic Availability in the Human Preterm Neonate*

Preterm infants do not tolerate full enteral feeding from birth onwards. This is why they are fed intravenously during the first few postnatal days, with enteral feeding being gradually introduced. Inevitably, enteral intake during the first few days to weeks is low, but the intestine is challenged with feeding and will exert its function. This might lead to a high utilization rate of enteral substrates with a low enteral intake, resulting in a subsequently low
systemic availability. Current practices to reduce parenteral amino acid intake as soon as enteral intake is established might thus lead to a reduced systemic availability of amino acids. Therefore we were also interested in learning whether enteral protein restriction would influence the fractional uptake of specific amino acids. In the piglets we found that the fractional utilization increased whenever dietary amino acid intake decreased. A similar finding was observed in preterm infants receiving 40% of their total amino acid intake enterally and 60% parenterally. While the fractional utilization rate of lysine almost doubled to 32% (18% during full enteral feeding), the first-pass uptake of threonine increased by 12% (82% of total threonine intake) during restricted enteral protein intake [20; van der Schoor et al., unpubl. data].

We measured glucose uptake to compare the splanchnic metabolism of different kinds of substrates [23]. Glucose intake is of course much higher than that of individual amino acids (in millimolar instead of micromolar quantities). Again we used the dual tracer methodology on two occasions, i.e. during full enteral feeding and during partial enteral feeding, as described above. Approximately one third of the glucose intake was utilized in the first pass during full enteral feeding, whereas the fraction increased to 44% during partial enteral feeding. So we can conclude that the fractional splanchnic utilization rate for both types of substrates (glucose and amino acids) increases whenever enteral intake is reduced.

**Intestinal Sources of Energy in the Human Preterm Neonate**

Amino acids are the predominant fuel source for the intestine in piglets. As hardly any such data are available in humans, we examined the intestinal

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Utilization during full enteral feeding</th>
<th>Utilization during partial enteral feeding</th>
<th>Oxidation during full enteral feeding</th>
<th>Oxidation during partial enteral feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>18</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Threonine</td>
<td>70</td>
<td>82</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>48</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glutamate</td>
<td>72</td>
<td>n.d.</td>
<td>63</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glucose</td>
<td>32</td>
<td>44</td>
<td>27</td>
<td>35</td>
</tr>
</tbody>
</table>

Data are expressed as percent of enteral intake.

oxidation rate of specific substrates in preterm infants (table 3). Although essential amino acids such as leucine and lysine were oxidized in piglets we could not show this in preterm infants. On the other hand, dietary glutamate was oxidized to a great extent. Almost 90% of the utilized glutamate in the first pass was oxidized. However, glucose oxidation contributed 5 times as much as glutamate oxidation to splanchnic CO$_2$ production, probably indicating that glucose is the major source of energy in the human neonatal intestine. Approximately three quarters of the glucose utilized by the intestine and liver was oxidized.

**Conclusions**

The visceral organs use great amounts of amino acids as is shown in both animal and human studies. The utilization rates for the various amino acids differ widely, probably reflecting the specific metabolic fate of the different substrates. Glucose and amino acids are major fuel sources for the intestine which metabolically is one of the most active organs in the body. Upon restriction of the protein intake, the intestine is capable of resorting to substrates other than amino acids, although the utilization rate is still high especially when expressed as a proportion of dietary intake. During continuous feeding part of the intestinally utilized amino acids become systemically available later on as a result of recycling of secreted (glyco-)proteins and/or proteolysis of intestinal constitutive proteins.

**Acknowledgement**

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Intestinal Amino Acid Metabolism in Neonates


Discussion

Dr. Rigo: I would like to ask a question about the amino acid requirement in preterm infants in oral and parenteral nutrition. Do you think that the requirement could be different in parenteral and oral nutrition? I ask this question because when we design a parenteral amino acid solution we calculate the intake plasma relationship and design the solution according to this relationship. We find that there is no significant difference between the intake plasma concentration relationship if the baby is fed parenterally or orally. With the exception of some amino acids, such as the aromatic amino acid, the plasma amino acid concentration differs in oral and parenteral nutrition if we give the same amount of this amino acid.

Dr. van Goudoever: I think that Dr. Pencharz is going to answer part of your question because there are some amino acids that clearly have different requirements whether you feed humans or piglets intravenously or enteraly, and he has shown that in
several studies. First of all I don't think that amino acid concentrations in the blood show the needs exactly. The gut is a very high amino acid consumer, and the requirements are different for infants fed iv or enterally. When babies are fed parenterally for a long time, basically the gut is not working so more amino acids are not needed. We like the intestine to work for several reasons so we start giving all our preterm infants some enteral nutrition. Minimal enteral nutrition is necessary but for optimal gut function (including appropriate mucus production, enzyme production, etc.) much more amino acids are needed and consequently the infant's requirements will increase quite dramatically during enteral nutrition. My suggestion to all neonatologists is when you start enteral feeding but are still giving parenteral nutrition that you should go on with high amounts of parenteral amino acid supplementation and have around 1.5–2 g enteral protein before tapering off parenteral amino acids supply.

**Dr. Pencharz:** What we have done in our piglets is to look at the difference between parenteral and enteral nutrition. Our work is parallel to the work that Dr. van Goudoever has talked about, and so we have worked in a similar way. What we have done is to define what the amino acid requirements are parenterally in the pig, and we are now trying to see how much of it relates to humans. If you have a patient with an atrophic gut on parenteral nutrition the levels are probably less; so I agree with everything Dr. van Goudoever has said in answering your question. Ultimately when we have the ideal amino acid solution for parenteral use we may need to give less than for enteral feeding because the gut is atrophic. As the gut starts to recover, then it is a different story. Dr. van Goudoever has talked mostly about essential amino acids, but among the semi-essential amino acids is arginine which we have been very interested in, and arginine is probably synthesized by the gut and is a very important one that we should not forget about.

**Dr. Lafeber:** Over the last 5 years it has been very popular to study glutamine and nutrition, particularly in preterm infants, because of a concept proposed by Neu et al. [1] that orally supplemented glutamine would help to mature the gut wall and have a special function in the gut. But looking at your studies in the pig and also in humans, do you think it is glutamine that has such a special position or should we rather be looking at glutamate?

**Dr. van Goudoever:** There are glutamine believers and glutamine nonbelievers so to say. I am in the not so believing in glutamine camp. We are currently doing studies, and I think that most of the glutamine is being oxidized by the gut in preterm infants. We have measured now about 5 or 6 infants, and almost 90% is being oxidized. But that stands aside from a signaling effect that glutamine might have. From adult studies in intensive care units many critically ill patients have shown some good effect when they were given glutamine. This was not so for the whole population, so there might be a function of glutamine in gut integrity and maybe on immune function, but I think that the majority of glutamine is being utilized via glutamate and being oxidized.

**Dr. Yun Cao:** From your lecture we can see that most of the amino acids was utilized in the first pass. What is the difference between bolus feeding and continuous feeding in the gut?

**Dr. van Goudoever:** We didn't do those kinds of studies. We looked at different gut hormones when we fed the piglets enterally or in a bolus way or continuously. If you then look at the area under the curve of these specific hormones, there was no difference. The same amount of hormones were being produced. We put in a stomach catheter and give them a drip in the stomach, and of course the pylorus will always lead some fluids from the stomach into the duodenum, so it is a semi-continuous way. To my mind there won't be any difference whether you feed them via bolus or continuously. I think that approximately half of the dietary protein is utilized by the gut, and
Dr. Axelsson: Do you think that consumption in the gut is higher in preterm infants than in term infants?

Dr. van Goudoever: That is also a nice question to which I don't know the answer. I would think that the requirements would be a little bit higher in preterm than in term infants as the gut is growing more rapidly.

Dr. Axelsson: What was the protein intake in these preterm infants?

Dr. van Goudoever: In total it was 3.6 g/kg/day. So in the first period one third was given enterally and 2.4 g were given in intravenously, and in the second period they were fully enterally fed.

Dr. Axelsson: Can you speculate if your results maintain the composition of preterm infant formulas?

Dr. van Goudoever: That is also a nice question and not only for preterm infants but also, for instance, for children suffering from diarrhea. We now have oral rehydration solutions to replenish the babies that suffer from diarrhea, and I would make an argument that it might be very good to have some amino acids in those solutions. The same applies when you start giving a preterm infant enteral nutrition; the protein composition of that could be a bit higher than a regular preterm formula in order to get optimal gut function.

Dr. Turck: Did you have a chance to perform experiments in piglets that had undergone small bowel resection?

Dr. van Goudoever: No I didn't. What we are doing currently is looking at babies with small bowel resection following necrotizing enterocolitis (NEC). We are using these infants to look at the metabolic fate of the threonine because threonine is a major substrate for mucin synthesis. We are measuring mucin synthesis in infants with ileostomas. But to come back to your question, if you remove part of the intestine I would think that the requirements would be much less because the gut is using so much amino acids.

Dr. Roggero: Could the intestinal utilization of amino acids differ if parenteral nutrition lasts longer than 7 days, for instance in babies who can’t eat by mouth for a long time?

Dr. van Goudoever: I agree completely with that. If the intestine is not being used then fewer amino acids are needed to maintain the function of the gut. Dr. Pencharz just said that parenterally fed animals and probably also infants have different requirements for specific amino acids depending on how they are fed. So if the infants are fed only intravenously then some of amino acid requirements will become much lower than when they are fed enterally.

Dr. Roggero: On the basis of your results, when you start with oral feeding, do you have to consider the loss related to amino acid utilization by the enterocytes? When the intestine is not used for a long time, is the absorption or utilization of the amino acids by the intestine different from the normal intestine?

Dr. Van Goudoever: Yes, I think if there is adequate intestinal growth the requirements will be a little bit higher, but of course there is a huge turnover. As I showed in one of the first slides, and actually Dr. Garlick did some studies on fractional synthetic rates of different organs, you can see that 100% of the protein mass in the gut is being renewed every day, and that is in normal living animals, and I think that will be about the same in humans. When you start up having to reuse the gut you probably need somewhat more, but I don’t think that is a huge difference from a fully enterally fed gut.

Dr. Hernell: You showed that when you reduce the protein intake you increase oxidation from glucose. Fat is the major source of energy in human milk. How much are these figures actually affected by the amount of fat given?
Dr. van Goudoever: We didn't measure fat oxidation by the intestine. When you lower the amino acids the gut has to switch to another fuel source, but we showed that basically glucose has not really taken over the place of amino acids. So I think it has to be either short chain fatty acids or even long chain fatty acids or ketone bodies; I don't know what is going to be utilized by the gut, but it might be fat.

Dr. Telmesani: I think more or less along the same line. Would using amino acids only with glucose reduce the utilization of amino acids for oxidation and fuel?

Dr. van Goudoever: The studies I showed you were with normal diets containing lactose and fat. This was a regular formula for piglets. If you only feed them amino acids, I think the fraction that would oxidize would be even higher but we didn't perform those studies.

Dr. Shiuh-Bin Fang: As we know the absorption of amino acids can be enhanced by peptides [2, 3]. Did you evaluate the effect of peptide, especially when enteral feeding was started in your study? Since intestinal growth is so rapid, do you suggest any method of monitoring intestinal growth, so that we can modify the introduction of amino acids?

Dr. van Goudoever: The first question was related to peptide absorption. What we measured here in those balance techniques across portal-drained viscera were intact proteins. Intact proteins were given to the animals and then we tried to find free amino acids in the portal vein. Barbara Stoll, one of the coworkers in Houston, tried to measure peptides in the portal vein; the peptides should be taken up and released as peptides in the systemic circulation, but she could not find any. Although we know that peptides are being absorbed from the lumen, we think that the majority of the protein substance is administered, appears as amino acids into the portal vein. Then your second question was regarding whether there is a measurement of intestinal growth, but basically there is none. There are of course techniques to look at total surface area with all kinds of tests, but I don't think that they are able to pick up small differences in growth. You have to see whether the infants tolerate the food and that is the basic way to step up enteral feeding.

Dr. Martinez: I have a comment on oral rehydration solution and the potential use of amino acids. We have been working since 1988 in a WHO task force for improving oral rehydration solutions, and unfortunately our experience is that neither alanine nor glutamine seem to improve oral rehydration solutions. We believe the reason for this is due to the impact of osmolarity when amino acids are added to the solution and often no compensate is made.

Dr. van Goudoever: I agree with the osmolarity issue. Since we have now done all these studies and if you look at the utilization rates of enteral amino acids, alanine and glutamine might not be the best choice. But there could be other amino acids or peptides if you want to reduce the osmolarity. Osmolarity is an issue, but in recovering a gut, amino acids are important.

Dr. Turck: Do you have any indication on the proportion of amino acids taken up by the gut that is used for the synthesis of mucins? And if so is there any influence of gestational age and the type of feeding as opposed to artificial feeding?

Dr. van Goudoever: The proportion being secreted, of which the mucins are the majority, is around 15% of total protein intake. That is basically by recalculating all the data. We are able now to separate mucins but to quantify the amount of mucins produced by humans is very hard because they are very sticky and it is very difficult to get them all. But by deduction of all numbers I think about 15% is being utilized for mucin synthesis. If you look at the peptide backbone of mucins there are three specific amino acids: threonine, serine and proline. They are abundantly present in mucins. With regard to the second question whether there is a difference in gestational age, basically I don't know because it is very difficult to do this kind of study.
Dr. Gia Tien Pham: Is there any difference in the digestion and absorption of amino acids in intact protein and modified protein?

Dr. van Goudoever: Metges et al. [4] have actually looked at this, and there is some difference in absorption but mostly in the metabolic fate. They looked at intrinsically labeled proteins versus free amino acids and what they observed was that the oxidation rate of free amino acids was higher than of an amino acid in a specific protein. If you look at absorption, especially in newborns and also in older children, almost all milk proteins are being digested completely. So I think that for milk proteins the digestion/absorption is virtually complete.

Dr. Mohd Suhaime Abdul Wahab: As in the first few days of increased growth of the gut the need to utilize amino acids is quite high, does an inadequate supply of amino acids play a role in the pathogenesis of NEC? If we give amino acids in the first days of life, would that reduce the incidence of NEC?

Dr. van Goudoever: This is another good question which I don’t know the answer to. We of course know that human milk seems in some way to lower the incidence of NEC. In our unit, we start enteral nutrition in preterm infants already on the first day of life and our incidence of NEC is rather low. But next year it might be very high because in some years there is very low incidence and then suddenly there is a high incidence of NEC. Basically it is a good question and it needs to be addressed, but the difficulty with these kinds of studies to lower the incidence of NEC is that you usually need about 1,500 infants, making these studies very expensive studies and difficult to conduct.

Dr. Agostoni: In the case of diarrhea do you have an idea of the proportion of amino acids that are consumed by bacteria and which type of bacteria?

Dr. van Goudoever: In these kinds of studies you cannot sort out what is being consumed by bacteria and what is being consumed by the gut because these animals were running around, they would have loads of bacteria in their intestine. From a methodological point of view it is a very interesting question. From a real life situation basically it doesn’t matter because we are feeding infants and they will also have intestinal bacteria and those bacteria will use some amino acids as well. But the other thing is that Metges et al. [5] also showed that lysine for instance is produced by intestinal bacteria to a great extent. They actually argued that approximately 15–20% of the systemic availability of lysine was derived from bacterial production. So on one hand bacteria can be big consumers but on the other hand they can be producers of essential amino acids.

Dr. Do Van Dung: You said that in developed countries when you perform oral rehydration, you provide more amino acids in the solution. But we are afraid that if we put more amino acids into the solution it will increase the osmolarity of the solution and have a negative impact on diarrhea, and it might create a better environment for the bacteria to develop. What is your advice?

Dr. van Goudoever: I agree with the osmolarity issue, but there are ways to solve this by giving dipeptides, tripeptides, so you would reduce the osmolarity by a factor of 2 or even 3. Also inducing a good bacterial load in the intestine would actually help reduce the severity of diarrhea. There are studies with pre- and probiotics in Honduras that actually show that they are good in lowering the incidence and the time of diarrhea. But I think the osmolarity can be dealt with, but it would make oral rehydration solutions far more expensive.

References

van Goudoever et al.


Amino Acid Requirements of Infants and Children

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Abstract

Nitrogen balances have been conducted in preterm infants, preschool children, and 6- to 10-year-old children to determine dietary indispensable amino acid. A recent review concluded that the data, being sufficiently uncertain, could not be used as the basis for defining amino acid requirements in infants and children. Therefore, it was decided to use a factorial approach (basal plus growth). This approach is based on the assumption that basal requirements are the same throughout the life cycle. Recently, using indicator oxidation, the requirements of the eight classical indispensable amino acids have been defined in adults. These values have been used as the basal component of requirement in childhood. The growth component was based on the changes in body protein with age. We have recently shown that the maintenance requirements for branched chain amino acids are similar in children and adults, thereby validating the factorial approach.

Introduction

This review is restricted to the 20 $\alpha$-amino acids for which t-RNAs exist and hence are part of protein. These amino acids can be divided into the classical indispensable (essential) amino acids, the conditionally indispensable amino acids and the dispensable (nonessential) amino acids \cite{1}. The 8 classically indispensable amino acids cannot be made by humans and hence must be obtained from the diet, hence dietary requirements have been defined for them. For the 9th classical indispensable amino acid in infants and children, histidine, it has proven impossible to determine its requirements in adults, since it takes $>40$ days to deplete body histidine stores \cite{2}. Conversely, in infants \cite{3} and in young pigs \cite{4} it has proven straightforward to determine the histidine requirement,
since body histidine stores are much smaller. The term ‘conditionally indispensa-
ble amino acid’ implies that the infant or child is unable to make sufficient
amounts of that amino acid and hence all or a part of the daily needs for that
amino acid have to be provided by the diet. For infants the literature evidence is
that only 5 α-amino acids are truly dietary dispensable amino acids, namely, ala-
nine, aspartate, asparaginine, glutamate and serine [1].

Components of Requirements

Requirements for α-amino acids are made up of components, namely how
much is needed for net incorporation into protein, plus that which is needed
for other biological processes. While biologically important, some of these
processes such as neurotransmitter synthesis from tyrosine and tryptophan
are quantitatively small [5, 6]. Conversely others require significant amounts
of the amino acids, namely: (a) cysteine, glutamate and glycine for glutathione
synthesis; (b) arginine for urea cycle activity, and (c) arginine, glycine and
methionine for creatine synthesis [6].

Determination of Amino Acid Requirements

The determination of dietary requirements in infants and children has
proven to be a challenging task. Whatever method is used, graded levels of
the amino acid under study have to be fed to the subjects, ranging from below
to above the requirement level [7–9], and changes determined in a biological
response. The biological responses which have been used include: nitrogen
balance; plasma amino acid level; direct amino acid oxidation and balance,
and indicator amino acid oxidation and balance [5, 8]. Plasma amino acid lev-
els have not proven to be useful except possibly for tryptophan [8]. Nitrogen
balances have been conducted in preterm infants [3, 10, 11], preschool chil-
dren [12], and 6- to 10-year-old children [13–17] to determine dietary indis-
pensable amino acid. As recent extensive review of this work concluded that
the data were sufficiently uncertain so that they could not be used as the
basis for defining dietary indispensable amino acid requirements in infants
and children [5]. Direct oxidation and balance are limited to the few amino
acids whose carboxyl group is released as soon as the amino acid is committed
to degradation and, in addition, there are several potential biological limitations
with direct oxidation/balance which have been reviewed in detail [8]. Hence,
indicator oxidation and balance are regarded as the optimal methods to
determine dietary indispensable amino acids [5, 8, 18]. There is some dis-
agreement as to how much adaptation time is needed prior to conducting a
indicator study at a particular level of intake of the test amino acid [5, 8]. It
has long been known that if nitrogen balance is used as the method to determine
the requirement it takes 7 days to achieve equilibrium in the body urea pool and in urinary urea excretion. Hence if nitrogen balance is used then subjects must be adapted on the test intake for a minimum of 7 days. It is unacceptable to place an infant or young child on a markedly deficient level of test amino acid intake for 7–10 days (7 days of adaptation plus 3 days to conduct the nitrogen balance study). Conversely the indicator method is based on the partitioning of the indicator amino acid between net incorporation into protein or oxidation. In which case the adaptation needed does not relate in any way to the urea pool but does relate to the turnover of acylated t-RNAs, which is a matter of a few hours [8]. Therefore we have developed a minimally invasive method to determine essential amino acid requirements in infants and children which uses fed state as an indicator of oxidation [7–9, 19, 20].

Since there are limited published data on dietary amino acid requirements in children using indicator amino acid oxidation (IAAO), and in light of the decision not to use the earlier nitrogen balance data [5], it was decided to use a factorial approach to define dietary indispensable amino acid requirements in infants and children [5, 18]. The factorial approach is based on the assumption that the basal requirements of a component are the same throughout the life cycle and that requirements in infants and children are higher than those in adults due to growth [5]. Using a combination of IAAO and indicator balance, the requirements of the 8 classical indispensable amino acids have recently been defined [5, 18] and these have been used as the basal component of requirement for infants and children. Recent data from the Children's Nutrition Research Center in Houston have provided much improved estimates of the growth component and this has been used to define the growth component for each indispensable amino acid [21, 22]. Because of all the assumptions involved in the factorial method, we are in the process of determining dietary indispensable amino acid requirements in children and comparing them with estimates obtained in adults using the same IAAO method. So far our studies support the use of the factorial method [8, 9, 23, 24]. Specifically for branched chain amino acids in 6- to 10-year-old children, their requirements approximate the maintenance (adult) requirement plus the small growth component.

**Requirement Estimates for Dietary Indispensable Amino Acids**

For infants <6 months of age estimates of dietary indispensable amino acids have been based on the average intake from mother's milk (table 1). These were calculated based on the average amino acid content of human milk protein [5, 18] multiplied by the average protein intake from mother's milk [5, 18].

For infants >6 months of age, children and adolescents amino acid requirement estimates were calculated using the factorial approach described above.
The protein deposition estimates used are shown in table 2. Since there were only small differences due to gender, it was decided to use an average of the results for the two genders [18]. Details are given in the footnote to table 3 as to how amino acid requirements were calculated. Briefly, the maintenance pattern derived from adult requirement estimates were multiplied by the maintenance protein requirement (0.686 g protein/kg/day) to calculate the maintenance need for each amino acid, to which was added the growth component. The growth component is calculated from tissue protein composition multiplied by the amino acid content of that tissue protein (table 3). Finally a correction is made for dietary protein utilization which has been estimated as being approximately 58% of the ingested protein. The efficiency factor is derived from the slope as regression analysis of nitrogen balance studies conducted in children with a variety of different protein sources [5, 18]. These new estimates of dietary indispensable amino acid requirements differ from the previous World Health Organization report [25] to a variable degree as shown in table 4. The variability is less when the estimates are compared as a requirement pattern, namely as milligrams of each amino acid per gram of protein intake. The biggest differences are for tryptophan and for the aromatic amino acid, the sum of phenylalanine and tyrosine. There is no doubt that the maintenance estimates for the dietary indispensable amino acids are based on more complete data than were available in 1985 [5, 8, 18]. Similarly the growth estimates are based on extensive new data [21, 22]. However, it is important to mention that the maintenance estimates for the aromatic amino acid are based on the averaging of two very different results and may be an underestimate. If that proves to be the case the requirement patterns will

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**Table 1.** Indispensable amino acid intakes in exclusively breast-fed infants

<table>
<thead>
<tr>
<th>Age, months</th>
<th>AAA(^1)</th>
<th>HIS</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA(^1)</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>162</td>
<td>36</td>
<td>95</td>
<td>165</td>
<td>119</td>
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<td>76</td>
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<td>2</td>
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<td>69</td>
<td>121</td>
<td>87</td>
<td>42</td>
<td>55</td>
<td>21</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>102</td>
<td>23</td>
<td>60</td>
<td>105</td>
<td>75</td>
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<td>4</td>
<td>93</td>
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<td>95</td>
<td>68</td>
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<tr>
<td>6</td>
<td>88</td>
<td>20</td>
<td>52</td>
<td>90</td>
<td>65</td>
<td>31</td>
<td>41</td>
<td>16</td>
<td>52</td>
</tr>
</tbody>
</table>

\(^1\)AAA = Aromatic amino acids, the sum of phenylalanine and tyrosine; SAA = sulfur amino acids, the sum of methionine and cysteine.

\(^2\)Values taken from the WHO/FAO/UNO Protein and amino acid report [18].

\(^3\)Protein content in human for each age group (75% crude protein) multiplied by the average amino acid content as mg/g of protein shown above.
only differ markedly for tryptophan. Clearly, there is a need to directly determine the tryptophan requirements of children.

**Requirement Estimates in Disease**

The development of the minimally invasive indicator amino acid oxidation model for the first time opened the way to directly determine requirements for inborn errors of amino acid metabolism: phenylketonuria [19, 20] and maple syrup urine disease [26]. In addition we have been able to use the indicator oxidation technique to determine essential amino acid requirements during intravenous feeding and compare the results with estimates obtained during enteral feeding. The work was conducted initially in neonatal piglets as a model for human neonates [27, 28] and has been partially confirmed in human neonates [29]. Overall this work has shown that the intestinal mucosa is active in amino acid metabolism. The key findings are that: up to 60% of dietary threonine in the neonatal piglet is taken up and used for the synthesis

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**Table 2.** Protein deposition for infants and children\(^1\)

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Protein deposition, g/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>0.5</td>
<td>0.266</td>
</tr>
<tr>
<td>1.0</td>
<td>0.168</td>
</tr>
<tr>
<td>1.5</td>
<td>0.108</td>
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<td>0.005</td>
</tr>
<tr>
<td>18.0</td>
<td>0.000</td>
</tr>
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</table>

\(^1\)Derived from Butte et al. [21] and Ellis et al. [22], data smoothed using non-linear regression. For details see [18].
### Table 3. Amino acid requirements of infants, children and adolescents

<table>
<thead>
<tr>
<th>AAA</th>
<th>HIS</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid content (mg/g protein)</td>
<td>73</td>
<td>27</td>
<td>35</td>
<td>75</td>
<td>73</td>
<td>35</td>
<td>42</td>
<td>12</td>
</tr>
<tr>
<td>Tissue amino acid pattern(^1)</td>
<td>38</td>
<td>15</td>
<td>30</td>
<td>59</td>
<td>45</td>
<td>22</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Maintenance pattern(^2)</td>
<td>59</td>
<td>22</td>
<td>36</td>
<td>73</td>
<td>64</td>
<td>31</td>
<td>34</td>
<td>9.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Protein requirements for maintenance(^3)</th>
<th>Amino acid requirements(^5), mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.686 0.46 59 22 36 73 64 31 34 9.5 49</td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>0.686 0.19 40 15 27 54 45 22 23 6.4 36</td>
<td></td>
</tr>
<tr>
<td>2–10</td>
<td>0.686 0.06 30 12 23 44 35 18 18 4.8 29</td>
<td></td>
</tr>
<tr>
<td>10–14</td>
<td>0.686 0.07 30 12 22 44 35 17 18 4.8 29</td>
<td></td>
</tr>
<tr>
<td>14–18</td>
<td>0.686 0.04 28 11 21 42 33 16 17 4.5 28</td>
<td></td>
</tr>
<tr>
<td>&gt;18</td>
<td>0.66  nil 25 10 20 39 30 15 15 4.0 26</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Amino acid content of whole body protein [18].
\(^2\)Maintenance amino acid requirements expressed as mg/g of protein per day.
\(^3\)Maintenance protein requirements in childhood and for adults (>18 years) determined as described by WHO/FAO/UNO Protein and amino acid report [18].
\(^4\)Calculated as an average value from the growth data in table 2 adjusted for a protein utilization of 58% [18].
\(^5\)Sum of the maintenance protein × the adult maintenance amino acid pattern and growth (tissue deposition adjusted for a 58% dietary efficiency of utilization × tissue amino acid pattern).

### Table 4. Comparison of the factorial requirement estimates for preschool children (1–4 years old) with previous values

<table>
<thead>
<tr>
<th>Requirement pattern, mg/g protein</th>
<th>AAA</th>
<th>ILE</th>
<th>LEU</th>
<th>LYS</th>
<th>SAA</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio New/1985 values</td>
<td>0.60</td>
<td>0.87</td>
<td>0.75</td>
<td>0.70</td>
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<th>LEU</th>
<th>LYS</th>
<th>SAA</th>
<th>THR</th>
<th>TRP</th>
<th>VAL</th>
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<tr>
<td>New WHO/FAO Report [18]</td>
<td>47</td>
<td>32</td>
<td>64</td>
<td>53</td>
<td>27</td>
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<tr>
<td>Ratio New/1985 values</td>
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<td>0.97</td>
<td>0.91</td>
<td>1.08</td>
<td>0.82</td>
<td>0.68</td>
<td>1.2</td>
</tr>
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</table>
of intestinal mucins [27, 28]; about 40% of the branched chain amino acids are oxidized, and the gut is necessary for arginine synthesis [27, 28].

**Future Directions**

More work needs to be conducted to verify whether the factorial method of calculating dietary indispensable amino acid requirements is valid in infants as well as in children. Chronic diseases such as liver and kidney failure are know to alter amino acid metabolism and hence may also alter dietary indispensable amino acid requirements [8, 9]. The minimally invasive indicator model makes studies possible in these chronically ill children. Of much larger public health importance, many of the world's children live in poverty and have chronic diarrheal illnesses. We believe that this increases their amino acid requirements and there is a need to define by how much.

**References**


Discussion

Dr. van Goudoever: Thank you very much and congratulations on all the work you have done in improving all these requirements. The figures you showed on branched chain amino acid requirements in adults versus 6- to 10-year-old children saying that 6 mg/kg/day is the amount needed for growth, have you calculated how much g/kg/day that is and whether it would fit the normal growth pattern of infants of that age?

Dr. Pencharz: Those were actually based on the data of Butte et al. [1] and Ellis et al. [2] in children 6–10 years of age and then corrected for utilization; so that is the number we came up with. Now the actual direct isotope breakpoint we think really reflects expenditure. In one day the growth component cannot actually be measured anymore than with doubly labeled water; you are just measuring the expenditure. In
our case it is an 8-hour period; in Dr. Butte’s case with the doubly labeled water it is 5, 3 days, whatever, and then adding the growth on top of that.

Dr. van Goudoever: But if I understood correctly, the requirements were 1.43 for adults and 1.47 for 6- to 10-year-olds, and those were measured by your indicator of amino acids?

Dr. Pencharz: That is correct.

Dr. van Goudoever: And so the 6 mg/kg/day is calculated separately.

Dr. Pencharz: The 1.43 and 1.47 are the numbers we actually found. I will admit that we did not initially interpret our data completely correctly. We said there is a difference there of 4, and 6 and 4 are very close. So we come to realize that in piglets with the rapid growth we are measuring the growth component as well as maintenance. In our studies in the slow growing children we are measuring only maintenance, we are not measuring the growth component.

Dr. van Goudoever: Because the 6 mg would not represent let’s say 2 g/kg/day of growth.

Dr. Pencharz: I can’t remember but you have to go back and see what they are growing and what proportion of that is the three branched amino acids, etc.

Dr. Dewey: One of the issues that I think is interesting to explore is the assumption that in the first 6 months human milk does meet all the essential amino acid requirements. Does that fit with your estimates of requirements from 6 months onward? I was just comparing your tables showing intake from human milk and the requirements. It looks as though for most of them intakes are higher than requirements, except for histidine, lysine and the sulfur amino acids it is very close. Could you comment on that?

Dr. Pencharz: It is a really good question, and we don’t know because the data just don’t exist. The human milk data are based on analysis of the true protein amino acid composition plus adding free amino acids on top, and so it is just like being an accountant and adding everything together. We are in the process of doing measurements using indicator oxidation techniques in neonates and we hope to have estimates for both the enteral and parenteral requirements of branched chains of threonine and so on. But that hasn’t been done yet, so we don’t really have good data. Peter Reeds and I have tried to subject the Holt and Snyderman data [3] to nonlinear regression but the data are too weak, so we don’t have a good estimate. At the moment, we need more data to be able to answer your question.

Dr. Garlick: The major difference between your approach with the indicator and the approach that other people have used previously, either with isotopes or nitrogen balance, is the adaptation issue. Even when using an indicator method, people have still used the traditional adaptation period of a couple of weeks on each diet because they felt that this was required for the enzymes to readapt to the new level. You don’t; you use the time it takes for the subject to start with the meals with the new amino acid content, a few hours rather than 2 weeks. Could you elaborate on why it is that you feel that you can dispense with this adaptation period?

Dr. Pencharz: I did mention adaptation briefly in my written paper but I haven’t brought any slides. The adaptation business really relates to the urea pool. The urea pool in the body is distributed within body water, so 60% of our body composition as adults is water. Rand et al. [4] showed years ago that the adaptation when you go from one level of protein to another or one level of amino acid to another is 5–7 days; so that is what is behind Dr. Garlick’s question. The big question is does this apply to enzyme adaptation with regard to oxidation of an indicator amino acid and to formation of transfer RNA and protein synthesis. We thought not, and in fact in our piglet studies suggested that this was not the case and it was not necessary. So in our first studies in humans we actually looked at two very different levels of phenylalanine intake and
showed what prior adaptation did to our estimates, and we found no effect. So that is where the data are at the moment. We are actually conducting a study right now and I am pleased to be able to tell you that we are finding no effects on indicator oxidation with our adaptation of 6h versus 3 days versus 7 days. I will also say that for three amino acids there are data from Kurpad and Young [5] and our own work, and there is no systematic difference in our estimate of sulfur amino acids of lysine or threonine. So in fact although the adaptation question is an important one, it doesn't affect the estimates we are obtaining in the breakpoint.

_Dr. Thi Thanh Binh Nguyen:_ Is there any difference between the amino acid requirement for preterm babies and term babies. If we use a total parenteral nutrition and the enteral route, is there any difference between the quantity and quality requirement of amino acids for preterm infants?

_Dr. Pencharz:_ The very small premature baby in utero is growing and accreting protein more rapidly than the term infant. So in terms of total protein there is a difference; a term baby needs less protein than the preterm baby. What we don't really know, and at the moment it is only theory, is there may be differences in different amino acids. There clearly are differences if you feed parenterally than enterally and that is the work that Burrin et al. [6] and our own group [7] in Canada have done. The piglet work is lead by Ron Ball and the human work by myself, but we work together. We have defined a pattern of essential amino acids. The one we haven't actually looked at is histidine. There are earlier data on histidine. So I do think that we are going to find some differences in very small premature infants, and we are embarking on studies looking at arginine and other amino acids in very small premature infants. So we don't know the answer, but perhaps in the next 5 years or so we might have the answer.

_Dr. Muhammad Heru Muryawan:_ You have shown that the essential amino acid requirements for the ages 2–10 and 10–14 years are the same for the 2 groups, except for threonine which is very different for these groups. Can you explain why?

_Dr. Pencharz:_ I may have to go back and check and see if we have got the right numbers because in entering it I may have made a mistake. It should not be systematically different from all of the others, so I appreciate you pointing out that potential error. Dr. Garlick and I will look at that and make sure that in the book it is correct. This is not directly from the WHO document; this is my transcription and so I could well have made a mistake.

_Dr. Yates:_ I can understand nitrogen balance, which is a fairly approximate evaluation methodology, when we talk about body weight based on kilograms. But when we are dealing with a nutrient that is so closely tied to lean body mass, how much of the decision to put these in terms of the amount/kg/day is due to convention and ease of determining body weight versus a real lack of concern about lean body mass as a better unit of measure?

_Dr. Pencharz:_ That is a really good question and you have actually answered it yourself. The reason that we chose both the dietary reference intake and the WHO is that measuring body weight is so much easier. Scientifically there is no doubt that nutrients like this will relate to lean body mass. We have repeatedly tried to see if we could show differences when we measure body composition in our studies and we haven't yet come up with anything, but perhaps in disease we may. Actually we did study this in liver patients and we still could not find any different interpretation using lean mass versus body weight.

_Dr. Rigo:_ When you extrapolate the data on factorial approach to the first months of life and compare them to human milk composition, is there a good agreement for all the amino acids?

_Dr. Pencharz:_ I haven't done that for quite a while, so I can't really answer that. It would be nice to go back and to see if this issue of maintenance applies in the first
6 months, and is our backward extrapolation correct, but the answer is we haven't
done it recently. When we have looked at it approximately rather than systematically,
it looked as though it was right, but we haven't done it systematically.

*Dr. Dewey:* You mentioned that the maintenance requirement was determined to
be similar between 6- and 10-year-old children and adults. How much evidence do we
have that this is also true under 12 or 6 months of age?

*Dr. Pencharz:* We don't know the answer. I can only fall back on animal studies
which we have done in pigs. It looks as though the maintenance in newborn pigs is the
same as in adult pigs, so it is likely true in humans as well.

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Effects of High Protein Intakes

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Abstract

Among other nutrients of breast milk, the amino acid pattern is considered normative throughout infancy. Exclusive breastfeeding by a healthy mother should be the standard from birth to 6 months. During the breastfeeding period the protein intake is low in the human being compared to many other animals. The protein content in breast milk is about 1 g/100 ml and the daily protein intake approximately 1 g/kg/day. When other foods are introduced during the weaning period the protein intake increases remarkably to 3–4 g/kg/day in spite of the fact that the protein requirement is decreasing. The long-term consequences of this phenomenon are obscure. A high protein intake may have both endocrine, metabolic, physiologic effects and may increase the risk of obesity.

Studies in humans are still surrounded by a number of uncertainties. Few studies have addressed the nutritional needs of infants at the time of weaning and the long-term consequences of the changes in the diet.
Here the methods by which the international recommendations for protein intake have been determined will be discussed. Furthermore the metabolic, endocrine and physiologic effects of a high protein intake will be considered.

**Requirement and Recommended Protein Intake of Term Infants**

Protein requirement during the first 6 months of life has been estimated using the healthy breast-fed infant as a model [1, 2]. Another approach to estimate the protein requirement of infants is the theoretical calculations used in the ‘factorial method’ [2]. This method for calculation of protein requirements is used especially in infants between 6 and 12 months of age who are not normally exclusively breast-fed. Different authorities give different recommendations depending on the method used for calculating the requirement. The factorial method consists of two parts, the requirement for maintenance and the requirement for growth.

The protein requirement for maintenance is that needed to replace losses through urine, feces and the skin. The factorial method uses many assumptions such as the adequacy of nitrogen balance data for calculating maintenance requirements, the method used for estimation of the needs for growth, the degree of intra-individual variation of growth and the efficiency of converting dietary protein to body protein. According to Dewey et al. [3] the protein requirement has been overestimated using this method. Revised estimates for the mean requirement and safe level of protein intake by Dewey et al. [3] are given in table 1.

In addition to the methods mentioned above, clinical investigations are useful in which protein intakes are accurately determined and measurements of growth and protein nutritional status are carried out.

**Calculation of Protein Content in Formulas**

Authors refer to protein content in infant formula in different ways. There are three possible methods to determine the protein content: calculation of nitrogen content, protein determination, and protein as a sum of amino acids.

Crude protein includes all non-protein nitrogen (NPN)-containing substances. In cow’s milk this NPN amounts to 25–30 mg/100 ml consisting of urea nitrogen and free amino acids. True protein has been defined most often as total nitrogen minus NPN multiplied by the appropriate conversion factor. This calculation excludes nitrogen that is partially metabolically available to the body. Levels of NPN and urea nitrogen in formulas are dependent on the
type of whey used. Ion exchange whey gives the highest concentration (26%) followed by electrodialyzed (14–18%) and ultrafiltrated whey (6–8%) as shown by Donovan and Lönnerdal [4]. The Scientific Committee for Food [5] proposes to determine the crude protein content of all types of infant formula and follow-on formula as total nitrogen × 6.25. In addition, the NPN content must not be higher than 15 of the total nitrogen content in formula based on intact proteins. The recommendation given by the Scientific Committee of Food for protein content in formula is 1.8 g/100 kcal (minimal protein content) for formula based on intact cow’s milk protein. The maximum value is 3.0 g/100 kcal based on all types of protein sources. The same values are proposed for follow-on formula.

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<th>Age months</th>
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<tr>
<td>0–1</td>
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<td>2.69</td>
</tr>
<tr>
<td>1–2</td>
<td>1.54</td>
<td>2.04</td>
</tr>
<tr>
<td>2–3</td>
<td>1.19</td>
<td>1.53</td>
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<tr>
<td>3–4</td>
<td>1.06</td>
<td>1.37</td>
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<td>4–5</td>
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<td>1.25</td>
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<td>5–6</td>
<td>0.92</td>
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</tr>
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<td>6–9</td>
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</tr>
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<td>9–12</td>
<td>0.78</td>
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</tbody>
</table>

From: Dewey et al. [3] with permission.

Metabolic Consequences of Different Protein Intakes

Most metabolic experimental studies of infants have been on formula-fed infants during the first 3 months of life. Several of these studies have shown that infants fed formula with a true protein level of 15 g/l or higher have shown significantly higher plasma levels of several amino acids and urea nitrogen than those found in breast-fed infants [6–12].

A true protein level of 13 g/l has been shown to result in a plasma amino acid pattern similar to that of breast-fed infants [13]. It should be noted that bovine casein and whey are quite different from human casein and whey. A casein-predominant (18:82) formula gives higher concentrations of plasma tyrosine and phenylalanine and lower values of threonine compared to a formula with a whey:casein ratio of 60:40 [14]. Picone et al. [13] showed that formula with a whey:casein ratio of 50:50 provided an amino acid pattern more
characteristic of that of human milk than any of the widely used infant formulae with whey:casein ratios of 60:40 or 20:80.

In a series of studies, a low protein formula (13 g/l or 1.8 g/100 kcal) given to infants from 3 to 12 months resulted in serum urea and plasma amino acid concentrations similar to that found in breast-fed infants [13, 15–17].

In a recent study by Räihä et al. [14, 18] the plasma concentrations of valine were 132 (breast-fed), 149 (experimental whey-modified) and 210 (standard formula) µmol/l, respectively. For threonine the figures were 124, 147 and 201 µmol/l, respectively. Even for cysteine the plasma concentrations in the infants fed whey-modified low protein were closer to that found in breast-fed infants [14] (formula closer to the reference Nestlé Workshop, vol 47, suppl, Bachman [14]). Thus an improvement in the amino acid metabolism was found for several amino acids in this study.

Protein Intake and Growth

Since the theoretical calculations regarding protein requirements are somewhat insufficient as pointed out above, clinical experiments are necessary as a complement.

Räihä et al. [9, 10] published studies in which normal term infants were fed either human milk, a standard formula (protein 15 g/l, 2.2 g/100 kcal), or a formula with reduced protein content of 12 g/l (18 g/100 kcal). Growth was adequate in all feeding groups from birth to 3 months of age. At 8 and 12 weeks the protein intake was significantly higher in infants fed standard formula when compared to breast-fed infants or the infants fed the reduced protein formula: 2.7 vs. 1.6 and 1.7 g/kg/day, respectively. Other studies confirm that formulas with protein concentrations of 11–12 g/l or 1.6–1.8 g/100 kcal result in adequate growth rates [13, 15, 19].

Between 4 and 8 months, the mean protein intake in exclusively breast-fed infants varies between 0.9–1 and 2 g/kg/day [20–22] and in partly breast-fed infants between 0.85 and 1.5 g/kg/day [16, 20, 23]. In this age group both formula feeding and cow’s milk feeding increased the mean protein to 2.74 and 4.75 g/kg/day, respectively [15, 19, 24–28]. After 9 months the corresponding protein intakes were 3.1 and 4.35 g/kg/day in formula and cow’s milk-fed infants [24, 26–28]. Thus, during the weaning period the protein intake increases remarkably although the requirement decreases.

Infants who were exclusively breast-fed, with a protein intake of 0.9 g/kg/day at 9 months [21], had a slower growth rate than formula-fed infants. Even if supplementary foods were given to breast-fed infants after 4 months of age, the weight gain was significantly lower in breast-fed compared to formula-fed infants between 4 and 18 months [29, 30]. Weight for length and skin-fold thickness were also found to be higher in formula-fed infants.
than in breast-fed infants during this age period [31]. The explanations for the difference in weight gain may be differences in protein intake together with differences in energy intake [24, 29]. If a formula with a protein content of 15 g/l is given together with supplementary foods during the first year of life, no deviation from current growth curves has been noticed in the studies that are currently available [9, 12, 15, 19, 23, 30]. In one published study, the growth pattern of infants who were gradually weaned between 3 and 12 months of age to a formula with a protein content of 13 g/l did not deviate from the standard growth curves [23].

Hormonal Effects and Relation to Growth

The effects of different protein intakes on weight gain, insulin secretion, and plasma concentrations of amino acids have been evaluated in a prospective study involving term infants fed breast milk, low protein formula, or high protein formula. The urinary C-peptide excretion in the infants fed the high protein formula was significantly higher than that in the infants fed the low protein formula or the breast-fed infants. Weight gain correlated with urinary C-peptide excretion and with protein intake [32].

These results were confirmed in another study [33]. Between 3 and 12 months, 71 healthy infants were breast-fed (BM group) or fed formulas with 13, 15 or 18 g/l of protein (F13, F15, F18 groups, respectively) and given the same weaning foods. Plasma BCAA (isoleucine, leucine, valine) and plasma C-peptide and urinary C-peptide were analyzed, and weight and length were measured at 6 and 12 months.

At 6 months, plasma BCAA was higher in F18 (p < 0.0001), F15 (p < 0.0001) and F13 (p = 0.022) than in BM and slightly higher in F18 than in F13 (p = 0.053; fig. 1). At 12 months, plasma BCAA was higher in F18 than in BM (p = 0.015). At 6 months, urinary C-peptide was higher (p = 0.017), and at 12 months slightly higher (p = 0.09) in F18 than in BM (fig. 1). Plasma C-peptide did not differ significantly between the groups at 6 or 12 months. At 6 months, protein intake in formula-fed infants was found to correlate with plasma BCAA (rs = 0.50; p = 0.016) and urinary C-peptide (rs = 0.48; p = 0.017). Plasma BCAA at 6 months in formula-fed infants was found to correlate with weight gain between 6 and 12 months (rs = 0.55; p = 0.0084).

In conclusion this study indicates that a higher protein formula induces higher plasma BCAA and higher insulin release at 6 months than if breast milk or lower protein formula is given. The effects of breast milk on plasma BCAA and insulin release persist, though attenuated throughout infancy. Positive correlations between plasma BCAA at 6 months and weight gain between 6 and 12 months may indicate that protein intake influences growth.

Furthermore in the same study we evaluated the effects of protein intake on insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-1 in
3- to 12-month-old infants fed breast milk or formulas with different protein concentrations [34].

During the period 3–6 months, plasma IGF-1 decreased (median 43.0 (range 60.0) vs. 37.0 (63.0) µg/l, respectively; p = 0.02) whereas plasma IGFBP-1 was found to increase (66.0 (164) vs. 90.0 (206) µg/l, respectively; p = 0.012) in breast-fed infants. Plasma IGF-1 and plasma IGFBP-1 then remained unchanged between 6 and 12 months in breast-fed infants. At 6 months, plasma IGF-1 tended to be lower in infants fed formula with 13 g/l protein than in those fed formula with 18 g/l protein (39.0 (8.0) vs. 43.0 (23.0) µg/l, respectively; p = 0.073). At 2 months, plasma IGF-1 was significantly lower in infants fed formula with 13 g/l of protein than in those fed formula with 18 g/l (34.5 (46.0) vs. 46.0 (59.0) µg/l, respectively; p = 0.009; fig. 2). Plasma IGFBP-1 was similar in breast-fed and formula-fed groups at 6 and 12 months.

Protein intake seems to influence plasma IGF-1 during infancy. Despite an increasing intake of weaning foods, the influence of breastfeeding on plasma IGF-1 remains during the second half of infancy.

Fig. 1. At 6 months, urinary C-peptide was higher (p = 0.017), and at 12 months slightly higher (p = 0.09), in F18 than in BM. At 6 months, insulin-releasing amino acids (plasma BCAA) were higher in F18 (p < 0.0001), F15 (p < 0.0001) and F13 (p = 0.022) than in BM and slightly higher in F18 than in F13 (p = 0.053). At 12 months, plasma BCAA were higher in F18 than in BM (p = 0.015). With permission from Åkeson et al. [33].
Conclusions

Diets high in protein offer no benefits and can theoretically have a number of adverse effects. High circulating blood levels of amino acids may exceed the capacity of the hepatic and renal systems to metabolize and excrete the excess nitrogen. This may lead to acidosis, diarrhea and elevated levels of blood ammonia and urea. The high potential renal solute load associated with diets rich in protein reduces the margin of safety related to the maintenance of water balance. Consequently, during periods of illness with associated dehydration, the reduced capacity to excrete waste products increases the risk of hypernatremia.

In addition to the risk that high protein intakes can compromise fluid balance, excess protein intake has also been linked to obesity in later life which can be related to the hormonal effects.

References

Axelsson

Discussion

**Dr. Haschke:** I have a short comment on the formula with lower protein content. The formulas that you are addressing, with 1.8 g of protein/100 kcal are now on the market, mainly due to the pioneering work of your group and Prof. Räihä. They are now available for infants who need formula feeding. Our company for example has introduced the 1.8 g formula/100 kcal starter formula in 68 countries around the world already, and in Viet Nam it will come during the next few weeks. The problem remaining is the regulatory framework for follow-up formulas. The protein level of follow-up formulas should go down as well as we all know, and you have demonstrated that. However, our regulatory framework, at least in the European Union and Codex Alimentarius, at present does not allow a reduction in the protein level.

**Dr. Axelsson:** But the new data from the Scientific Committee for Food say that you can use the same protein content in a standard infant formula as in a follow-on formula.

**Dr. Haschke:** Yes, but this is an expressed opinion; it has not been translated in European law so far.

**Dr. Axelsson:** I think there are still problems with the follow-on formulas in the United States.

**Dr. Haschke:** I am not so sure about the follow-on formulas because in the United States they play a minor role. It is clear that you can have formulas above 1.8 g/100 kcal as far as I am aware.

**Dr. Ziegler:** May I comment on this? The regulations in the United States are that every formula has to be suitable for the entire first year of life. So with a follow-on formula you still have to be able to meet the nutrient requirements of very young infants. That is the current rule.

**Dr. Axelsson:** I think that is good. But don’t you use follow-on formulas later?

**Dr. Ziegler:** In the United States we do not have any follow-on formulas.

**Dr. Haschke:** I think there are soy formulas that have stage 1 and stage 2. Good Start has just one stage. Maybe one more comment which is also important: in Europe there is the regulation that partially hydrolyzed protein formulas should have a higher protein concentration than regular formulas, and we are also working towards bringing down the protein content in partially hydrolyzed formulas. We feel that the high protein intake with partially hydrolyzed formulas is unnecessary and once the safety of reduced protein partially hydrolyzed formulas can be shown, they should also come onto the market with a protein level of 1.8 g/100 kcal.

**Dr. Giovannini:** Could you speculate on the possible association between higher protein intakes in older formulas in the later development of type-1 or type-2 diabetes and the possible link with reduced glucose tolerance? Because everybody speaks about obesity but glucose intolerance may be interesting.
Dr. Axelsson: I think it is very difficult to speculate. We have a very high frequency of obesity, even in Sweden. In 4-year-old children we find overweight and obesity in about 20%, and we have a very high frequency of breastfeeding. I don't know if a high protein intake would increase the risk of diabetes. There is so much speculation about that, and in the studies by Åkerblom et al. [1] from Finland they used Nutramigen hydrolysate of casein in infants who have diabetes in the family.

Dr. Giovannini: In the future young investigators must study glucose tolerance in the older people in our population, especially people born in the 1950s when human milk was not used very much. There are a lot of people who receive hypoglycemic drugs nowadays, and the clinicians forget to ask whether they were breastfed or not. So we need more data, and for this reason we need young investigators to check this in the future. Years ago there was a study on protein intake at 1 year of age in Italy where supposedly the Mediterranean diet is tradition [2]. But the Mediterranean diet exists only on paper because in the north of Italy the diet is similar to the middle European diet. In this study we found a protein intake 1.5 times higher than the allowance. Now in every part of the world you see mothers who think that giving more protein to children is healthy. This is a problem, it is important not only to think of weight but also of all the metabolic parameters.

Dr. Pencharz: I just wanted to touch on Dr. Haschke's question. In the report that Koletzko et al. [3] put together the recommendation to the Codex Alimentarius Commission is to basically not have a different protein intake, but it has not yet been agreed upon. First of all it has to be accepted by the Codex and ultimately in terms of European regulations, but it is moving in that direction.

Dr. Bozo: I have a comment and a question about hypernatremic dehydration and its relation to sodium in infant formula. To make this relation I think it is very important also to note the sodium concentration in the oral rehydration solution utilized during acute gastroenteritis. There are some recommendations to have a lower sodium concentration in the oral rehydration solution. Was this mentioned in the study you presented?

Dr. Axelsson: The sodium content in this formula was also high; both sodium and protein.

Dr. Bozo: What was the sodium concentration in the oral rehydration solution used during acute diarrhea? Was it high or 60 or 90?

Dr. Axelsson: It was 60, and the baby was given a very small amount, about 25 ml.

Dr. Gomes-Pedro: Kidney growth seems to be related to increased IGF-1 production with high protein diet. Have you got some data on urinary IGF-1 and kidney growth?

Dr. Axelsson: No, I have not.

Dr. Yates: When you were talking about complementary feeding, you mentioned that the high protein intake in the young child was perhaps too high because of the types of foods that were being given to the children or the very young child. What types of food would you suggest as a better source of weaning foods?

Dr. Axelsson: I can speculate. I think it is very difficult to come down to the requirement and the recommendation. You have to change a lot in the diet. Cow's milk is good for calcium and perhaps we should fortify it. You need meat for iron. It is difficult; it is a paradox.

Dr. Shiuh-Bin Fang: I am quite interested in what you said about the changes in kidney size between 3- and 18-month-old infants [4]. If there is an adaptation of kidney size, is it possible to find any functional changes or long-term defects after a transient renal adaptation? If it is an anatomic phenomenon, do you perform any functional evaluation on those babies who are formula-fed or partially formula-fed, such as hormonal tests or their long-term outcome?
Dr. Axelsson: In studies in rats for example, more has been shown about the function and the long-term effects. I can't answer regarding the long-term effects in children.

Dr. Shiu-Bin Fang: Some changes in the kidney size are seen, however we don't know whether or not there are any long-term effects such as a hormone defect; hypertension may even develop in these babies.

Dr. Axelsson: Changes in the glomerular infiltration rate have been shown in rats for example, and also we have shown higher values of creatinine in urine. But I can't say more about that.

Dr. Turck: The Danish group of Hoppe et al. [5] showed that there was a strong relationship between protein intake in infancy and final height. On the other hand, even if the literature is contradictory, it has been suggested that there is a relation between growth rate, meaning length not weight, and an increased risk of cancer. Do you think that there might be a bridge between high protein intake and infancy and risk of cancer in adulthood?

Dr. Axelsson: I forgot to mention the study from Denmark. We are looking at these children who are now 7 years old, but I can't answer your question.

Dr. Ziegler: Could I ask you a sort of philosophical question? Why is there such a strong tendency in most cultures to provide older infants and toddlers with unduly high protein intakes? Is it that the mothers think it is good, or why is it?

Dr. Axelsson: I think it is tradition.

Dr. Telmesani: A question for Dr. Haschke. In the formulas that are partially hydrolyzed, why do we need to go higher on the proteins? Are we losing some of the proteins in the process of hydrolyzation?

Dr. Haschke: The EU initially recognized that measurements such as nitrogen balance in animals indicated lower protein retention from hydrolyzed formulas. However this was the first generation of hydrolyzed formulas. In the meantime, considerable progress has been made in the process of protein hydrolysis. Indeed, Dr. Ziegler has done a balance study with such a new formula showing that with 1.8 or 1.9 g/100 kcal, protein nutrition is perfectly adequate. So it was an improvement in technology that now allows us to decrease the protein content in hydrolyzed formulas.

References

Complementary Foods


Physiology of Food Intake Regulation: Interaction with Dietary Components

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Abstract

Food intake is regulated in both the short- and long-term by a complex physiological system that involves neuroendocrine pathways that are both distinct and overlapping. The underlying causes and mechanisms of the dysregulation of food intake in obesity is poorly understood; however, it is clear that dietary components interact with the physiological determinants of food intake and can cause profound alterations during the development of control mechanisms. The objective of this review is to discuss possible food solutions to the obesity epidemic based on our current understanding of food intake regulation and its interaction with dietary components. First, the physiology of long- and short-term food intake regulation is reviewed. The effects of dietary components on food intake, satiety and intake regulatory markers are then discussed with particular emphasis on macronutrient class and source. Finally, the impact of nutritional manipulations during the early stages of development on food intake and metabolic regulation is examined, followed by a brief description of the possible genetic and epigenetic mechanisms involved.

Introduction

Food intake is controlled by an extraordinarily complex system. The urgency to understand the system is obvious in order to determine how physiology might contribute, or be harnessed, to reverse the current epidemic of obesity. Progress is being made in understanding the complexity of the system, but it is clear that evolution has built in sufficient room for conservation of energy so that in an environment of plentiful food and limited activity the majority of individuals accumulate excess energy.
A genetic or pharmacologic solution does not appear likely in the near future. Although more than 600 genes that play a role in obesity and its disorders have been identified [1], a fundamental change in the genome would not have occurred concurrently with the increase in obesity over the past 30 years. In addition, the connectivity and integration of brain and peripheral circuits associated with feeding is characterized by redundancy. That is, the feeding circuit can rearrange itself even if one of the components of the system is removed or enhanced [2], suggesting that a single and safe drug solution might be unlikely for either prevention or treatment of obesity.

Of some certainty, however, is the fact that the interaction of genes with lifestyle and the food supply is a strong contributor to the obesity epidemic. There is very limited understanding, yet, of how environmental factors affect the development and function of physiological mechanisms of food intake control [3]. Thus, it is unclear whether obesity and its associated disorders develop in susceptible individuals because the physiological mechanisms of food intake control are compromised first, beginning in utero, or if they are simply overridden by the environment and become compromised later. Because the focus of this review is on the physiology of intake regulation, lifestyle will not be considered. Rather the goal is to determine if there is a food solution to intake control. Food triggers multiple physiologic responses that induce satiety, and its components may cause profound alterations during the development of control mechanisms.

The question to be examined is: Arising from the new advances of understanding the circuitry, anatomy, and neurochemical processes involved in intake control, do we have new insights on food characteristics that interact with the intake regulatory system? If not, what might be emerging as a solution?

To encourage a discussion of this question, a background review of the intake regulatory system is provided first, followed by an examination of the interaction of dietary components with intake control mechanisms.

**Physiology of Intake Regulation**

Food intake is regulated in the central nervous system, which receives input from sensory properties of food, mechanical and chemical receptors in the gut, circulating metabolites, and hormones. A complex neuronal circuitry involving the hypothalamus, brainstem and cortex integrates these signals and translates them into information regulating meal size and duration, interval to the next meal, the amount of food consumed over the day or several days, weeks and months, and possibly the composition of food as well as the intake of total energy (fig. 1). The hypothalamus regulates both long-term and short-term intake. The long-term regulation of food intake is mediated by leptin and insulin secreted in proportion to the adipose tissue mass and exerting their action in the hypothalamus [4, 5]. These hormones affect the amount of food
consumed over the day or several days, weeks and months, body fat energy stores, basal metabolism, thermogenesis and eventually body weight. They have also been shown to act synergistically with gut hormones in the regulation of short-term food intake as well [4], thus emphasizing the intertwining and redundancy of the intake regulatory system.

Energy imbalance could arise from errors in the regulation of many aspects of short-term intake as well as in the long-term monitoring of intake [3]. As described in the following, many physiologic signals have been shown to contribute to short-term intake regulation. At present the relative importance of each of the signals in contributing to satiety is unclear. Furthermore, it is possible that due to plasticity of the regulatory system, a decreased sensitivity to satiety signals occurs during maturation or in adulthood, thus contributing to obesity. Some research has led to the suggestion that compensation for the energy content of preloads consumed before test meals is highly variable among children but less precise in older compared with younger children [6]. Much more research is required to determine the genetic and physiological origins of variability in short-term intake.

### Short-Term Intake Regulation

Many of the signals that result in a decrease in food intake in the short-term are activated by gastrointestinal (GI) responses to food ingestion and are transmitted to feeding centers in the brain, primarily via the vagus nerve. The interactions between the gut and the food ingested depend on the macronutrient composition of the food. In addition to being populated by receptors that respond to the physicochemical properties of food, the gut has evolved to recognize the composition of the food ingested and to send signals

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**Fig. 1.** Short- and long-term input signals influencing energy balance, components of the central nervous system network that integrate these signals, and the resulting outputs of the system.

<table>
<thead>
<tr>
<th>Input</th>
<th>Central nervous system</th>
<th>Output</th>
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<tbody>
<tr>
<td><strong>Long-term regulation</strong></td>
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<td><strong>Long-term regulation</strong></td>
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<tr>
<td>Adipose tissue, insulin, leptin</td>
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<td>Size of energy stores</td>
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<td><strong>Short-term regulation</strong></td>
<td></td>
<td>Seasonal food intake</td>
</tr>
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<td>Sensory signals (sight of food,</td>
<td></td>
<td>Day to day</td>
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<tr>
<td>smell, taste)</td>
<td></td>
<td><strong>Short-term regulation</strong></td>
</tr>
<tr>
<td>GI signals (chemical, mechanical,</td>
<td></td>
<td>Satiation</td>
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<tr>
<td>hormones)</td>
<td></td>
<td>Meal size</td>
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<tr>
<td>Circulating nutrients, metabolites</td>
<td></td>
<td>Meal duration</td>
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<td>and hormones</td>
<td></td>
<td>Satiety</td>
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<tr>
<td></td>
<td></td>
<td>Intermeal interval</td>
</tr>
</tbody>
</table>

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**Input**

- **Long-term regulation**
  - Adipose tissue, insulin, leptin

- **Short-term regulation**
  - Sensory signals (sight of food, smell, taste)
  - GI signals (chemical, mechanical, hormones)
  - Circulating nutrients, metabolites and hormones

**Output**

- **Long-term regulation**
  - Size of energy stores
  - Seasonal food intake
  - Day to day

- **Short-term regulation**
  - Satiation
  - Meal size
  - Meal duration
  - Satiety
  - Intermeal interval
in anticipation of their metabolic effects by release of peptide hormones to
different organs involved in the processing of the nutrients derived from
digestion and absorption [7]. Whereas long-term food intake is controlled by
adiposity signals, the regulation of short-term food intake is dictated mainly
by food signals arising from both their preabsorptive action in the gut and
their postabsorptive metabolism.

Preabsorptive Signals

The ingestion of food and the passage of its subsequent digestion products
through the GI tract prior to absorption gives rise to a myriad of signals that
are transmitted to the brain, primarily by the vagus nerve, and are integrated
with long-term energy signals to ensure an appropriate food intake response
[8]. Mechanoreceptors, osmoreceptors and chemoreceptors in the stomach
and the small intestine provide direct signals to the brain. In addition, nutri-
ents stimulate the release of GI hormones that act directly on receptors in the
vagus nerve and in the brain.

Slower gastric emptying is associated with increased satiety [9]. Many fac-
tors contribute to the rate of emptying, including the physical state and tem-
perature of the meal, volume ingested, osmolality, calorific content, released
digestive products and hormonal interactions. Solid foods are emptied more
slowly from the stomach than liquid, increased volume accelerates the rate of gas-
tric emptying, and solutions of high osmolality slow gastric emptying. However,
while gastric distension contributes to food intake regulation, it alone cannot
explain the state of satiety that typically lasts for several hours after a meal [10].

The secretion of hormones controlling food intake is regulated by the pres-
ence of food in the GI tract. The gut is a source of numerous peptides that con-
tribute to the regulation of intake and metabolism. These include, from the
small intestine, cholecystokinin (CCK), glucagon-like peptides (GLPs) 1 and 2,
bombesin, gastrin-releasing peptide, neuromedin B, glucagon, apolipoprotein
A-IV, amylin, somatostatin, enterostatin and peptide YY (3–36) and from the
stomach, ghrelin and leptin [7, 11]. Many of the GI hormones and/or their recep-
tors are also expressed in the central nervous system, underlining their impor-
tant role in appetite control [11]. Some enter the central circulation via leaky
areas (brainstem and hindbrain) in the blood-brain barrier, or send signals
through vagal afferents that are relayed to the hypothalamus. The macronutri-
ent-dependent release of gut hormones might explain, at least in part, differ-
ences in the satiating and satiety effect of macronutrients. For example, fat and
protein are the main CCK secretagogues in humans and rats, respectively [12],
whereas carbohydrate and fat are stronger stimulants of GLP-1 release [13].

While the emergence of knowledge of the multiple satiety signals arising
from the GI tract adds to the understanding of intake control, it has not yet
led to an integrative picture of the action of these peptide hormones or to an
understanding of their relative importance in response to food ingestion.
Similarly, the role of postabsorptive signals remains unclear.
Postabsorptive Signals

Postabsorptive signals are generated after nutrients have been digested and have entered the circulation where they stimulate satiety centers in the brain by endocrine and metabolic actions. The glucostatic, aminostatic and lipostatic hypotheses of intake regulation have been the main theories describing how absorbed nutrients generate and influence satiety signals [14].

The glucostatic theory postulates that fluctuations in blood glucose levels trigger an appropriate change in food intake. In support of the hypothesis, transient declines in blood glucose of the correct magnitude and time course have been associated with meal initiation as they are detected by peripheral and central glucoreceptive elements.

Similar to the glucostatic theory, the aminostatic hypothesis is based upon the brain monitoring of nutrients, in this case amino acids derived from protein ingestion, and consequently shaping consumption patterns. An inverse relationship between serum amino acid concentration and appetite in humans has been observed. It has been further postulated that amino acids act on food intake regulation through their ability to act as precursors to certain neurotransmitters known to influence food consumption. But as reviewed elsewhere, this may be a mechanism determining later food selection and the inter-meal interval rather than within meal satiation [15].

The lipostatic theory, advanced over 50 years ago, was based on signals arising from the metabolism of fats. In recent years, new evidence has emerged to support the hypothesis. Transport mechanisms and enzymes for both fat oxidation and synthesis are present in the brain and inhibitors of fatty acid oxidation increase food intake. Although this could be a peripheral effect, it is clear that the hypothalamus senses a nutrient surfeit in fatty acid metabolism arising from circulating lipids from either dietary sources or adipose tissue [16].

In addition to glucose, fatty acids and amino acids, a number of intermediate products of metabolism associate with satiety. Ketones, lactate, and pyruvate suppress food intake in animals [8].

Dietary Components and Intake Regulation

Food and its components contribute to both short- and long-term regulation of food intake. The challenge is to understand the relative importance of food components and how to optimize their interaction with intake regulatory systems.

Energy

At the physiological level, energy requirement is a powerful determinant of food intake [14]. Thus growth, increased activity, and exposure to cold increase intake. Physiological systems in both experimental animals and humans are remarkably precise in regulating energy intake in relation to requirements. For example, exposure to cold or to exercise, or alterations in
the energy density of the diet or to food availability and choice result in rats quantitatively adjusting their food intake, thus maintaining energy balance when provided with their usual diets [14, 17]. However, it is also clear that experimental animals will become obese when provided a variety of palatable foods, or high fat diets, showing that factors other than the physiological drive toward energy balance determine food intake [18].

Similarly, humans adjust their energy intake to meet their energy expenditure in response to changes in activity, or ambient temperatures. As with experimental animals, humans consume excess energy when they are exposed to an environment of low activity [19] and highly palatable energy dense foods [20].

Although all macronutrients provide energy, their effect on food intake cannot be predicted simply from their energy content. Each macronutrient possesses unique properties that provide signals to the central nervous system independent of their energy content [14].

**Macronutrients**

Protein suppresses food intake more than carbohydrate, which in turn suppresses food intake more than fat. This hierarchy has been shown in both humans and rats [15]. Less appreciated is that even within a macronutrient class, the source is a factor influencing short-term food intake and appetite. However, it is not possible at present to identify the primary biomarkers of satiety that arise from proteins, carbohydrates and fats.

**Proteins**

The mechanisms by which proteins stimulate intake regulatory systems are many, making them unique compared with carbohydrates and fats. Furthermore, the systems stimulated are dependent on the source. The satiety cascade arising from protein is as follows. First, protein initiates satiety through its digestion and the subsequent release of biologically active peptides (BAP) encrypted within the protein. These BAP affect feeding through their actions in the GI tract. They activate receptors, thus providing signals via the vagus nerve either directly, or by interacting with gut hormones that are involved in intake regulation. Second, free amino acids arising from digestion activate neurochemical systems, thereby contributing not only to satiety, but also to macronutrient choice. Finally, the end products of amino acid metabolism, ammonia and urea, probably play a part in determining intervals between meals, but act primarily to signal excess intake or errors in metabolism [14].

Protein source, in addition to protein quantity, is a determinant of satiety [15]. Greater subjective satiety was reported by young men fed a 50-gram meal of lean fish compared with an equivalent amount of either beef or chicken [21]. Whey and soy protein drinks (45–50 g protein) suppressed food intake 1 h later compared with the energy-free control and with sucrose, whereas egg albumen did not [22].
The differential effect of protein source on food intake and subjective appetite might be explained by the action of BAP released during protein digestion. These peptides are unique to the protein source and dependent on its tertiary structure and amino acid composition. Among the most extensively studied BAP are those derived from milk digestion. A glycosylated form of caseinomacropeptide (GMP), a peptide derived from the in vivo and in vitro digestion of casein, is a potent CCK secretagogue [23], and preliminary studies from our laboratory show that GMP is a potent inhibitor of food intake in rats [24]. In addition to GMP, opioid peptides derived from the digestion of casein (casomorphins) suppress food intake through opioid receptors located in the GI tract [25].

Carbohydrates

Consistent with the glucostatic hypothesis are the observations that carbohydrate consumption and the resulting increase in blood glucose are associated with satiety. In the short-term, high glycemic carbohydrates suppress food intake (up to 2 h) more than low-glycemic carbohydrates [26]. Although there is much indirect support for the hypothesis that satiety is associated with the glycemic effects of carbohydrates, a primary role for blood glucose in determining satiety remains uncertain [27], perhaps because the glycemic response to carbohydrates primarily depicts their absorption characteristics. Many other mechanisms, including those based on the rate of gastric emptying and gut hormones, may explain the different effects on satiety of slow compared to rapidly digested carbohydrates. For example, a rapid increase in the stimulation of glucoreceptors would be expected following ingestion and digestion of carbohydrates, but this stimulation does not last long enough to account for the prolonged satiety effect. A more extended effect of carbohydrates on satiety could arise from the stimulation of a multitude of gut peptides, such as GLP-1 and CCK [28, 29]. Gastric emptying is slowed by GLP-1, a putative satiety peptide whose release is stimulated by carbohydrates in the small intestine and that regulates carbohydrate metabolism [13]. A rise in blood glucose concentrations is also a factor that slows gastric emptying [30].

Fats

In general, fat suppresses food intake less than carbohydrate or protein on a calorie for calorie basis. However, this ranking may also depend on the source. Among fats, both the chain length and the degree of saturation have been shown to impact short-term food intake and subjective appetite. Medium-chain triglycerides and polyunsaturated fatty acids suppressed food intake more than long-chain triglycerides and monounsaturated or saturated fatty acids, respectively. These effects have been attributed to the CCK and apolipoprotein A-IV releasing properties of these types of fats [31].
It is clear that an understanding of the interaction between food components and the intake regulatory mechanisms continues to develop. However, as noted earlier, there is little information on the stability of the system once the development of the circuitry has been completed. Similarly lacking is information on the role of environmental or nutritional factors in the development of the intake regulatory system in utero and in early life.

**Nutrients and Development of Intake Control Mechanisms**

Although it is clear that increased risk of chronic disease and obesity are associated with exposure to both deficiencies and excesses of energy and nutrients in utero and in early life [32], there has been very little examination of the role of nutrients in the development of food intake regulatory mechanisms in the etiology of metabolic disease. As with many physiological systems, the development of intake control mechanisms would be expected to occur both in utero and in the early life of the offspring.

The interaction among nutrients and development of regulatory systems in determining effects in later life is complex and there are likely multiple mechanisms to explain the outcome. Altered gene expression is a likely factor. In addition, the development of regulatory systems in the GI tract continues after birth and depending on the composition of the food may have long-lasting effects.

Altered expression of genes regulating insulin has been offered as an explanation of the development of obesity through loss of intake control early in life. Insulin is intimately involved in both long- and short-term intake control and the actions of both insulin and leptin are modified by malnutrition. Programmed development of obesity and adipogenic diabetes in rats has been attributed to a permanent dysregulation of the adipoinsular feedback system, again amplified by a hypercaloric diet, leading to hyperleptinemia, leptin resistance, hyperinsulinemia, and compensatory leptin production by pancreatic δ cells [33]. The offspring of undernourished mothers (30% of the ad libitum intake of the control mothers), cross-fostered during lactation to the control mothers, had lower birth weights than the offspring of control mothers [34]. After weaning, they exhibited higher food intake, systolic blood pressure, and fasting plasma insulin and leptin concentrations than control pups. These effects were amplified by a hypercaloric diet (30 vs. 5% fat), prompting the authors to conclude that hyperphagia is programmed in fetal development and exacerbated by environmental factors.

Epigenetic effects of nutrients and other dietary factors (e.g. antioxidants) during embryonic development, not gene mutations, have recently provided a plausible link between genetic makeup and susceptibility to development of chronic diseases [35]. DNA methylation is a major modifier of the genome,
repressing transcription and thus a ‘gene silencing’ mechanism. Early in development, the genes are not methylated, but this process is thought to occur after implantation of the zygote. Methylation occurs through the action of the DNA methyltransferase, a process in which many vitamins participate. Therefore, certain dietary supplements given during pregnancy and early postnatal life may have an unintended silencing or deleterious effect on gene expression [36] as shown by the effect of feeding a diet high in vitamins involved in methyl group metabolism on the expression of coat color in the viable yellow Agouti mouse (A<sub>vy</sub>). The expression of the Agouti gene is characterized by a yellow coat color, obese, diabetic and cancer-prone phenotype. A 3- to 5-fold higher intake of methyl donors and methylation cofactors in the form of choline, betaine, B<sub>12</sub> and folate during pregnancy led to a shift towards offspring with the brown coat color phenotype, indicating an epigenetic regulation of the A<sub>vy</sub> gene through methylation [36]. The resulting phenotype is characterized by a brown coat color, lean body weight, and normoinsulinemia [37].

The development of the gene expression of gut hormones involved in the regulation of food intake is at present unknown, as is the influence of the composition of food consumed in early life, but this requires exploration. The ability to potentially modify different populations of enteroendocrine cells may become an important therapeutic strategy for treating and/or preventing excessive food intake and obesity [38].

Preliminary data support the importance of epigenetic events in the development of intake control mechanisms [39, 40]. Increasing the vitamin content of the diet fed to Wistar rats, only during pregnancy and after implementation of the zygote, predisposes the offspring to impairment of intake control, altered gut hormone response, insulin resistance and obesity. Altered phenotypic expression due to epigenetic changes appears to be a likely explanation.

**Conclusion**

The advances in understanding the physiology of intake control mechanisms has not led to a food solution for the obesity epidemic. However, they have provided incentive for testing new hypotheses of food- and diet-based strategies and the interaction between food components and physiological responses. Continued research at the level of physiologic and molecular mechanisms shows promise to contribute to a food-based solution to intake control.

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Anderson/Aziz/Abou Samra


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Physiology of Food Intake Regulation


Discussion

Dr. Butte: After the digestion of human milk is there evidence of guanosine monophosphate (GMP)? My second question is, if I followed you correctly in the studies by Waterland and Jirtle [1] increased manipulation lead to leaner animals, but in your study the increased vitamin intake lead to obesity [2]. Could you clarify that?

Dr. Anderson: I can't give the reason for the contrasting effect. The Agouti mouse and Wistar rat are different genetically. However, we expected that the increase in vitamin intake would be protective for the offspring in the development of insulin resistance and perhaps enhancing appetite regulation. But we got quite the opposite. Scientists in Nestlé research have done some preliminary studies on the release of GMP during digestion in infants.

Dr. Butte: I was just wondering when you were talking about cow's milk. Is there any evidence regarding the release after human milk digestion?

Dr. Anderson: There was some discussion of that at the whey protein conference in Chicago in September. GMP appears but the duration, which is what I am uncertain about and whether it has functional significance, was not looked at.

Dr. Dewey: In terms of the development of appropriate appetite regulation and control of food intake, is there evidence that overfeeding, i.e. from animal studies, has a negative effect on control of food intake later in life?

Dr. Anderson: There are some data that support the notion that overfeeding has health consequences [3], but the effect on food intake control has not been examined.

Dr. Desjeux: You mentioned that during fetal life the nutrient could alter genetic programming and it could be an epigenetic phenomenon. We also know that there are direct interactions between nutrients from amino acids to glucose and fatty acids, and direct interactions with genes. Do you know if these interactions could be permanent or could have long-term effects?

Dr. Anderson: I think there is very little mechanistic exploration that would explain what is happening to the phenotypic expression. What I neglected to mention is that we have given these mother rats a complete vitamin mix but not at the high amount that Waterland and others have done in order to get the methyl addition to the DNA. From our own studies we don’t know which of the vitamin groupings might be
Anderson/Aziz/Abou Samra

generating the effect. Other mechanisms need to be explored, as you suggested, but currently DNA methylation and histone modification are the favored mechanisms [4].

Dr. Desjeux: Did you purposely not speak on energy density as a regulatory mechanism?

Dr. Anderson: No, I am quite familiar with the volumetric idea and energy density as a factor determining food intake. However, there has been little exploration of physiological mechanisms. Because I am in the center of the glycemic index universe at the University of Toronto, I would like to point out that a high energy-dense, rapidly digestible, high glycemic index food is much more effective in shutting down short-term food intake than is a low glycemic index food [5].

Dr. Pencharz: As I listen to your talk it sounds to me like studying rats is studying rats, and we around this table are mostly interested in people, particularly children, and as you mentioned in fact children behave differently to adults. Thinking for the future, should the focus be on studies in humans or might some other animal model help us understand the mechanisms, or are we stuck with the difficulty of human studies?

Dr. Anderson: I believe that to explore mechanisms you need animal models. For example, with our studies on the effect of high vitamin intakes in the rat and the increase in insulin resistance, surely that provides a motivation to explore whether or not this applies to the human population. But otherwise you would have no justification for entering into a human study.

Dr. Fan Yang: You talked about fetal programming. In our clinic we have found that some babies born with intrauterine growth retardation do not show catch-up growth in later life. Is there any evidence that in utero influences have some effect on the long-term and short-term food intake regulation by the baby which may contribute to the baby losing catch-up growth in later life?

Dr. Anderson: It is assumed that there is a distortion in food intake regulation, but I am unaware of any evidence for this. I think that our own studies provide some justification for trying to understand whether the satiety hormone responses are different in obese vs. normal children and whether or not there is a developmental aspect.

Dr. Yi-hung Choi: Can you comment on the role of other food components including appetizers? Some vitamin or mineral components can have an influence on the food intake of children. Can you also comment on the very early feeding behavior of the caregivers in relation to the food preferences of young infants from a behavioral aspect?

Dr. Anderson: I am certainly no expert on the subject of the impact of caregivers; Dr. Dewey and others could probably answer that question much better than I. The question of whether caregivers giving extra calories influences long-term food intake and the regulatory system is a good one and needs to be explored. Your other question addressed vitamins and minerals; we gave vitamins but one of the reasons for starting to look at milk protein and the biologically active peptides, at least here in North America, is that there have been published associations between dairy consumption and healthier body weight. One of the popular hypotheses relates to calcium and vitamin D metabolism in adipose tissue. Perhaps increased calcium intake is the factor, but it is certainly not the only answer, because the studies that have been conducted suggest that the dairy product itself is more beneficial than simply calcium. We have shown that whey protein reduces food intake more than soy or egg protein. So I think milk proteins and their bioactive peptides are important factors [6]. However, there are minerals that affect food intake regulation, and zinc is a classic example, but that usually is a deficiency that causes anorexia.

Dr. Ziegler: The obesity epidemic seems to be worldwide and concerns us all, and seems to have occurred in parallel with an increase in the use of non-caloric or
low-caloric foods and drinks. Would you care to comment on the effect of non-caloric foods on the regulatory systems?

*Dr. Anderson:* In our own studies, if you sum up the calories in the preload with what is eaten at the meal, you very often get a net gain in calories over and above what you would get if the preload was a high intensity sweetened beverage. Not only that, we often see that just a sweet taste from a high intensity sweetener tends to suppress food intake at a meal a little more than just the water preload [7]. If you go around the world, there are many dietary patterns that lead to obesity and are associated with economic advancement. So I don't think it is a factor. Generally, calorie-free sweetened beverages lead to less caloric intake than those containing calories [8].

**References**

Complementary Food: International Comparison on Protein and Energy Requirement/Intakes

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Abstract

The possible role of early dietary habits as the origin of later consequences on health has raised questions on the optimal macronutrient intakes of the growing infant. Infants and toddlers in developed countries usually show a high dietary protein:energy ratio during the complementary feeding period, averaging 2.5–3, because of the protein density of solid weaning foods and the low percentage of mothers still breastfeeding beyond the first 6 months of life. In conditions of very high protein intakes, those in the higher classes of consumption seem to carry a higher risk of becoming obese later on. Over the limit of 14% energy from proteins in the 8- to 24-month period, some mechanisms may begin to operate leading young children towards an early adiposity rebound and overweight development. On the other hand, in many developing countries the only available weaning foods are cereals, with a low protein:energy ratio value. When the protein concentration of weaning foods falls below the limits of human milk (that is, from 1 g/100 kcal to lower levels), the infants’ dietary requirements cannot be met. In planning interventions, the coverage of infants’ dietary needs through all the various world regions should be considered together with the opportunity not to exceed higher limits.

While exclusive breastfeeding is strongly recommended for the first 6 months of life [1], solids should progressively be introduced afterwards in order to complement those nutrients becoming insufficient if supplied only by human milk (particularly energy, protein, iron, zinc and fat-soluble vitamins). The process is called ‘weaning’, and represents the progressive introduction of solid foods into children’s diet in order to fulfill their changing nutritional needs [2].

The recent interest in the role of dietary proteins at early ages and the possible origin of later consequences on health (first of all the connections with
overweight and obesity development) has raised questions regarding the optimal macronutrient intakes in terms of total proteins, energy and protein:energy ratio (PER; grams protein per 100 kcal energy) required by the growing infant.

**Why the Protein:Energy Ratio?**

As a general rule, higher energy intakes may allow more efficient utilization of nitrogenous sources derived from catabolic processes and intakes. Therefore, protein and energy are inversely associated for recommendations within definite ranges.

An example of the operational approach to calculate the changing protein requirements in relation to different energy intakes and applied to young, healthy male adults is given in figure 1 (here PER is given in grams per 1,000 kcal) [3]. To derive a reference PER in this way, one should divide the +2SD protein requirements (i.e., the safe level of protein intake) by the average

![Fig. 1. An example of calculation of protein:energy ratios. Distributions of both protein requirements and energy requirements are portrayed on the axes. The published average energy need and recommended protein intake are marked by arrows. The values shown are based on 1983 Canadian requirement estimates for young male adults. Partially modified from Beaton [3].](image-url)
energy need (mean requirement for energy) at all ages and then compute
PER values, as portrayed schematically, by selecting various arbitrary points on
the distributions. Since these factors are difficult to estimate, the results are
based on assumptions. This approach, even if convenient, might not seem to
be logical, since it does not convey the meaning of either the protein require-
ments (sufficient for almost all individuals) or the average energy needs (suf-
ficient for half the individuals). The consequent ratio involves two factors,
each with its own variance [4]. Finally, the example does not include the fur-
ther variable of the additional protein/energy requirements of a growing indi-
vidual, which would make the model even more complicated.

Pragmatic and physiological considerations justify expressing the protein
requirements in relation to energy intake for situations as diverse as weaning
diets, catch-up growth, and hypercatabolic states [4]. Infants and toddlers in
developed countries usually show a high dietary PER during the comple-
mentary feeding period, averaging 2.5–3, because of the protein density of
solid weaning foods and the low percentage of mothers still breastfeeding
beyond the first 6 months of life [5]. In conditions of very high protein intakes,
those in the higher classes of consumption seem to carry a major risk to
become obese later on. On the other hand, in many developing countries the
only available weaning foods are cereals, with a low PER value. When the pro-
tein concentration of weaning foods falls below the limits of human milk (with
a PER of <1, i.e., <1 g protein/100 kcal to lower levels), the infants’ dietary
requirements cannot usually be met [6].

Protein intakes and PER values show great variability in the 6- to 24-month
period, starting from around 7% energy as protein (PER = 1.7) in 6-month-
old infants at the end of the exclusive breastfeeding period. Given the vari-
ability of the average protein content of human milk, in those cases in which
protein supply represents less than 6% energy (PER = 1.5), within a limited
energy supply, even fully breast-fed infants are likely to enter a status of neg-
ative nutrient balance [7]. Indeed, Wharton [4] has already underlined that it
is ‘probably unsafe to adopt the very low protein/energy ratio in breast milk as
a minimum safe standard, since the nitrogen present is utilized with unusual
efficiency’ and ‘there should be caution in accepting also very low ratios of
1.7’ for those who are not breast fed.

On the other hand, high protein intakes in the complementary feeding period
(from the 8th up to 24th month) are even more frequently reported in both
developed and developing countries considering children from both rich and
poorer classes [8–10]. From the collected data, it seems that over the limit of
14% energy from proteins (PER = 3.5) in the 8- to 24-month period some mech-
nisms may begin to operate leading young children towards an early adiposity
rebound and overweight development, beyond any genetic predisposition.
Preliminary data seem to indicate a causal role for whole cow’s milk proteins.
In the following the limits mentioned here will be discussed as far as causes, potential consequences and measures to prevent low or high dietary PER values.

**Protein, Energy and PER in Developing Countries: Old Issues and New Questions**

In most developing countries the main (or even only) weaning foods are still represented by local staple cereal foods such as maize, rice or cassava. The daily servings supplied with these cereals should match an adequate amount of milk in order to keep both the PER value higher than 1.5 and enough energy to promote anabolic processes and prevent catabolic states, also considering the rates of infections in these child populations. Since different milk sources are theoretically available, schemes have been developed in order to check the adequacy of the source of complementary foods according to the type of milk supplied in the complementary feeding period. According to Wharton [4], possible options are human milk (advantageous for the high quality of the protein content, the anti-infective properties and the functional effects, including the close mother-infant relationship), a follow-on formula (balanced as far as nutrient composition but with more protein compared to human milk and lacking its anti-infective and functional properties), and whole cow’s milk (unbalanced as far as nutrient composition for a growing infant and with the highest PER ratio). For instance, in the case rice is the (almost) exclusive staple food, and considering the PER of the milk being supplied, the protein requirements may be met when human milk provides around 70% energy, follow-on formula 50%, and cow’s milk 30%. When oil is added to rice, the energy requirements may be met at lower intakes, but at the expense of the protein intake, since also the PER value of the complementary foods is proportionally reduced.

Recently, dietary enrichment with ‘ad-hoc’ designed complementary foods and/or drinks with enhanced protein and also enhanced (even if less marked) energy content has been considered to improve the PER value of diets and prevent malnutrition. Indeed, in communities where malnutrition is endemic the 6- to 24-month period is the most critical for nutritional interventions and the introduction of specifically designed supplementary feedings can prevent the onset of wasting in a large proportion of children [11].

In progressing (‘transition’) countries the PER values of diets are higher than 2 also in disadvantaged groups (for instance, those living in rural areas) [10, 12], but they generally result from an adequate protein intake and a lower than recommended energy supply with nutritionally poor food sources. Therefore, a ‘second level’ adjustment in terms of both total energy and micronutrient balance (vitamins, minerals, trace elements) is needed. Preliminary, ‘qualitative’ reports from China raises strong suspicious that large
groups of children (either deprived of complementary foods at appropriate ages or complemented by means of low PER foods, with proteins of low biologic value) are in a negative protein/energy balance [13, 14]. Since the simple addition of micronutrients does not reverse the effects of malnutrition on growth, energy intakes should be increased by supplying foods with a high energy density and/or more frequent meals. Care should be taken to prevent the energy addition through fat and/or sugars from further reducing the protein and micronutrient density below critical limits. Therefore, interventions to improve the quality of protein sources are still urgently required also in these environments in an advanced stage of socioeconomic transition, besides specific dietary enrichments (e.g., with iron, zinc and/or liposoluble vitamins).

Finally, child populations from Asian and African countries, still suffering from both protein and energy malnutrition, should progressively be involved in processes leading to an improvement in both the supply and adequacy of foods. Within these processes, the improvement in the infants’ general health conditions (including the prevention of vertical viral transmission from mothers) should be considered in order to improve their general nutrition status.

Economic conditions (e.g., the cost of complementary foods), environmental backgrounds (e.g., availability of additional foods, microbiological safety of waters and milk), family traditions and sociocultural convictions may all influence the final dietary schedules and intakes of children in developing countries during the complementary feeding period, the discussion of which goes well beyond the scope of this presentation.

**High Protein Intakes, High PER and Obesity**

Over the last 10 years, there has been a common consensus that the average PER in the diets of children from Western countries is around 2.5–3 [3, 7, 15], in some cases reaching values close to 5 [16] (table 1). Reports from low income urban families confirm that in Western countries the protein

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**Table 1.** Reports of nutrient intakes in European countries in the 8- to 24-month period

<table>
<thead>
<tr>
<th>Country</th>
<th>Age months</th>
<th>Protein g/kg</th>
<th>Protein %</th>
<th>Lipid %</th>
<th>Carbohydrate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>9</td>
<td>4.4</td>
<td>15.7</td>
<td>26.4</td>
<td>58</td>
</tr>
<tr>
<td>France</td>
<td>10</td>
<td>4.3</td>
<td>15.6</td>
<td>27.1</td>
<td>57</td>
</tr>
<tr>
<td>Italy</td>
<td>12</td>
<td>5.1</td>
<td>19.5</td>
<td>30.5</td>
<td>50</td>
</tr>
<tr>
<td>Denmark</td>
<td>12–36</td>
<td>3.3</td>
<td>15</td>
<td>28</td>
<td>57</td>
</tr>
</tbody>
</table>

Partially adapted from Agostoni et al. [7].
requirements are easily met and exceeded also under poor conditions [9],
even if we lack data from communities of immigrants, whose nutritional con-
ditions should be investigated in the near future.

In general high protein intakes are believed to have an impact on the
glomerular filtration rate and to increase the renal solute load, while leading
to elevations in certain plasma amino acid levels with possible effects on body
metabolism and the production of neurotransmitters, which has not yet been
sufficiently investigated [17].

Rolland-Cachera et al. [18] were the first to propose an early adiposity
rebound as a first predictor of later obesity in infants. They speculated that
the age at body mass index rebound may be influenced by the amount of pro-
tein supplied in the 10- to 24-month period [19]. They investigated 112
French children who were measured for weight and length and skin-fold
thickness (two sites) at 10 months, 2, 4, 6 and 8 years of age. At 2 years of
age, protein intake as percent of energy was <14.8% (PER 3.7) in the lowest
quartile, 14.8–18% (3.7–4.5 PER) in the next two quartiles and more than
18% (4.5 PER) in the highest quartile. Significant correlations were found
between the percentage of protein and both body mass index and subscapu-
lar skin-fold thickness at 8 years after adjusting for energy intake at 2 years
and parental body mass index. The percentage of protein at 2 years was also
negatively associated with the age at adiposity rebound. Similar results were
reported in 150 Italian children, followed from birth through 5 years [20]. At 5
years, children with a body mass index above the 90th percentile had a higher
protein intake (as energy percentage) at 1 year than those who were not
overweight (<90th percentile). The protein intakes at 12 months were very
high, 22 and 20% of energy (equivalent to 5.5 and 5 PER) in both the over-
weight and the non-overweight groups, respectively.

On the other hand, Dorosty et al. [21], who attempted to reproduce the
results of the relatively small study of Rolland-Cachera et al. [19], followed a
cohort of 889 British children born in 1991 and 1992 from birth through 5
years. Ten anthropometric measurements were performed in 5 years and two
3-day dietary records were taken at 8 and 18 months of age. Dietary proteins
as percent of energy intake were on average 14 ± 2% (3.5 ± 0.5 PER) at 18
months in each of the three categories in which the participants were subdi-
vided on the basis of age at adiposity rebound (very early, early, later). Only
parental body mass index or obesity were significantly correlated to an early
adiposity rebound. In a Danish cohort, protein intakes averaging 13 (females)
to 14% (males) of energy intake (PER around 3.5) were not associated with
the percentage of body fat at 10 years of age [22]. Similar findings have been
reported in children treated for hyper-phenylalaninemic syndromes (classic
and mild forms), who showed an association between overweight at age 8
years and early adiposity rebound, but no associations with early protein
intakes [23]. Also in this case the average protein intake in the mild form
(with the higher protein supply) averaged 14% of the daily energy intake (3.5
and overweight was more likely in children with, rather than without, parental overweight.

**Biologic Plausibility of the Protein Hypothesis:**
**Role of Quantity and Quality**

Commenting on the results from the study by Dorosty et al. [21], Rolland-Cachera et al. [24] have underlined that, in spite of the contrasting results on proteins, a common finding is represented by the lack of any relationship between earlier energy and fat intakes and age at adiposity rebound. Indeed, human milk, the reference food, contains a low proportion of protein (around 7% energy) and a high proportion of fat (around 50% energy). Accordingly, a rapid metabolic adaptation to low fat intakes in the complementary feeding period would make the child unprepared to face a high-fat diet later on [24].

The first explanations on the high protein–early adiposity rebound association were almost speculative and hypothesized a possible stimulating effect of dietary proteins on insulin-like growth factor-1 (IGF-1) [25], inducing both adipocyte differentiation and adipogenesis [26]. Accepting this hypothesis, we could speculate that adipocytes, differentiated early and over-stimulated, would be more prone to be filled through the years by fats deriving (either directly or after endogenous synthesis) from the high energy, high saturated fat diets commonly reported in Western children. Then, according to our previous observations, the critical point would be represented by the dietary unbalance at 8–24 months of age, when dietary proteins approaching 4–5 g/kg weight/day (around 16–20% of total energy intake, PER = 4–5) are associated with later overweight [8], while with dietary proteins below 14% energy an association is not found.

A Danish group has recently published a series of reports partly expanding these hypotheses. At 2.5 years of age the serum IGF-1 concentration of 90 Danish children was positively associated with intakes of animal proteins and milk, but not with those of vegetable proteins or meat [27]. Height was also associated with serum IGF-1 and the intakes of animal proteins and milk. The 90th percentile of protein intake in this study was 4.0 g/kg/day (around 16% of energy, PER = 4), and presumably this level matched the maximum effect on IGF-1 secretion. Further randomized studies in 8-year-old children have shown that 7 days of increased protein intakes (13–20% of the daily energy, 3.2–5 PER) by additional milk supply (but not meat) increased serum IGF-1 by around 20% [28] and fasting serum insulin levels by 100% [29]. These observations, while raising debate on the possible long-term effects in older children, put more light on the mechanisms possibly linking early over-intake of proteins (particularly from milk and dairy products), synthesis of endocrine mediators, age at adiposity rebound and later overweight.
Solutions for the high PER levels are in line, but opposed to those for malnourished children: first of all, supporting breastfeeding at least up to 12 months in order to balance not just the excess protein but also balance the other macronutrients and micronutrients; then using less protein-dense weaning foods (with a PER ideally ranging 2–3), and finally introducing an appropriate formula with ‘adjusted’ PERs when human milk is insufficient. Within this context, producers and regulatory bodies should consider the opportunity to further modulate the PER level of follow-on formulae, to counteract the apparently unavoidable trend to a high protein, high PER diet. According to recent reports, follow-on formulae with 1.3 g/l protein and 67 kcal/100 ml (with a PER close to 2) are adequate for diets in Swedish infants [30, 31]. The protein content and PER levels of follow-on formulae could be tailored further according to dietary habits differing in usual weaning foods [32].

Conclusions

Protein intakes should be maintained in the safe range of 8–12% calories (equivalent to 2–3 PER) with a diet adequate in energy and balanced for both macronutrients and micronutrients. In planning interventions, the coverage of infants’ dietary needs through all the various world regions should be considered according to local traditions, food availability and the socioeconomic backgrounds. The maintenance of breastfeeding during the introduction of solids may help to modulate the trend towards excessive dietary proteins in developed countries and the declining protein quality in the case of poorer countries. In this case, a dietary PER ranging from 1.5 to 2 could be acceptable for providing adequate energy, for the high efficiency of human milk protein utilization. For developed countries, a reassessment of the composition of complementary foods, and also follow-on formulae in the case human milk is lacking, should be considered to prevent the overload of dietary proteins. Available data suggest that above the limit of 14% energy derived from dietary proteins (3.5 PER) in the 8- to 24-month period, some mechanisms may begin to operate, beyond, or unmasking, a genetic predisposition towards the phenomenon of early adiposity rebound and overweight development.

Finally, it is tempting to speculate that differences in early feeding habits (breastfeeding vs. formula feeding) could be associated with different schedules for the introduction of solids, and differences in suggested PER values of the complementary foods.

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Complementary Food

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Agostoni/Riva/Giovannini


Discussion

Dr. Haschke: I was most interested in your studies. I think for the work from India that you cited at the beginning, and as far as I understood they were evaluating the effect of the so-called low and high energy density supplementary foods in relation to breast milk intake, the outcome was that there was no effect. But it is my understanding that an energy density of 35 kcal/100 g is not a high-density complementary food. So the question would be: if you were to take 100 kcal/100 g, which would be really energy dense, whether there would be an influence? So were the results influenced by the study design?

Dr. Agostoni: These two studies [1, 2] have been thoroughly discussed within the editorial committee of the Journal of Pediatric Gastroenterology and Nutrition, and we finally decided to publish them due to the clear differences in intakes on the short-term (24 h), even if data on the medium or long-term effects were lacking. Nevertheless, it could represent a good starting point to consider the effects of complementary feeding on breastfeeding for developing (or better, ‘transition’) countries. Perhaps Dr. Dewey could add some information from their previous studies in Central America.

Dr. Pencharz: What you have described is really important in infancy which is great, and we have actually done some work in children with cystic fibrosis (CF). But because they are compelled to thrive, the question is how to approach that. So we did one relatively long-term study [3] published a number of years ago where the children were under the classical fat-restricted diet. We followed them for a whole year because children grow at different rates at different times of the year, and then we gave them basically high caloric milk shakes made from grocery foods of 1 cal/ml or 100 cal/100 ml, and they grew faster. In that undernourished group who were taking a low fat feeding, we were able to get them to gain weight. Working later at the Hospital for Sick Children in Toronto we did a study which we called the boost study [4], that is a proprietary product which is 1 cal/ml which can be bought from the pharmacy in North America. Quite classically undernourished CF children in clinic would be given this boost, and all we found when we studied it was that it replaced food. In the clinic this pharmacological product boost, which was high in fat, in other words about 35% of calories as fat, and is complete nutrition, just displaced food. It was only when we went to tube feeding at night that we were able to increase the total calories in 24 h.

Dr. Agostoni: I am really now interested in CF because it is a very challenging model. I think that for CF infants we now are on the way to change some older beliefs, as you already underlined. First of all, there are very good examples of the functional benefits of human milk [5]. We have unpublished data showing that prolonged breastfeeding in CF patients, regardless of the genetic form of the disease, is associated with a 20% improvement in lung function years later. Once human milk was suggested not
to be good for CF, but today we know that it is also a good source of n-3 long-chain polyunsaturated fatty acids [6] that may be beneficial for CF patients [7]. Regarding weaning in breastfed CF patients, we also have some preliminary indications [unpublished] that those who got better are those who received complementary food earlier, before 6 months, perhaps preventing some marginal form of undernutrition, in association with their increased demand.

**Dr. Dewey:** I want to come back to the energy density issue. Our study that you were referring to was comparing intakes of infants whose mothers' milk had different fat contents and therefore different energy density. The infants were quite able to compensate for that by changing the volume that was consumed, which is consistent with what Dr. Ziegler was describing for formula-fed infants in those early studies. But I think when it comes to complementary foods we still don't completely understand the consequences of different energy density of complementary foods on intake. My colleague Ken Brown has done quite a few studies on this, most of them with non-breastfed children. In that case, increasing energy density of the foods does tend to increase the overall energy intake, even the volume consumed decreases a little bit. But in breastfed infants there is very little information. The study you showed from India was for only 24h. There is a small study in Bangladesh [8] which used a crossover design with higher and lower energy density complementary foods. The higher energy density did increase the total energy intake but when the children in that group crossed back over to the lower energy density diet, we expected that the breast milk intakes would respond by going back up, and that didn’t occur. I think we certainly need to keep in mind that although lactation is probably very flexible in early infancy we are not sure that it is quite as flexible later on. If you have a reduction in breast milk intake you may not be able to rebound quite as easily as when the baby is young. So adding fat or increasing the intake from other foods may permanently reduce breast milk intake, which may not be a desirable consequence.

**Dr. Agostoni:** Do you mean in the second part of the first year of life, for example?

**Dr. Dewey:** Yes, after 6 months, but I would like to ask a question about the studies that you mentioned on the relationship between breastfeeding and child obesity. You mentioned the most recent meta-analysis by Owen et al. [9] and I want to make two points about that. It is very different from the other meta-analyses because it is on the mean body mass index, not the percentage overweight, and that is important because breastfeeding may be affecting both ends of the distribution, both the percentage overweight and the percentage underweight. If that is true then the mean body mass index may not change at all even if you have a reduction in the percentage overweight. The other point is that they group all the ages together in terms of when body mass index was measured later on. From my reading of the literature the relationship between breastfeeding and later obesity is strongest when you look at overweight between, for example, 6 and 14 years of age. The relationship is not very clear in early childhood and it is not very clear after about 18 years. So by putting the age groups all together I think you dilute any relationship.

**Dr. Agostoni:** First of all, I would like to take the opportunity to give my opinion. Yesterday I mentioned that there are glutamine believers and glutamine non-believers. We can say the same about breastfeeding; we are humans and therefore have our opinions, and it is sometimes difficult to adjust for this. The second point, you mentioned correctly that the preventive effects of breastfeeding on obesity are more evident in the first 6–14 years. Some years ago in the New England Journal of Medicine, there was a paper showing that overweight in adolescence predicted a broad range of adverse health effects that were independent of adult weight after 55 years of follow-up [10]. Even if we still do not know when overweight has its major negative impact on the later outcome, a prevention of overweight in adolescence could also have relevant preventive effects.
Dr. Barclay: I have a question about the protein-energy ratio in complementary foods. For rural regions in developing countries, if I understood you correctly, you said that there is a risk of protein deficiency during weaning. You showed on one of your slides that the minimum protein-energy ratio required during weaning is about 6–7%. Now if you look at the protein-energy ratio of cereal-based diets, rice has a protein-energy ratio of about 7–8% and cereals such as wheat about 12–15%. A large multicentric study in Mexico, Egypt and Kenya published by Beaton et al. [11] in the early 1990s showed that for young children consuming cereal-based diets, protein deficiency was unlikely as long as the energy requirements were covered, since the protein-energy ratio of the diet would be at least 8–12%. Although the protein requirements could be somewhat higher in these regions due to higher infection rates, can we conclude that there is a risk of protein deficiency?

Dr. Agostoni: This is a complex question. First of all I was referring to a mixed diet, including animal and vegetal proteins. Moreover, reaching the correct limits of energy requirements could also be positive with regard to the nitrogenous balance, since the re-utilization rate could be more efficient. Third, I would like to emphasize that when you add minimal amounts of animal protein, as a source of essential amino acids, you automatically improve the nutritional efficiency of vegetable proteins, because you also supply some essential amino acids otherwise limiting if you just supply vegetal proteins. The present indications for developing countries are to find local solutions combining some animal sources of ‘exceeding’ essential amino acids together with staple cereals. It is a matter of present research to optimize interventions in this way; it is not simply the question to increase the protein-energy ratio of foods.

Dr. Axelsson: You showed a slide about the recommendations for Italian infants on how to introduce different complementary feedings. We start with human milk and standard infant formula, and then when we have standard infant formula we try to make it similar to human milk. Then why do we adapt it to cows’ milk, and why do we need long-term formula?

Dr. Agostoni: There are some discrepancies between expert opinions and the political conclusions from regulatory bodies. My personal opinion would be to put much effort into the formula for the first weeks of life, to plan a sort of ‘functional formula’ very rich in bioactive compounds, followed by a different formula for the 4–6- to 52-week period, reduced in proteins compared to actual standards. Human milk composition in Italy seems always to be the same, starting from 10–15 days of lactation up to 12 months of age, as we have shown for fat content and composition [6].

Dr. Axelsson: Since evolution we have been adapting; we were hunters. But why can’t we make formulas for the later part of infancy on meat?

Dr. Agostoni: It is interesting because this is a typical question from Sweden or Norway, also considering the changing genetic pattern with regard to lactose tolerance from the north to the south. On the evolutionary standpoint there are some basic questions still unanswered: who is the best human to adapt himself; the most intelligent; the most beautiful for reproduction? Perhaps those who were stronger adapted themselves to meat; those who were more pacifist raised cows; so we would speculate that what people have done in the evolutionary process represents the basis of some different dietary habits.

Dr. Roggero: I was impressed by one of your slides showing that insulin-like growth factor-1 (IGF-1) doesn’t increase when you give meat to the children. It is known that IGF-1 increases when the protein intake increases and, from the lectures of this meeting, it appears that IGF-1 is related to the level of proteins. Can you comment on that?

Dr. Agostoni: I can just comment on the work from Hoppe et al. [12] who didn’t find any association between insulin secretion and blood levels of branched chain amino
acids. This is the key point as leucine (the main branched chain amino acid) is a strong promoter of insulin secretion and also IGF-1. Perhaps the tertiary structure of proteins plays some role, or something else that we still don’t know. So we are still far from the explanation; we only have these preliminary, yet very interesting observations.

References

What Is the Optimal Age for Introduction of Complementary Foods?

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Abstract

In 2001, a WHO Expert Consultation concluded that waiting until 6 months to introduce complementary foods to breastfed infants confers several benefits for both infants and mothers. Nonetheless, there is still controversy about this issue. In developing countries, the reduced risk of infant gastrointestinal illness and increased duration of maternal lactational amenorrhea associated with exclusive breastfeeding for 6 months make the benefit-risk ratio of this recommendation highly favorable. In industrialized countries, the case is less clear-cut, but the benefit-risk ratio is also likely to be favorable with regard to infant infectious morbidity, motor development and maternal weight loss postpartum. For outcomes such as infant growth, food acceptance and iron or zinc status, the evidence for industrialized countries suggests no particular benefit but also very little risk of following this recommendation. Some exclusively breastfed infants may become iron- or zinc-deficient before 6 months, but this can be prevented more effectively by targeted iron and zinc supplementation to high-risk infants than by introducing complementary foods. On the whole, the evidence to date supports the WHO recommendation to introduce complementary foods at 6 months, but further research in industrialized countries would be useful.

Introduction

Complementary foods are defined as the foods that are provided along with breast milk [1]. In the past, such foods were often called ‘weaning foods’. However, the term ‘complementary foods’ is preferred because weaning implies the cessation of breastfeeding, whereas the goal is that such foods should complement breast milk, not replace it. In May 2001 the 54th World Health Assembly urged Member States to promote exclusive breastfeeding for 6 months as a global public health recommendation [2]. This recommendation
followed a report by a WHO Expert Consultation on the optimal duration of exclusive breastfeeding [3], which considered the results of a systematic review of the evidence [4]. The Expert Consultation concluded that waiting until 6 months to introduce complementary foods to breast-fed infants confers several benefits on the infant and the mother. Prior to 2001, the WHO recommendation had been to introduce complementary foods at ‘4–6 months’ of age, but by the time of the systematic review, enough evidence had accumulated to warrant changing the wording to ‘6 months’. The WHO recommendation is meant to apply to all countries, but its ramifications are probably greatest in developing countries. A large percentage of developing countries have now adopted a government policy in concordance with the WHO 2001 recommendation.

In several industrialized countries, however, there is a difference of opinion among professionals about whether to recommend 6 months or 4–6 months as the optimal age for introduction of complementary foods. For example, in the most recent American Academy of Pediatrics Pediatric Nutrition Handbook [5], both views are expressed. In the chapter on Complementary Feeding (p 108), the main text states, ‘It seems reasonable, therefore, to recommend that in developing countries, where the use of potentially contaminated and/or low-nutrient dense foods puts infants at risk for diarrhea and undernutrition, infants should be exclusively breast fed for 6 months. If this is not the case (either in developing or, in particular, developed countries), complementary foods may be introduced between ages 4 and 6 months. This is a population-based recommendation, and the timing of introduction of complementary foods for an individual infant may differ from this recommendation*.’ The asterisk refers to a footnote at the bottom of the page, which states ‘There is a difference of opinion among AAP experts on this matter. The Committee on Nutrition acknowledges that the Section on Breastfeeding recommends exclusive breastfeeding for at least 6 months’. In fact, the footnote has an error, and should have stated ‘recommends exclusive breastfeeding for about 6 months’, not ‘at least 6 months’ [Gartner L, personal commun.]. This error will be corrected in an erratum.

This lack of agreement among health professionals in some countries is in part due to the paucity of evidence on this issue from randomized trials in industrialized countries. In the systematic review conducted for WHO [4], studies were included if they were clinical trials or observational studies that compared outcomes between full-term infants and their mothers who had breast fed exclusively for ≥6 months (EBF) vs. those who had breast fed exclusively for at least 3 months, with continued mixed breastfeeding and complementary feeding until at least 6 months (MBF). The literature search identified 2,668 unique citations, but only 36 met the selection criteria for the review. These 36 citations were from 20 separate studies, of which 9 were carried out in developing countries and 11 in industrialized countries. However, of these 20 studies, only two were controlled clinical trials of EBF vs. MBF.
Both of these were conducted in Honduras by our research team [6–13]. In the first study, 141 low-income mother-infant pairs who were exclusively breastfeeding at 4 months postpartum were randomly assigned to begin complementary foods at 4 months or continue exclusive breastfeeding until 6 months. Nutrient-rich, commercially prepared baby foods were provided in jars to the former group. The second study used a similar design but was restricted to low birth weight (but full-term) infants (n = 119). To date, there have been no published randomized trials of EBF vs. MBF in industrialized countries.

Several health outcomes may be influenced by the age at introduction of complementary foods, including infant growth, iron and zinc status, infectious morbidity, behavioral development and food acceptance, and maternal duration of lactational amenorrhea and the rate of postpartum weight loss. The following sections address each of these categories of outcome, with emphasis on studies relevant to comparing 4–6 months vs. 6 months as the optimal age for introduction of complementary foods.

**Infant Growth**

The authors of the systematic review concluded that there was no evidence for any deficit in weight or length gain among infants who are exclusively breast fed for 6 months compared to those given complementary foods [4]. This is probably because the energy provided by complementary foods largely displaces breast milk during the 4- to 6-month age interval. For example, in the first of our controlled trials in Honduras, breast milk intake decreased by \(\sim 100 \text{ g/day}\) in the group that received complementary foods and were breast fed ad libitum between 4 and 6 months, but was unchanged in the EBF group [6]. Similar results were found in the second controlled trial of term, low birth weight infants in Honduras [12]. In a previous observational study in the US, the breast milk intake of infants given complementary foods at 4–6 months was significantly lower than that of infants who were exclusively breast fed for 6 months, with the difference being \(\geq 150 \text{ ml/day}\) at both 6 and 9 months of age [14]. As a result, total energy intake (from both breast milk and complementary foods) and growth status did not differ significantly between these 2 groups at 6, 9 or 12 months.

Other observational studies in industrialized countries have also shown little or no growth difference between EBF and MBF infants [15–17]. For example, in a pooled analysis of data from 7 studies in North America and northern Europe, infants given complementary foods (but not formula) in addition to breast milk between 4 and 6 months (n = 122) did not differ from EBF infants (n = 200) in weight gain (936 ± 330 vs. 925 ± 283 g) or length gain (3.48 ± 0.96 vs. 3.44 ± 0.86 cm) during the 4- to 6-month interval, with or without controlling for initial size at 4 months [1]. In a large cohort study
nested within a randomized trial in Belarus, Kramer et al. [17] found that weight and length gain from 3–6 months were slightly greater in the 2,862 infants who were exclusively breast fed for 3 months and then mixed-fed through ≥6 months (MBF) than in the 621 infants who were exclusively breast fed for ≥6 months (EBF). However, the EBF group had greater length gain from 9 to 12 months and a larger head circumference at 12 months than the MBF group. In subsequent analyses of the same cohort, the MBF group was subdivided based on the types of complementary foods consumed: formula or other milks, cereals, juices or other liquids, and other solids. These analyses revealed that formula and other milks had a growth-accelerating effect on weight and length gain throughout infancy (a result that is consistent with the differences in growth observed between breast-fed and formula-fed infants [15]), whereas the intake of cereal at 3–6 months was associated with substantially lower weight, length and head circumference gain during that interval (z-score differences of –0.29, –0.24 and –0.29, respectively), compared to the EBF group [18]. Thus, the type of complementary food consumed appears to influence the nature of the growth response.

**Infant Iron and Zinc Status**

There is very little information on the effects of the age at introduction of complementary foods on iron or zinc status of breast-fed infants. These two nutrients have been identified as the most likely limiting nutrients among EBF infants during the first 6 months of life [19]. Although the adequacy of certain vitamins during the period of exclusive breastfeeding may also be of concern, depending on maternal diet and nutritional status, these concerns can generally be addressed by assuring that the mother's intake is adequate (e.g. for vitamin A, B6, or B12). By contrast, the concentrations of iron and zinc in human milk are not altered by maternal supplementation.

During the first 6 months, infant iron status is largely dependent on iron stores at birth, which are influenced by gestational age, birth weight, maternal prenatal iron status, and the timing of clamping of the umbilical cord. After birth, infant iron needs are influenced by the rate of growth and certain types of infections. Thus, although full-term, normal birth weight infants whose mothers had adequate prenatal iron status can generally maintain adequate iron status through ≥6 months of exclusive breastfeeding, certain subgroups of infants may be at risk of iron deficiency prior to 6 months.

In the first controlled trial in Honduras, very few of the EBF infants with a birth weight of >3 kg had low hemoglobin (5% < 103 g/l) or plasma ferritin (0% < 12 μg/l) at 6 months of age, but in those with a birth weight of <3 kg, the EBF infants were at higher risk of iron deficiency than the infants who received complementary foods (~49 vs. 27% for low hemoglobin; ~26 vs. 10% for low ferritin) [10]. This is not surprising given that the complementary
foods were fortified with ferrous sulfate. However, the provision of free iron-fortified complementary foods during the age interval of 4–6 months did not eliminate iron deficiency at 6 months. Evidence from a separate study of iron supplementation in the same population [20] indicates that iron supplements given to high-risk infants are likely to be more efficacious for preventing iron deficiency than feeding iron-fortified complementary foods prior to the age of 6 months. In the second Honduras trial, with term, low birth weight infants, there was a significant interaction effect between the provision of complementary foods and iron supplementation. Among infants not given medicinal iron drops, iron status was higher in the group given iron-fortified complementary foods than in the EBF group. However, in those given medicinal iron drops, iron status was higher in the EBF group, suggesting that complementary foods interfered with iron utilization [11]. Given the recommendation that low birth weight infants should receive iron supplements beginning in early infancy, these results suggest that exclusive breastfeeding for 6 months, together with iron supplementation, is likely to optimize iron status for such infants.

There is a paucity of data on the effects of age at introduction of complementary foods on the iron status of breast-fed infants in other countries. In a small observational study of breast-fed infants in Italy [21], the EBF group had significantly higher hemoglobin concentration than the MBF group (117 vs. 109 g/l) at 12 months of age. Although iron-fortified cereals are usually one of the first complementary foods given to infants in the US, the bioavailability of the electrolytic iron typically used in such foods is estimated to be quite low [22]. Among the breast-fed cohort (n = 173) in an intervention trial in Chile in which infants were randomly assigned to receive iron-fortified (55 mg electrolytic iron/100 g dry cereal) or unfortified rice cereal at 4 months [23], there was no significant difference in iron status (e.g. serum ferritin) between groups at 8 months of age, but by 12 months the group receiving unfortified cereal had a lower iron status and was more likely to be anemic (10.8 vs. 1.4%). This suggests that the iron in the cereal was absorbed, but that the breast-fed infants did not benefit from the extra iron until after 8 months of age.

As is the case for iron, the zinc concentration of human milk is relatively low, and it is thought that low stores of zinc at birth may predispose certain subgroups of infants to zinc deficiency [24]. In disadvantaged populations, zinc supplementation during infancy has generally had positive effects on growth and morbidity [25]. However, in the Honduras study of low birth weight infants, there was no impact of complementary foods on plasma zinc, even though the mean zinc intake of infants in the complementary foods group was twice that of the EBF group [11]. In a randomized trial in the US in which infants at 5 months of age were given either beef or iron-fortified rice cereal as the first complementary food [24], no effect on growth, development or biochemical indices of iron or zinc status at 9 months was observed despite significant differences in zinc intake at 5–7 months. Thus, there is no evidence
that complementary feeding prior to 6 months would enhance zinc status. High-risk infants, however, may benefit from zinc supplementation.

**Infant Infectious Morbidity**

Observational studies in developing countries show a much higher risk of diarrhea in breast-fed infants exposed to complementary foods at 4–6 months than in those who were exclusively breast fed [1]. This difference was not observed in the two controlled trials in Honduras [6, 12], presumably because the complementary foods were provided in sealed jars and leftovers were discarded after use, thus eliminating the risk of bacterial contamination. In industrialized countries, the study in Belarus demonstrated a significantly lower risk of gastrointestinal infection during the first year of life in the EBF group than in the MBF group (adjusted incidence density ratio 0.35 (0.13, 0.96)), even though the overall rate of gastrointestinal infection was very low [17]. Pooled results from studies in Australia [26], Arizona [27] and Belarus [17] showed no significant differences between EBF and MBF groups in the risk of upper or lower respiratory infection or otitis media [4]. However, a recent analysis of data from a US national survey indicated a reduced risk of respiratory infection in infants who were fully breast fed for 6 vs. 4 months [28], though it is unclear what percentage of infants in the latter group continued to be breast fed (while receiving complementary foods) through 6 months.

**Infant Behavioral Development**

Motor development was assessed in the two controlled trials in Honduras. In both studies, infants in the EBF group crawled at an earlier age than infants in the group given complementary foods (6.3 vs. 7.3 months in the first study; 6.8 vs. 7.4 months in the second study) [13]. In the first study (but not in the second study), infants in the EBF group were also more likely to be walking by 12 months of age (60 vs. 39%, p = 0.02). The mechanism by which exclusive breastfeeding during the 4- to 6-month age interval might affect motor development is unknown. Certain constituents of breast milk (e.g. docosahexaenoic acid) are known to be associated with infant mental development, but there is little evidence that they affect motor development. On the other hand, Vestergaard et al. [29] reported that achievement of two motor skills (crawling and pincer grip) was linked to the duration of breastfeeding in a large sample of Danish infants, even after adjustment for potentially confounding variables. Thus, it is possible that a greater consumption of breast milk by EBF infants could contribute to enhanced motor development. To date there have been no published studies evaluating the effect of age at introduction of complementary foods on infant cognitive development.
**Infant Food Acceptance**

Some parents and health care providers believe that there is a ‘critical age’ for infants to be introduced to complementary foods, and that waiting too long will interfere with the infant’s acceptance of foods at a later age. This is linked to the notion of developmental ‘readiness’, an ill-defined concept that nonetheless has a strong influence on parental behavior. In an effort to address this concern, our first study in Honduras included an assessment of infant dietary intake at 9 and 12 months, as well as the mother’s report of infant acceptance of 20 commonly consumed foods [8]. There were no significant differences between intervention groups in breastfeeding frequency, amount or number of foods consumed, percentage of food offered that was consumed, usual daily number of meals and snacks, number of food groups consumed, or overall food acceptance score. In our study of breast-fed infants in the US [14], 4-day weighed intake records of all foods and fluids (including breast milk) were completed every 3 months. Infants in the EBF group had significantly higher intake of breast milk and lower intake of energy from complementary foods at 9 months, compared to those introduced to complementary foods before 6 months, but the differences were no longer significant at 12 months of age. As stated above, there were no significant differences between groups in total energy intake at any age. Thus, delaying the introduction of complementary foods until 6 months does not appear to adversely affect subsequent infant appetite or food acceptance.

**Maternal Duration of Lactational Amenorrhea**

In the systematic review conducted for WHO [4], the authors concluded that exclusive breastfeeding through 6 months is associated with delayed resumption of menses, which can promote more optimal birth spacing in populations with relatively low rates of contraceptive use. Their conclusion was based on the results of the two Honduras trials. The difference in the percentage of mothers who were amenorrheic at 6 months postpartum was not statistically significant in the first trial (though in the expected direction), but was significant in the second trial (89% in the EBF group vs. 68% in the MBF group, p = 0.02) [13].

**Maternal Weight Change Postpartum**

Maternal weight change was also assessed in the two Honduras studies [13]. Maternal weight loss between 4 and 6 months was significantly greater in the EBF group than in the complementary feeding group in the first study (−0.7 ± 1.5 vs. −0.1 ± 1.7 kg, p < 0.05), but not in the second study. The dif-
ference between the two studies is probably related to the fact that the net difference between intervention groups in milk volume, and thus in maternal energy demand, was larger in the first study than in the second. In fact, the estimated additional energy burden of exclusive breastfeeding during the 4- to 6-month interval was in close agreement with the between-group weight difference in both studies (assuming that the weight lost was nearly all fat). Thus, the results support the conclusion that the degree of breastfeeding during this time period affects the rate of maternal weight loss. It should be noted that only about 10% of the mothers in the Honduras studies had a low body mass index (<19 kg/m²), so these results may also apply to women in industrialized countries. In observational studies, the effect of lactation on maternal weight loss is most evident between 3 and 6 months postpartum, when breast milk volume is still high but prolactin levels (which are thought to stimulate appetite) are lower than they are in early lactation [30]. Given the high rates of obesity among women in many countries, promotion of exclusive breastfeeding for 6 months may thus be beneficial to maternal health.

Conclusions

The evidence to date supports the WHO recommendation to introduce complementary foods at 6 months. In developing countries, the reduced risk of infant gastrointestinal illness and increased duration of maternal lactational amenorrhea associated with exclusive breastfeeding for 6 months make the benefit-risk ratio of this recommendation highly favorable. In industrialized countries, the case is less clear-cut, but the benefit-risk ratio is also likely to be favorable. In these settings, waiting until 6 months to introduce complementary foods is likely to be of benefit with regard to outcomes such as infectious morbidity, motor development and maternal weight loss postpartum. With regard to other outcomes, such as infant growth, food acceptance and iron or zinc status, the evidence suggests no particular benefit but also very little risk of following this recommendation. Some EBF infants may become iron or zinc deficient before 6 months, but this can be prevented more effectively by targeted iron and zinc supplementation to high-risk infants than by introducing complementary foods. Further research is needed to document the impact of age at introduction of complementary foods on infants in industrialized countries.

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What Is the Optimal Age for Introduction of Complementary Foods?

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Discussion

Dr. Dewey: With regard to the effects of iron supplements, Dr. Lönnerdal, Dr. Hernell and I conducted a joint study [1]. The two countries studied were Sweden and Honduras, with the goal being to have a very wide range in iron status of the infants. It was a double-blind randomized controlled trial of babies who were exclusively breastfed for 6 months and then given other foods while continuing to be breastfed until 9 months or longer. They were randomized to receive iron from 4 to 9 or from 6 to 9 months of age, with a placebo from 4 to 6 months or from 4 to 9 months of age. We were interested in both the 4- to 6- and the 6- to 9-month intervals. We found that in Honduras, where iron deficiency is common, iron supplementation had a beneficial effect on iron status. In Sweden, where iron deficiency is not common, there was no impact on the already low rates of iron deficiency anemia. Giving iron supplements to babies who had normal hemoglobin levels at 4 months of age had an adverse effect on linear growth and it also increased the risk of diarrheal morbidity. Even in Honduras, where most of the babies were at a greater risk of iron deficiency, we found the same thing among those who had normal hemoglobin. Thus there was an interaction effect between the initial iron status of the infant and the effect of the supplement: beneficial for those who need it but risky for those who don’t need it. That is why we don’t recommend routine iron supplementation to breastfed infants.

Dr. Turck: I have two questions. The first is related to the Kramer study in Belarus. You said that infants exclusively breastfed for at least 6 months had a larger head circumference at 12 months of age, which is very interesting. Could you comment on that further? The second question is related to the allergy issue. Obviously the literature is controversial in that. Could you give us your own opinion; how do you feel on the issue of the optimal time of complementary feeding in infants at risk?

Dr. Dewey: Those are both very good questions. For the first one, the head circumference data were analyzed as an observational study [2]. In other words, even though it was a randomized trial of the baby friendly hospital initiative, the growth data were examined by feeding mode controlling for socioeconomic status, educational level and several other variables. We have not found differences in our own population in head circumference between breastfed and formula-fed infants, but in the Belarus study they had a very large sample size, more than 17,000 infants. So it is probably a very small difference in head circumference that they detected. Whether that is biologically meaningful, I don’t know. Regarding the second question on allergy,
I am going to ask others in the audience to comment. I think the allergy area is so complicated that it is difficult to know where things stand in terms of duration of exclusive or any breastfeeding because of the potential exposure both in utero as well as from breast milk and other foods. I would like Dr. Hernell to talk a little bit about some issues regarding the age at introduction of complementary foods and celiac disease, because that is one issue that has come up recently.

**Dr. Hernell:** We have studied the effect of breastfeeding on the introduction of gluten with respect to the risk of developing celiac disease [3]. In the case referent study that we did in Sweden, we found that the most beneficial way of introducing gluten was to introduce it in small amounts and gradually, while the mother is still breastfeeding. With respect to the question whether there is a particular age window when it is more favorable to introduce gluten, the results are conflicting. We found that it was perhaps a greater risk to introduce gluten-containing foods between 4 and 6 months than before or after that age. However, age was not found to be an independent risk factor in multivariate analysis adjusting for breastfeeding and the amount of gluten. There are two other more recent studies on the development of celiac disease in infants with heredity for diabetes mellitus type-1. With respect to age at introduction being a risk factor, these two studies come to different conclusions. Hence, I don't think that we know whether there is a particular age when it is beneficial to introduce gluten. For the moment we can conclude that more important than the age at introduction is that the introduction occurs under the umbrella of breastfeeding. The question arises if, for one reason or another, a mother decides to stop breastfeeding before 6 months, the recommended duration of exclusive breastfeeding, should we recommend that the mother introduce gluten-containing foods before she stops breastfeeding? I believe it is routine in most cultures to introduce other foods during breastfeeding. Whether or not that is beneficial also in terms of developing oral tolerance to other food antigens than gluten is an unsolved question. There are very few studies that have addressed this question.

**Dr. Lafeber:** What always puzzles me in this type of study is how the randomization has been organized because it is difficult to have a good randomized control trial when dealing with the implementation of breastfeeding followed by weaning foods. How sure are you about the fact that the groups have been randomly divided regarding the introduction of complementary foods?

**Dr. Dewey:** Of course the studies in Honduras could not be double-blind because the mothers obviously knew that they were feeding other foods to their babies. I will say though that the mothers were not aware of what our objectives were or whether we had any hypothesis about what the outcomes would be. The two groups were not significantly different in any variables that would have affected the outcomes. In terms of adherence to their assignments, we had virtually 100% adherence in the solid foods group because they were given the foods free of charge. We did have a few mothers in the group assigned to exclusive breastfeeding who didn't necessarily completely adhere to that, but they gave very small amounts of food so that was not a major issue.

**Dr. Giovannini:** Do you have any data on 1-year lactational amenorrhea and on nipple erosion in this period? In some countries there is a risk of nipple erosion and infection due to hygiene problems. Have you any data about this from 12 to 24 months?

**Dr. Dewey:** For the first question, the data that I presented were for lactational amenorrhea at 6 months postpartum, when there was a significant difference. The difference was no longer significant at 12 months, which is not too surprising because we were looking for what happened right after that period of 4–6 months, and there are many other influences on what is going to happen after that. You could ask if there is any longer term consequence of sustaining amenorrhea a little bit longer during that period.
In any case this is what we found. I have not heard of nipple erosion as a problem in the populations that I have worked with. In most cases breast milk has some anti-infective properties that usually protect the mother’s nipple as well. Unless there is some underlying immune disorder I would think it would be relatively uncommon. We do have problems with yeast infection but that is more common in industrialized countries to my knowledge than it is elsewhere.

Dr. Telmesani: I always tell the mothers that when Eve came to earth she didn't have anything but her breast milk. I believe your talk is in keeping with nature. You mentioned that in areas where there is not enough sun, vitamin D needs to be added. In Saudi Arabia we have plenty of sun but occasionally we see rickets because people there are afraid of the sun and tend to cover their babies to protect them, and so they get rickets. I think this is an issue that needs to be taken into consideration in certain societies where people think that the sun is harmful.

Dr. Dewey: I agree with you. I think the phrase I used was insufficient exposure to sunlight, not necessarily insufficient sunlight. Certainly in cultures where people are covered or use sunscreen they will not get adequate vitamin D conversion in the skin, and that is why the American Academy of Pediatrics recently recommended vitamin D supplementation for breastfed infants, beginning around 2 months of age. It is an issue that developing countries really have to struggle with because that is a difficult recommendation to implement in countries with poor resources. I think one has to evaluate very carefully whether sunlight exposure is or is not adequate in those places.

Dr. Pencharz: You fully persuaded me and provided very good data that support the WHO recommendation. I work at the Hospital for Sick Children where the first precooked infant cereals were developed by Tisdale, Drake and Robertson. At that time infant mortality in Toronto was something like 100 per 1,000 live births, and the professor of pediatrics took on these researchers to develop a precooked infant cereal with added iron and vitamins to reduce infant mortality rates. In fact they did reduce the mortality rates down to about 17–20 per 1,000 live births. So you told us when, I rather now challenge you but it wasn’t your mandate to say what should we be dealing with complementary foods from 6 months on.

Dr. Dewey: Let me refer you to some documents that have been published. First there was a book published by the WHO in 1998 [4] and then an update to the book and a document published in 2003 [5]. The latter was meant for health care workers. There are 10 guidelines and for each of them there is a scientific rationale. Several of those deal with what types of food should be given, with particular emphasis on nutritional quality. The most limiting nutrients in most populations are iron, zinc, vitamin B6, possibly calcium and some other B vitamins. One of the conclusions is that animal source foods need to be a part of the diet as much as possible because they are the richer sources of those nutrients. If that is not possible or the amounts consumed are too small, then some sort of fortified product or some sort of supplementation is recommended. We have been doing several studies on various strategies to achieve that, either with fortified foods or with micronutrient supplements that can be added to food in the home. You are familiar with Sprinkles which Dr. Zlotkin developed, and there are other products as well. I think Dr. Desjeux is going to talk about a fat-based product developed in France that is very promising. We used it recently in a randomized trial in Ghana and it had very positive effects on infant growth and motor development. So I think we are moving forward in terms of how we approach that issue.

Dr. Butte: I want to emphasize one point that you made from WHO statement, which I think is very important, that the recommendation for exclusive breastfeeding to 6 months is a public health recommendation at the population level. You alluded to but didn’t show the list of warnings that we also put in our report that it doesn’t preclude growth monitoring at the individual child. So the recommendation at the clinical setting is different and we can’t lose sight of that. Do you have any experience with
iron drops in the clinical setting in the developing world, and is there a potential for misuse in illiterate populations?

Dr. Dewey: Let me comment on your first point and then answer the second. I think it is important to emphasize that on the individual level one always has to monitor whether exclusive breastfeeding is working. In my experience when there are problems with exclusive breastfeeding they usually occur early on, in the first few months of life, and it is not so common that a mother would breastfeed exclusively for 4 months and then have a problem with sustaining exclusive breastfeeding. Even mothers who are moderately malnourished tend to be able to continue to produce an adequate amount of milk. But I do agree that growth monitoring is important and I want to mention the new growth charts that are going to be put out next year, which were developed by the WHO based on children who were breastfed. To answer the second question, we did give iron supplements using drops in the trial in Honduras. We were worried about either misuse or toxicity, but we didn't have any problems at all. I was pleased that they used them as instructed and we had no accidents or poisonings. Now certainly it can happen and has happened, but I think the supplements are manufactured so that the dose one would get from an entire bottle would not be fatal. So I think they are safe products. The Sprinkles that I just alluded to are one possibility after the age of 6 months when you are giving other foods, but prior to 6 months we don't have too many options.

Dr. Margolis: The Canadian Pediatric Society went with the WHO recommendations, and my question relates to the American Academy of Pediatrics and practice in terms of what you presented and the clear lack of evidence in developed countries. Could you comment on why the American Academy of Pediatrics did what it did, and how do we look at the changes based on the fact that there is no good evidence contrary to using exclusive breastfeeding to 6 months?

Dr. Dewey: I am not sure I can comment on the American Academy of Pediatrics document, as I am not a member of either of those committees. In the US there is a long history of recommending 4–6 months and we have a large government program, the Women, Infants and Children Nutrition Program, that has been doing that for decades. So I think there is a lot of inertia around making a change and I think the attitude is that until there is really strong evidence to change it, they would rather stick with what they had before.

Dr. Baker: I am in the committee so I will try to make a comment. We had exactly the same literature that you had to review and we came up with different conclusions. There just didn't seem to be a significant amount of negative effects from introducing complementary feeding between 4 and 6 months. It seems as though there would be a subgroup of babies with definitely detrimental effects so that is why we came up with that recommendation of 4–6 months. It was argued long and hard and that is the reason we could not come to an agreement between breastfeeding group and the committee on nutrition. I would like to ask another question about your presentation. You presented a slide on the Honduras study where you showed that breastfeeding plus complementary feeding, the amount of breast milk went down in the mothers who were giving complementary foods, but stayed level in the mothers who were exclusively breastfeeding. At the same time those babies were growing significantly between 4 and 6 months. How do you explain that, because they weren't getting more breast milk during that time?

Dr. Dewey: We have also observed in the US and in other populations that the breast milk volume changes very little between around 2 and 6 months of age, even in exclusively breastfed infants. I think the reason is that growth becomes a lower and lower proportion of the energy requirements. I can't tell you the exact percentage at 6 months, but by that time you can grow normally with a small amount of additional energy. We have calculated whether they meet the new energy requirements that have
been published, and they were right on the mark. We have observed the same phe-
omenon in the US too, that when babies are given solid foods they reduce their
intake of breast milk and there is very little net increase in total energy intake. That
has been observed in Texas as well. What is interesting though is that when formula-
fed infants were given solid foods, they did not reduce their formula intake, which is
very different from breastfed infants. I think this raises some questions about the self-
regulation of energy intake in the formula-fed group.

Dr. Macé: In the case of insufficient delivery of human milk, what will be your rec-
ommendation, to complement with infant formula or complementary foods?

Dr. Dewey: What I would say is when there is a problem, first try to understand
why there appears to be a low transfer of breast milk, and work with the mother to
resolve that problem. If there is no resolution after doing that (without waiting too
long), before 6 months I would recommend supplementing with infant formula and
not with foods. The reason for that is that the nutrient composition of infant formula is
usually much better than the composition of most complementary foods. The findings
from the analysis by Kramer et al. [2] in Belarus showed that cereal intake at 3–6
months was associated with potentially adverse consequences. I hesitate to make too
much out of that because it was an observational study, but infant cereals in large
quantities are not really what human infants were designed to consume. In evolution-
ary times, mothers pre-masticated meats and other food such as nuts, to feed their
infants. Grains have only become a major part of our diet in the last 10,000 years or so.

Dr. Mohd Suhaimi Abdul Wahab: Do you think that in the future the recommen-
dation will go up to 9 months rather than 6 months?

Dr. Dewey: No I don't, and the reason is that after 6 months of age infants really do
need to get nutrients from other sources because by that time a larger and larger per-
centage of them will be running low on iron stores and probably also zinc. In addition,
giving foods is important for oral development and speech because moving food in the
mouth is part of normal oral development. From a practical point of view, even if you
wanted to delay giving other foods to a baby beyond 6 months, it is really hard to do
that because then they start grabbing and putting things in their mouths. So I don't
see much of push to go beyond 6 months.

Dr. Hilmanto: Do you recommend giving iron supplements in the early infancy for
low birth weight infants even though they are being exclusively breastfeeding for 6
months? If yes, when do you recommend to start giving iron supplements?

Dr. Dewey: I will do my best on that, but there may be other people in the room
who would also like to answer. There is a WHO recommendation to give iron supple-
cements to low birth weight infants beginning at 1–2 months of age. There is good evi-
dence that low birth weight infants start running low on iron stores by around that
time. The definition of exclusive breastfeeding that the WHO uses includes the use of
vitamin and mineral supplements as needed. So when you are giving iron supplements
to babies who are getting only breast milk, they can still be considered exclusively
breastfed. In terms of when exactly to begin those supplements, I don't think there is
enough research right now to know the answer to that question. Perhaps Dr. Hernell
would like to comment.

Dr. Hernell: There is a study going on in Sweden right now on preterm infants and
iron supplement, a control trial. There is no information as to when you should start,
how much iron should be given, and for how long.

Dr. Bozo: I have a comment about the age at introduction of solid foods. For exam-
ple, in developing countries a real problem is the reaction of the family to the program
of introduction of solid foods. At 6 months of age we have to start introducing nutri-
ents fortified with iron, zinc, etc., but we cannot be sure that the family will comply
when we make this recommendation. There could be a risk of developing zinc defi-
ciency and iron deficiency if the age at introduction of solid foods is delayed.
Dr. Dewey: As I indicated I think that one of the outstanding issues is how to identify which exclusively breastfed infants might be at risk of iron deficiency before 6 months of age. My recommendation would be that if a baby is a full-term normal weight breastfed baby whose mother did not have iron deficiency during pregnancy, you would not need to worry about that. Otherwise I personally would recommend doing assessments at perhaps 4 months of age to determine if the baby has low iron status at that time. But as I mentioned I am not sure providing foods to them at that point would solve the problem even if they do have iron deficiency, because we still had a substantial percentage in Honduras who were iron-deficient at 6 months despite getting iron-fortified foods. But I think in terms of iron supplementation some sort of testing for those who might be at high risk would be worthwhile.

References

Abstract

Thirty years ago, protein deficiency was perceived to be the major nutritional problem of children in developing countries. Later on increasing the energy intake of young children during the complementary feeding period became a priority. Early studies on the pathophysiology of malnutrition are now turned into strategic and practical consequences for the prevention and treatment of severe malnutrition, four of which are presented. (1) Almost half of the deaths worldwide are due to being underweight. Nowadays, well-defined preventive and curative interventions have been identified. (2) An efficient and rigorous technique based on linear programming is now available to design a diet suitable for the complementary feeding period using locally available foods with a minimum budget to cover the nutritional requirements of at least 97% of the children. (3) Managing acute malnutrition in emergencies has greatly improved by the use of a spread that a child can eat directly without the addition of water (often called Ready-to-Use Therapeutic Food) and Community Therapeutic Care that treats the majority of severely malnourished children at home. (4) Recent data strongly suggest that it is possible to avoid death due to careful and rapid rehydration despite the high purging rate even if many of the risk factors for mortality are present in these severely malnourished children. Recovery from malnutrition was achieved in 7 days.

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the energy intake of young children during a complementary feeding period then became a priority. In the meantime, kwashiorkor and marasmus, the two main clinical forms of severe malnutrition, were found to be associated with a change in body composition: essentially loss of fat, muscle and cellular proteins; changes in body water distribution (increased extracellular but decreased intracellular water), and potassium and magnesium deficiency. It is remarkable that in the 1960s the Tropical Metabolism Research Unit in Jamaica obtained a small whole body counter with which total body potassium could be determined from the radiation of the natural isotope $^{40}\text{K}$. This is how it was firmly established that potassium deficiency is a common and important feature of energy-protein malnutrition [5–7]. Then trace element deficiencies, essentially vitamin A and zinc, were also recognized. Thus, energy-protein malnutrition is by far the most lethal form of malnutrition.

In addition to the deficit in food intake, other factors can also contribute to malnutrition. They could broadly be classified as medical and environmental. In the medical or nutritional category one can include: associated diseases which are essentially bacterial, viral or parasitic infections, and a selective deficit in nutrients in the daily monotonous diet. It is not uncommon that the food found in the local market does not provide all the nutrients required for child growth. Therefore a protein and energy deficit is often associated with a deficit in minerals and vitamins, and an imbalance between the nutrients is also common.

In the environmental category the list is long, including a lack of hygiene and clean water, overcrowding, illiteracy, poverty, an unstable political situation, and war. Malnutrition has been known to affect a large population of children throughout the history of humanity. In Europe, until the end of the 19th century, it was one of the leading causes of mortality, even though breastfeeding was the rule [8]. One of the most appalling accounts of malnutrition can be found in the celebrated lecture of Dickens who for the benefit of the Great Ormond Street Hospital described an Edinburgh slum [9]. In the rest of the world, the epidemic was later ‘discovered’ by Europeans.

The nutritional and medical aspects of infantile malnutrition have been studied in detail and presented in reference books [9–13] and journal articles in the case of famine [14, 15]. Furthermore, in 1999 the World Health Organization published a manual for physicians and health workers on the management of severe malnutrition [10].

The aim of this presentation is not to repeat what has already been communicated on infantile malnutrition, rather I would like to stress four recent issues of practical importance for preventing and treating malnutrition: (1) assessment of the magnitude of the problem; (2) assessment of the nutrients in food markets; (3) home-based therapy for malnutrition, and (4) rehydration therapy in dehydrated and malnourished children. It appears that the early studies on the pathophysiology of malnutrition have now turned into strategic and practical consequences for the prevention and treatment of severe malnutrition.
The Magnitude of the Problem

More than 10 million children younger than 5 years of age die every year. When facing such statistics it seems that malnutrition is beyond the nutritionists' reach. In the last 5 years an overall estimate of the determinants of child mortality has been published with specific targets identified [16, 17]. To summarize, it is notable that six countries account for 50% of worldwide deaths and 42 countries for 90%, this implies that national or regional policies should be implemented. Even if the causes of death differ substantially among countries, almost half the deaths are due to being underweight, which indicates that malnutrition is a key determinant, in addition to genetics and the infectious environment. It is well known that malnutrition alters the immune response, which leads to two main groups of diseases, respiratory infection and diarrhea (20% each), with neonatal disorders taking approximately 35% of the death toll. Also according to the prevalence of other diseases, essentially malaria, measles and AIDS, five different main profiles emerge in the 42 countries with 90% of global child deaths.

Fortunately, well-defined preventive and curative interventions exist for a large series of identified causes of death [18]. Those interventions can be delivered through the health sector with maternal education or birth control. In addition to immunization and specific treatments, nutritional interventions include appropriate use of breastfeeding and complementary feeding, and supplementation such as a pharmacological dosage of zinc and vitamin A. It is of note that other nutritional interventions are not considered in the analysis, and for this reason they will be discussed below. As a result of extensive analysis, 23 interventions were proven to be effective in a context of middle or low income countries identified. They were then put into an economic model where 18 contacts between the child or mother and a health care provider were incorporated into a delivery timetable from birth to 5 years of age [19]. The conclusion was that approximately USD 5 billion in new resources is required to save the lives of 6 million children annually in the 42 countries where 90% of the deaths occur. The average cost per life saved is USD 900.

Assessment of Nutrients in the Food Market

Ideally malnutrition should be prevented by using locally available food. However, this approach is restricted to the availability and cost of food. Even in industrialized nations the recommended daily allowances in toddler diets, especially for iron, is difficult to achieve [20]. During complementary feeding, children require a nutrient-dense diet to meet their high nutritional requirements. In addition, the cost of food may also limit proper feeding. Traditionally, research was conducted using a trial-and-error approach or by expert
guessing. Nowadays, an efficient and rigorous technique based on linear programming offers solutions to previous questions [21]. This method operates on widely available programs, including Microsoft Excel, and it aims to answer the following question: is it possible to design a diet suitable for complementary feeding periods using locally available foods? If this is possible, what is the minimum budget for designing a diet covering the nutritional requirements for at least 97% of children?

To understand the principle of this method one can look at a simple example, though nonrealistic and theoretical: a complementary diet based on cow’s milk and maize flour. Any nutritionist would say that the calcium requirement could be met, but not iron. However, linear programming further indicates graphically how far iron is out of the present regimen, and if designing a proper diet is feasible (fig. 1).

The second question is the determination of a maize flour and cow’s milk combination that provides enough energy for a 12- to 23-month-old breastfed child (746 kcal/day according to FAO table and 196 of calcium at the lowest cost). Linear programming again presents a basic and immediate answer. It can help design a graphic solution for a local diet problem taking into account both the availability of food and its cost (fig. 2).

Linear programming is much more efficient than the empirical trial-and-error approach currently used for formulating diets. It could become an indispensable tool for pediatricians and nutrition program planners. Several examples are already available [22, 23] and short courses on linear programming are available on the internet: http://www.nutrisurvey.de/lp/lp.htm

Fig. 1. Graphical illustration representing no solution for the diet problem. From Briend [personal commun.].
Recent Issues in Energy-Protein Malnutrition in Children

**Home-Based Therapy for Malnutrition and Ready-to-Use Therapeutic Food**

A review of the literature over the past 5 decades indicates that the median case fatality from severe malnutrition has remained unchanged over this period and was typically 20–30%, with the highest levels (50–60%) being among those with edematous malnutrition [24]. The authors indicated that a likely cause of this continuing high mortality was faulty case management. In the 1990s, malnutrition was revisited. Most recently, the management of severe acute malnutrition in refugee camps has been examined. It was proposed that a formula containing 100 kcal/100 ml, and called F100, was used in a well-defined setting called the therapeutic feeding center (TFC) [11, 25]. This diet is prepared as a liquid formula by mixing together dried skimmed milk, oil, sugar, and a vitamin and mineral complex. Similar diets have been used in relief operations for more than 25 years. In this case the practice was tested with striking results on several hundred children in refugee camps situated in sub-Saharan regions [26], and the mortality was often found to be below 5%. In its 1999 manual, the WHO recommended the use of F100 during the rehabilitation phase of severe malnutrition [10].

However, TFCs are difficult to establish, expensive to operate, and have very limited coverage. They do not build on the capacity of the community, and at times they can undermine traditional coping strategies. Mothers are often required to stay with their malnourished children in the TFC for several weeks. In addition, F100 has some disadvantages as bacteria grow very

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**Fig. 2.** Optimal solution presented by linear programming analysis taking into account the presence of beans and rice on the market. From Briend [personal commun.].
Desjeux

**Table 1.** Daily energy and macronutrient intakes from F100 and RTUF

<table>
<thead>
<tr>
<th></th>
<th>F100 group</th>
<th>RTUF group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>F100 local recipe</td>
<td>total</td>
</tr>
<tr>
<td>Energy, kJ/kg body weight</td>
<td>275*</td>
<td>298</td>
</tr>
<tr>
<td>Proteins, g/kg body weight</td>
<td>2.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Lipids, g/kg body weight</td>
<td>3.3*</td>
<td>1.6</td>
</tr>
<tr>
<td>Carbohydrates, g/kg body weight</td>
<td>7.2</td>
<td>10.0</td>
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*Significantly different between groups.

rapidly in it and safety becomes an issue when prepared under unhygienic conditions. Plus, before water is added, F100 looks like any milk powder, and may undermine efforts to promote breastfeeding if given to families.

In 2001 a new approach to managing acute malnutrition in emergencies and other situations was proposed by Collins and Sadler [27]. Community therapeutic care (CTC) aims to treat the majority of the severely malnourished children at home, build local capacity to better manage the care of acutely malnourished children, and address repeated cycles of relief and recovery. Recently, the use of the F100 formula has been revised by replacing part of the skimmed milk with peanut butter. Instead of dissolving a powdered product in water, this new recipe gives a spread that a child can eat directly without the addition of water. This is often called ready-to-use therapeutic food (RUTF). The nutritional composition of RUTF in relation to its energy content is very similar to F100. Since its energy density is five times higher (5.4 vs. 1 kcal/g), its micronutrient content in relation (per 100 g) is also five times higher [28] (table 1). It can be prepared locally at approximately USD 15–20/kg. Also, it has been tested against F100 in the TFC and proved to be well accepted and comparable to F100 with regard to weight gain [29].

Together, CTC and RUTF have proved to be a major innovation in the treatment of severe malnutrition. Mainly in Africa, RUTF has increasingly been used in emergency and non-emergency situations. The objective has been to shift the treatment of severe malnutrition from the TFC towards the community. To date, CTC programs have treated over 7,000 children in Africa. Results indicate lower mortality rates (perhaps as a reduction of cross-infection), fewer dropouts and better coverage than standard center-based approaches. As a whole, the CTC program has demonstrated that children suffering from severe acute malnutrition without complications can be safely treated at home. According to these data, a new classification of severe malnutrition has been proposed [30].

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Rehydration Therapy in Dehydrated and Malnourished Children

There is a scope to further reduce the case fatality rate and enhance recovery from malnutrition by improving the recovery from common associated illnesses including diarrhea. Malnutrition predisposes to an increased incidence and duration of diarrhea. As it is associated with further loss of weight, early recovery and lessening the severity of diarrhea will help to prevent further weight loss and deficiency of the nutrients in severely malnourished children. In addition to the active secretion and defective absorption of water and electrolytes in the small intestine, colonic dysfunction has also been demonstrated in cholera due to a lack of short-chain fatty acids. Recently, we initiated a clinical study to test the hypothesis that the addition of an amylase-resistant starch to a glucose-based oral rehydration solution would ferment in the colon to stimulate salt and water absorption in order to alleviate the severity of diarrheal illness and enhance recovery from it. Additional energy from the absorption of short-chain fatty acids through the colonic

**Fig. 3.** Weight gain in the first 72 h in severely malnourished and dehydrated children receiving one of the three oral rehydration solutions (ORS), containing 40 mEq/l potassium and glucose (G), glucose and amylase-resistant starch (ARS), or rice. Weight gain was significant in all three groups but it was greater in the rice group. From Alam et al. [unpublished data].

Rehydration Therapy in Dehydrated and Malnourished Children

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mucosa would also be supplied to assist nutritional recovery [Alam et al. unpublished] (fig. 3).

In a prospective randomized study, 174 children (mean ± SE age = 27.6 ± 0.9 months) were included as severely malnourished (weight/height = 68.5 ± 5.5 with or without pedal edema) and dehydrated because of cholera. After intravenous fluid therapy (602 ± 46 ml) for 84% of them, they were randomly assigned one of the three high potassium (40 mEq/l) oral rehydration solutions containing glucose, rice or amylase-resistant starch. On the main parameters, there was no significant difference among the groups at inclusion. Although the death risk factors were frequently present (55% not breastfed; 84% severe dehydration; 9% severe hypoglycemia), all the children recovered from dehydration. In the first 2 days, stool volume and oral rehydration solution intake were significantly different in the 3 groups, but the number of unscheduled intravenous therapies (15%), duration of diarrhea (66.3 ± 2.2 h), weight gain (0.8 ± 0.1 kg), and time to recover 80% of the weight/height ratio (7.1 ± 0.2 days) were not different.

Despite the high purging rate and although many of the risk factors for mortality were present in these severely malnourished children, in this study we were clearly able to avoid any death by using careful and rapid rehydration. After recovery from life-threatening dehydration, we expected that an amylase-resistant starch would have had a beneficial effect on malnutrition recovery, but this was not found. We are now testing the possibility that the intestinal microflora may not appropriately metabolize starch into short-chain fatty acids in severe malnutrition.

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Discussion

Dr. Desjeux: Feeding children with a high protein diet could be dangerous especially for kidney function. Therefore, at the suggestion of Prof. John Waterloo, when F100 was initially proposed as treatment in emergency situations, we also added F75 which is less dense, that is 75 kg cal/0 ml. But if you look at the results, F100 already reduces mortality to almost zero. It is of course given progressively. I know that some centers still use F75, but I think it would be extremely difficult to show a beneficial effect of F75 on mortality because, as seen under extreme conditions when there is severe malnutrition and dehydration, none of the children died and they were fed with F100 according to the WHO protocol.

Dr. Dewey: I want to thank you for bringing up linear programming. It is a technique that sounds intimidating to some people but I can testify that it is very simple to use. We have used it extensively for a document on feeding of the non-breastfed child from 6 to 24 months of age that the WHO has published. As you said, it involves is having information on the types of foods consumed and their costs. One of the cautions that I would like to mention is that when you work with the youngest age...
group, which is 6–9 months approximately, usually when the local foods are entered into this program there will be no solution. In other words you cannot meet all their nutrient needs with local foods, and that is usually because of iron, sometimes zinc as well. So what you can do is include in the program another entry for supplements and put in a cost that is very high, artificially high, so you minimize how much of the supplement is required in the solution. This is one way we determine what the gaps are and how they need to be met in the diet of any particular local area. I strongly encourage people to go to the website that you mentioned and try it out. The other comment I want to make is that the ready-to-use therapeutic food (RUTF), as you said, was originally developed to treat malnourished children. We have recently used a different formulation of it for complementary feeding of healthy normal children in Ghana, and in this case we used a very strongly fortified concentrated version and we only need 20 g/day, about 4 teaspoons. So far the results look very encouraging for improving growth as well as other outcomes. I think this is one product that deserves a lot more attention.

Dr. Desjeux: Thank you very much for your very appropriate comments. For linear programming, a tutorial can be found at www.nutrisurvey.de/lp/background_info.htm. You can enter what you have bought at the market, together with the cost, and then you get this kind of graph that I showed you. It also shows you how much of the requirements have been covered and, as you mentioned, iron is the most difficult micronutrient to fulfill. You can decide to add iron from a source other than local food and see if the diet is now appropriate according to the recommendations. So it is an extremely simple linear program, and extremely powerful for designing and checking the quality of the diet according to the cost. It goes from food to nutrient and cost, and includes all these items together.

Dr. Telmesani: Some studies suggest adding some lactobacillus or bifidobacteria to the oral rehydration solution (ORS), and report a positive effect in reducing the duration of diarrhea. What is your view?

Dr. Desjeux: We already discussed adding amino acids, adding bacteria. Many things can be added, but as you have seen very simple ORSs are quite effective, and a reduction of 6 h in the duration of acute disease that last 2–3 days does not matter. From the clinical point of view I don't think it is very relevant, and therefore I think we have to keep it simple and teach people how to use it, but still there is a need to improve its use all over the world. In my view it is more a matter of marketing than composition [1]. For probiotics there are some clinical trials showing a statistical effect. Now to answer the question that was raised earlier about amino acids; amino acids are provided with ORSs because after 4 h the children are fed so they receive amino acids. That is probably one reason why the addition of amino acids in clinical trials doesn't show any striking effect, if at all. Adding glucose to glucose doesn't help very much and adding glutamine to a protein diet doesn’t add very much either.

Dr. Macé: Do you have an idea which of the short-chain fatty acids could be the most efficient? Is it acetate butyrate?

Dr. Desjeux: It depends; if you want to rehydrate it is butyrate that stimulates sodium absorption [2]. But the other amino acids are also important because they would provide energy and could help to improve colon function which is very frequently altered in infectious diseases including cholera, and also in malnutrition [3].

Dr. Noor Khatijah Nurani: How long do you use RUTF and when do you start introducing other proteins from locally available food?

Dr. Desjeux: It starts in the rehabilitation phase, and I am referring to the WHO manual for the definition. It depends on the local circumstances but a weight for height of 80% of the National Center for Health Statistics can be obtained in 7 days, even in severely malnourished children.
**Dr. Barclay:** You have shown that the higher nutrient density refeeding formulas were useful for malnutrition. I was wondering about the same concept for refeeding during diarrhea. As you probably remember about 10 years ago, based on the results of studies mainly in the Indian subcontinent and some which you cited, we developed an improved rice-based ORS containing about 50 g/l of rice and showed that this rice-based ORS improved diarrhea outcome vs. standard glucose ORS. Nestlé R&D developed a ‘super’ rice-based ORS containing 160 g/l of rice, electrolytes and α-amylase. The product has to be cooked just prior to administration by the caregiver so the product is actually sterilized preventing further microbial contamination. Studies in Ecuador and Columbia showed that children treated with this new rice-based ORS had a significantly shorter duration of diarrhea, a lower number of stools and a greater weight gain during treatment vs. standard glucose ORS [4]. So my question is, do you think that there is any need for more energy-dense, more nutrient-dense ORS, keeping osmolarity below say 280 mosm, or that standard ORS with normal feeding schedules are sufficient?

**Dr. Desjeux:** First of all you showed very clearly is that it is essential to give enough energy as soon as the child is rehydrated. I don’t know how it is in your country, but in my country the doctors would prescribe no food to the child for 3 or 4 days, which of course would be impossible for the mother to follow. So feeding is really essential in the treatment of diarrhea. Now which type of feeding? I would be a bit reluctant to use RUTF in this condition for two reasons. One is that RUTF has been designed specifically for malnutrition. It is used in different kinds of malnutrition; for instance in mentally retarded children with malnutrition the effect of RUTF is absolutely spectacular. But to have another energetically dense diet for diarrhea could be interesting. This is really a paste and we were a bit afraid that giving such a high energy-dense paste would dehydrate the child, but it does not. I would be afraid of giving that diet to dehydrated children, at least at first.

**Dr. Butte:** You were focusing on the recovery of weight for height. Is there any new progress being made in treating the stunted child, in recovering linear growth?

**Dr. Desjeux:** In therapeutic feeding centers growth is faster than in home-based therapy. I would say that growth is really the proper indicator of efficacy, but does it really matter if the gain is 15, 16 or 12 g/day? After all if they are happy and healthy and alive, this is already quite an achievement. Now can we get a better indicator for recovery from malnutrition, does it matter, does the first phase of rehabilitation from malnutrition have long-term consequences? I know there are some people trying to address that question, but it is very difficult to have a follow-up in these populations.

**Dr. Huerta:** How do you give infants with marasmus, weight for height below 2 standard deviations and a mean age of 13 months, this amount of protein, 6.5/kg/day? How many days do you use it?

**Dr. Desjeux:** We are often afraid of giving too much protein, but this is not only protein. This diet also contains a high density energy and lipids, carbohydrates, mineral and vitamin mix. I think we have more from the clinical results than the theoretically expected results to base our recommendation on. I am not absolutely sure how it works, but for the time it works. For instance, as a gastroenterologist I am not too much concerned with protein but with fat. We have learned, and it is true, that in severe malnutrition the pancreas does not function well, and although we provide a huge amount of lipids, it works. So there are some discrepancies between what we have learned and what we see now with this treatment. It is a major breakthrough in the treatment of these children.
Desjeux

References

Protein Quality and Quantity in Cow’s Milk-Based Formula for Healthy Term Infants: Past, Present and Future

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Abstract

The development of infant formula with optimized protein quality and quantity has been, and still is, the subject of intense investigation. A better understanding of the protein composition of breast milk and infant needs in association with technological breakthroughs in cow’s milk fractionation, has led to the development of infant formulas with a protein content that is closer to that of human milk. Today, infant formulas with a protein/energy ratio of 1.8 g/100 kcal are commercially available. These formulas have been shown to be safe and nutritionally adequate for term infants. However, the short-term and potentially long-term metabolic benefits of formulas with reduced protein content have still to be elucidated and are currently under investigation. In addition to providing amino acids as building blocks for growth, milk is the source of numerous bioactive factors/hormones which are involved in multiple physiological processes. Continuous efforts are being made to identify new bioactive compounds in human milk. However, a better understanding of their biological functions in suckling infants as well as a comparison with their bovine counterparts are needed. Technological processes, which preserve some bioactive factors in cow’s milk already exist. These processes could be applied to infant formulas.

Introduction

Since the first commercially prepared infant formulas were available as powders in the late 1800s, constant improvement in the formulation has been done to match the composition of human milk more closely and, most importantly, to mimic the functional outcome of breast-fed infants (i.e., appropriate growth, development and health). In this context, the development of infant formula
with optimized protein quality and quantity has been, and still is, the subject of intense investigation.

**From Casein-, Whey-Predominant to Modified Whey Infant Formula**

In 1919, Gerstenberger and Ruh [1] developed the first commercially available formula with cow’s milk as the exclusive source of proteins. The original protein content of the manufactured formula was 1.8 g/100 kcal, but was increased to 2.2 g/100 kcal in 1945 [2]. In the middle of the 20th century, it was considered that formula-fed (FF) infants require a considerably greater intake of protein than breast-fed (BF) infants. This recommendation was based on an overestimation of both the nutritionally available protein content of human milk and the protein intake requirements of the infants [3, 4]. Furthermore, cow’s milk protein quality and digestibility was considered far inferior to that of human milk for satisfying the amino acid needs of infants. In the 1960s, the protein content of a number of widely used formulas ranged from 3.3 to 4.0 g/100 kcal and some formulas, designed for managing diarrhea, even provided up to 6.7 g/100 kcal [5]. Subsequently, national health institutes and pediatric associations defined standards for protein content in infant formulas (table 1). While there is now a consensus for the minimum required values (1.8 g protein/100 kcal), a broader range of recommended maximum values can be found (2.8–4.5 g protein/100 kcal). In 1991, a European Directive set the maximum protein content to 3.0 g/100 kcal. However, the Food and Drug Administration still recommended a maximum required intake level of 4.5 g/100 kcal, in spite of the revisions proposed by a task force of the American Academy of Pediatrics [5]. A current revision of the Codex
proposes a maximum protein content of 3.0 g/100 kcal, for both starter and follow-on formulas, based on new recommendations from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (table 1).

The intrinsic superiority of human milk protein over that of cow's milk is due to its higher whey to casein protein ratio (approximately 60:40 in human milk and 20:80 in bovine milk). In 1961, a major technological breakthrough (i.e. demineralization of whey protein through an electrodialysis process) allowed the addition of equal parts of casein and whey in formula, and by the mid 1990s, whey-predominant formulas prevailed in the US and Europe. Nevertheless, both the whey and casein fractions of cow's milk are quite different from those of human milk (fig. 1). Consequently, the amount of amino acids delivered by breast milk and formula differ. Compared to breast milk, the levels of most of the essential amino acids are, per gram of protein (or nitrogen), lower in the casein-predominant formulas, while leucine, phenylalanine, tryptophan, and to a lesser extent valine, are limited in whey-predominant formulas (fig. 2). In order to compensate for these quantitative differences, the amount of proteins per energy content must be higher in formula than in human milk. A casein- or a whey-predominant formula containing 2.5 g protein/100 kcal, provides, an excess of most of the essential amino acids found in breast milk. Nevertheless, tryptophan and the conditionally essential amino acid cystine are limiting factors for further reducing the protein quantity in cow's milk formula (fig. 3). Indeed, Janas et al. [6] demonstrated that term infants fed formulas with reduced protein content (1.8 g/100 kcal) and various whey/casein ratios had normal plasma cystine levels but depressed levels of tryptophan when compared to those fed human milk. New approaches/processes were therefore needed to adjust the protein/energy ratio to the minimal recommended value (1.8 g/100 kcal) and, thereafter, to that more closely resembling human milk.

In principle, the easiest way to avoid lower plasma levels of tryptophan in FF infants is to supplement the formula with free tryptophan. In 1992, studies demonstrated that term infants, fed a casein-predominant formula, reduced in protein (1.9–2.0 g/100 kcal) but fortified in free tryptophan, had similar plasma tryptophan levels to BF infants [7, 8]. However, taking into account the absorption kinetics of free and protein-bound tryptophan, as well as toxicological and economical considerations, supplementing with free tryptophan is not the most favorable option. Addition of α-lactalbumin (αLA) may provide a promising alternative [9]. The αLA fraction is rich in tryptophan (5.9%) but the proportion of αLA in bovine milk (4%) is considerably lower than in human milk (28%; fig. 1). Whey protein concentrates, or isolates enriched in αLA and produced by either ion exchange or membrane fractionation, became commercially available in the late 1990s [10]. The effect of αLA enrichment on tryptophan supply has been studied in healthy term infants fed a whey-predominant formula containing 2.0 g protein/100 kcal and 2.2 g tryptophan/16 g N over a 2-week period [11]. It was demonstrated that the
Fig. 1. Protein composition of human and cow's milk. Adapted from Heine et al. [9].
serum tryptophan levels of the FF infants did not differ significantly from that of an exclusively BF group [11]. A growth and safety study was recently performed in term infants fed a whey-predominant control formula or a reduced protein experimental formula with added bovine αLA [12]. The αLA/βLA ratio was 0.4 in the control formula and 1.6 in the αLA-enriched formula. Growth and serum albumin were comparable in both groups of infants for the first 12 weeks of life, suggesting adequate protein delivery using the reduced protein formula with added bovine αLA. However, as pointed out by Raiha [13], the difference in protein content between the control (15.1 g/l) and experimental formula (14.4 g/l) was in fact rather small (2.25 and 2.15 g protein/100 kcal, respectively, assuming an energy density of 67 kcal/100 ml). Additional studies are still required to address the safety of formulas with an even lower protein content (e.g. 1.8 g/100 kcal).

In the meantime, other approaches have been explored. Caseinoglycomacropeptide is released from casein during the enzymatic precipitation of κ-casein and remains in the sweet whey fraction commonly used for the production of whey-predominant infant formulas. An original process of whey

Fig. 2. Amino acid composition (g/16 g N) of human milk (□), casein- (■) and wheypredominant (■) formulas. *Essential amino acids.
fractionation was developed to first of all remove the caseinoglycomacro-peptide fraction, which is rich in threonine but poor in tryptophan, and then to increase the proportion of the αLA fraction which is rich in tryptophan (patent WO 01/11990). The resulting modified sweet whey (MSW) allowed the development of an infant formula with an essential amino acid profile that was closer to human milk (table 2; g amino acids/16 g N). In theory, this new formula allows the reduction of the protein/energy ratio to the minimum recommended value (1.8 g/100 kcal), without a deficit in essential and conditionally essential amino acids (table 2; mg amino acids/100 kcal). The protein adequacy of the MSW formula (1.83 g/100 kcal) was established with metabolic balance studies. The results showed that, despite the lower protein content, infants had similar nitrogen retention to those infants fed a formula with higher protein content (2.24 g/100 kcal) [14]. The nutritional adequacy of long-term feeding with a MSW formula (whey/casein ratio 70/30, protein content 1.8 g/100 kcal) was tested in healthy, term infants from birth to 4 months and compared to a conventional whey-predominant formula (whey/casein ratio 60/40, protein content 2.2 g/100 kcal) and to breast milk [15]. No differences were found among the 3 feeding groups for weight or length gains or for body mass indices. Protein intakes were lower in the infants fed the MSW

![Fig. 3. Amino acid composition (mg/100 kcal) of human milk (□), casein- (■) and whey-predominant (■) formulas. *Essential amino acids.](image-url)

Fig. 3. Amino acid composition (mg/100 kcal) of human milk (□), casein- (■) and whey-predominant (■) formulas. *Essential amino acids.
formula than in those fed the classical whey formula, however the energy intakes were identical in the 2 groups. Finally, both urea [15] and plasma amino acid levels (table 3) of MSW FF infants were closer to those found in BF infants. The adequacy and safety of a similar formula developed with partially hydrolyzed proteins have also been demonstrated [16].

Table 2. Amino acid composition of human milk (HM), whey-predominant formula (WPF) and modified sweet whey formula (MSWF)

<table>
<thead>
<tr>
<th></th>
<th>g/16 g N</th>
<th>mg/100 kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HM</td>
<td>WPF</td>
</tr>
<tr>
<td>Isoleucine\textsuperscript{a}</td>
<td>6.4 6.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Leucine\textsuperscript{a}</td>
<td>11.5 9.8</td>
<td>13.1</td>
</tr>
<tr>
<td>Lysine\textsuperscript{a}</td>
<td>7.9 8.7</td>
<td>10.4</td>
</tr>
<tr>
<td>Methionine\textsuperscript{a}</td>
<td>1.7 2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Phenylalanine\textsuperscript{a}</td>
<td>4.6 3.9</td>
<td>4.6</td>
</tr>
<tr>
<td>Threonine\textsuperscript{a}</td>
<td>5.6 6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Tryptophan\textsuperscript{a}</td>
<td>2.3 1.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Valine\textsuperscript{a}</td>
<td>6.8 6.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.3 1.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.7 4.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.2 2.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.8 2.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Protein content: HM, 1.5 g/100 kcal; WPF, 2.46 g/100 kcal; MSWF, 1.83 g/100 kcal. \textsuperscript{a}Essential amino acids.

Table 3. Plasma concentrations (\textmu mol/l) of amino acids of 120-day-old infants breast-fed or fed either a whey-predominant formula (WPF) or a modified sweet whey formula (MSWF)

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>WPF 2.2</th>
<th>MSWF 1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine\textsuperscript{a,b}</td>
<td>47.8 ± 12</td>
<td>73.1 ± 13.6</td>
<td>55.6 ± 10.1</td>
</tr>
<tr>
<td>Leucine\textsuperscript{a,b}</td>
<td>87.8 ± 24.3</td>
<td>121.7 ± 22.6</td>
<td>106.2 ± 18.6</td>
</tr>
<tr>
<td>Lysine\textsuperscript{a}</td>
<td>132.9 ± 35.4</td>
<td>194.2 ± 42.9</td>
<td>170 ± 35</td>
</tr>
<tr>
<td>Methionine\textsuperscript{a}</td>
<td>21.2 ± 4.2</td>
<td>27.9 ± 5.2</td>
<td>27.1 ± 5.7</td>
</tr>
<tr>
<td>Phenylalanine\textsuperscript{a,b}</td>
<td>38.9 ± 7.7</td>
<td>51.5 ± 8.8</td>
<td>47.9 ± 10.2</td>
</tr>
<tr>
<td>Threonine\textsuperscript{a}</td>
<td>103.8 ± 23.6</td>
<td>164.2 ± 44.8</td>
<td>132.8 ± 27.4</td>
</tr>
<tr>
<td>Tryptophan\textsuperscript{a}</td>
<td>66.5 ± 12.5</td>
<td>63.3 ± 11.6</td>
<td>75 ± 11.5</td>
</tr>
<tr>
<td>Valine\textsuperscript{a,b}</td>
<td>139.2 ± 31.2</td>
<td>206.8 ± 29.3</td>
<td>151.6 ± 19.6</td>
</tr>
<tr>
<td>Arginine\textsuperscript{b}</td>
<td>81.6 ± 20</td>
<td>86.3 ± 22.1</td>
<td>110.6 ± 26.6</td>
</tr>
</tbody>
</table>

Protein/energy ratio of 2.2 and 1.8 g protein/100 kcal for WPF and MSWF, respectively. Values are given as mean ± SD.

\textsuperscript{a}Essential amino acids. \textsuperscript{b}Insulin-secretagogue amino acids.
Metabolic Advantage of Reduced Protein Formula

Infants fed ‘classical’ casein- or whey-predominant formulas have higher levels of some amino acids and consistently elevated urea levels in their blood when compared to BF infants [17]. The short-term effects of these differences on infant growth and health have been carefully assessed and considered as safe. Nevertheless, the impact of both the quantity and quality of protein intake during early life on the incidence of disease later in life is, as yet, not known.

Dehydration and Kidney Functions

Potential renal solute load refers to solutes of dietary origin that would need to be excreted in the urine if not utilized by the body. It represents the sum of dietary nitrogen, sodium, potassium, chloride and phosphorus [18] and is a suitable parameter to measure the risk of dehydration illness. When ingested in excess, proteins constitute a considerable part of the solutes that must be excreted by the kidneys. Taking this into consideration, Ziegler and Fomon [18] recommended reducing the maximum protein content of infant formula from the level of 4.5 g/100 kcal, specified by the Food and Drug Administration, to 3.2 g/100 kcal. As an adaptive response to a high solute load, glomerular filtration rate and kidney size increase. While the adverse effects of a high protein intake in patients with kidney disease have been documented, there is to date no clear evidence of such detrimental effects in healthy individuals. The effect of formula- vs. breastfeeding on kidney growth was recently investigated in a cohort study of 631 healthy children examined at 3 and 18 months of age [19]. The results showed that kidney size and serum urea nitrogen were greater in the FF infants. Nevertheless, the differences in relative kidney size were temporary, as they were no longer apparent at 18 months of age [19]. The consequences of such increased kidney growth on kidney functions later in life are unknown.

Growth and Adiposity

Growth velocity seems to differ between BF and FF infants, especially when infants are breast fed for more than 6 months. Exclusively BF infants tend to grow more rapidly in the first 2–3 months of life, but from 6 to 12 months have a body weight, body length or body weight for length that are slightly lower than FF infants [20–23]. A significantly higher protein and/or energy intake [24–26] is associated with the faster growth rate observed in FF infants, but conflicting results exist [27, 28]. To date, the long-term impact of this moderate difference in growth on obesity risk later in life, is unknown. An association between high dietary protein intake during early childhood and subsequent adiposity has also been proposed [29]. In a longitudinal study a positive correlation was found between protein intake at the age of 2 years, but not at 10 months of age, and the body mass index and subscapular skin-fold at 8 years of
age [29]. Although some studies [30, 31] have described an association between protein intake at ≥9 months of age and adiposity in later childhood, other studies have reported an association with body size but not with body fat mass [32]. Interestingly, the influence of excess protein intake during the first year of life on weight gain in infancy and the risk of obesity later in life is now being studied in a large European Childhood Obesity Project [33].

A high protein intake is likely to have endocrine effects. It has been suggested that the higher growth velocity reported in FF vs. BF infants is due to a high protein intake early in life that promotes secretion of insulin-like growth factor-1 (IGF-1), a trophic hormone involved in longitudinal growth as well as muscle and fat mass development. Although there is limited knowledge about neonatal endocrine responses to milk feeding, there is increasing evidence that at 2 [34] and 6 months of age [35] FF infants have greater serum IGF-1 levels than BF infants. In a recent study, Savino et al. [34] observed that plasma IGF-1 levels are directly correlated with the Z score for weight, body mass index and tricipital skin-fold thickness in 2-month-old infants. Interestingly, we have observed that, during the first 4 months of life, infants fed a MSW formula (1.9 g protein/100 kcal) have a different evolution of their IGF-1 plasma levels to infants fed a normal sweet whey formula (2.4 g protein/100 kcal). IGF-1 levels decrease significantly (p = 0.013) between 28 and 112 days in infants fed formulas with a reduced level of protein (fig. 4).

**Fig. 4.** IGF-1 levels (µg/l) in infants fed a modified sweet whey protein formula with a reduced protein content of 1.9 g protein/100 kcal (▲) or a normal sweet whey formula containing 2.4 g protein/100 kcal (■).
IGF-1 levels average 79.3 ± 34.0 ng/ml (mean ± SD, n = 35) at 28 days and 58.9 ± 37.8 ng/ml (n = 41) at 112 days of age. On the other hand, in infants fed control formula IGF-1 levels did not decrease. IGF-1 levels average 77.5 ± 31.0 ng/ml (n = 21) at 28 days and 80.8 ± 37.8 ng/ml (n = 27) at 112 days of age (fig. 4). This confirms the strong influence of protein intake on IGF-1 levels. Interestingly, IGF-1 levels with a reduced protein formula are similar to those reported for BF infants [36].

Insulin acts as a growth factor during development and is considered as an anabolic factor of lean and fat mass. Moreover, protein intake is known to potentiate glucose-stimulated insulin secretion [37]. The amino acid profile of the ingested protein itself plays a role in the insulin response to feeding. In this respect, lysine, leucine, phenylalanine, valine and arginine are the amino acids which are the most potent insulin secretagogues [38]. Interestingly, FF newborns show higher postprandial plasma insulin levels than their BF counterparts [39]. Up to 6 months of age, urinary C-peptide (a marker of insulin secretion) correlates with plasma valine levels and is 2 to 3 times greater in FF infants than in BF infants [40]. Similarly, infants fed a formula containing 2.5 g protein/100 kcal between 4 and 6 months of age have higher levels of urinary C-peptide than BF infants or infants fed a formula containing 1.8 g protein/100 kcal [24]. In this study, weight gain is reported to correlate with C-peptide excretion, protein intake and plasma concentrations of the branched-chain amino acids, valine, leucine and isoleucine [24]. Interestingly, infants fed a modified sweet whey formula (1.8 g protein/100 kcal) vs. a classical whey-predominant formula (2.2 g protein/100 kcal) displayed significantly lower plasma levels of a number of amino acids, such as valine, leucine and isoleucine (table 3), which are considered to be insulin secretagogues. While the arginine levels were higher with the modified formula, the phenylalanine levels were unchanged (table 3). It would be of interest to investigate whether these differences are associated with decreased insulin levels in the plasma and/or C-peptide excretion. Additional studies are also needed to determine the short- and long-term metabolic consequences of these differences.

**Future Development for Improving the Protein Quality in Infant Formula**

New technological processes have allowed the development of nutritionally adequate and safe infant formulas with a protein content closer to breast milk. The benefits of further decreasing the protein/energy ratio of infant formulas to <1.8 g/100 kcal are probably limited. However, providing the required protein and energy intake is not the only means to prevent malnutrition or disease. In addition to its nutritional qualities, human milk provides a diversity of specific bioactive proteins such as hormones, cytokines, and growth factors
which have physiological relevance beyond purely nutritional properties [41]. These factors are implicated in growth and development, protective defense mechanisms, energy metabolism and immune homeostasis in multiple tissues. Despite the improvement in infant formula development, differences in response to infection and the development of allergy and atopic disease have been reported for FF and BF infants [42]. Although some of these bioactive proteins are also present in bovine milk [41], it has generally been assumed that they do not survive the technological processing used in the manufacture of infant formulas. As such, infant formulas are considered to lack some of the protective factors which human milk inherently provides.

With the advent of new methodology, the identification of milk proteins and their biological activity has received more attention. Indeed, we have recently reported that human milk contains proteins such as transforming growth factor (TGF) \(\beta\) [43], osteoprotegerin [44] and soluble CD14 [45], which are involved in immune homeostasis, innate responses to bacterial components and bone metabolism. TGF-\(\beta\) is a multifunctional polypeptide. It acts upon a variety of different cells to regulate their growth, differentiation and survival, and plays a crucial immunoregulatory function in mechanisms of tolerance and the prevention of disease and autoimmunity [46]. TGF-\(\beta\) is present in milk and may be activated by acidification or mild enzymatic treatment [47], thus it is feasible that it is activated during intestinal transit to exert a biological effect in the intestinal epithelium of the host. Furthermore, since TGF-\(\beta\) is present in bovine milk, we considered that it may remain biologically active after exposure to some manufacturing processes. To address this possibility, we analyzed a range of milk-based preparations and raw materials for their TGF-\(\beta\) content. As expected, its presence is dependent on the milk protein source and the processing conditions [43]. Interestingly, we showed that a casein-based formulation, with acceptable preserved levels of TGF-\(\beta\), prevents diarrhea in animal models of inflammation [48, 49] and induces remission and mucosal healing in children with small bowel Crohn’s disease [50, 51]. It is acknowledged that the technological process used to make the TGF-\(\beta\)-containing casein may also have preserved some other bioactive molecules. Nevertheless, this work on TGF-\(\beta\) casein, although carried out for a different application, is one illustration of how further improvement in infant formula products may be possible. It remains to better characterize the bioactive molecules in human milk and more importantly to demonstrate their biological activities and their benefits for infant growth and development.

To mediate optimal biological activity some factors will require binding to specific host receptors. In this respect, supplementation with recombinant human proteins may be advantageous. Indeed, the potential benefits of using recombinant human lactoferrin expressed in plants [52] or transgenic cows [53] is currently being investigated. However, such approaches have legal and regulatory issues, which are unlikely to be resolved in the immediate future.
In the interim, new biotechnology is preferred which enables the use of naturally occurring molecules in physiological doses and in an environment which is more likely to exert optimal biological activity.

**Conclusions**

Human milk composition is unique and breastfeeding provides components that nourish, protect and develop infants in the best way. Since the beginning of infant formula development, much effort has been made to try to mimic the characteristics of human milk. A better understanding of the protein composition of breast milk in association with technological breakthroughs in cow’s milk fractionation, have led to the development of infant formulas with a protein content that is closer to that of human milk. Today, infant formulas with a protein/energy ratio of 1.8 g/100 kcal are commercially available. These formulas have been shown to be safe and nutritionally adequate for term infants. However, the short-term and potentially long-term metabolic benefits of formulas with reduced protein content have still to be elucidated and are currently under investigation. Further improvement in the protein quantity in infant formula and the health benefits for the infants will be difficult. However, in addition to providing amino acids as building blocks for growth, milk is a source of numerous bioactive factors which are involved in multiple physiological processes. Continuous efforts are being made to identify new bioactive compounds in human milk. However, a better understanding of their biological functions in suckling infants as well as a comparison with their bovine counterparts are needed. Technological processes which preserve some bioactive factors in cow’s milk already exist. These processes could certainly be applied to infant formulas. Finally, the use of recombinant proteins is an interesting option for the development of infant formulas, which provide functions that resemble even more those provided by human milk.

**References**


Discussion

Dr. van Goudoever: Does your study mean that other factors are more rate-limiting than tryptophan or what explanation do you have, although you find a better metabolic tolerance by lowering the nitrogen excretion rate and the urea levels?

Dr. Macé: Perhaps I misunderstood but there is no deficit in tryptophan today in infant formula I believe there are casein-predominant formula or whey-predominant formula or this modified sweet whey. They are all going to supply an adequate amount of tryptophan. But if you want to reduce the whey-predominant formula to 1.8 g protein/100 kcal, here you will have an issue. But today there is no whey-predominant formula on the market at this level for this reason. With the modified sweet whey protein formula you can lower the protein to 1.8 and there will be no deficit in tryptophan or essential amino acids.

Dr. van Goudoever: Let me rephrase the question then. Do you think by giving this specific formula, you said 1.8 g/100 kcal, that by giving 2.0 g/100 kcal, you will improve the weight gain rates?

Dr. Macé: I don't know because we have not tested this. We have tested 1.9 g/100 kcal with partially hydrolyzed formula, but we didn't make a comparison between growth in these two studies.

Dr. Haschke: May I comment on this? We are fully satisfied with the weight gain rates because they correspond to those of breastfed infants. So there is no reason to make them fatter.

Dr. Pencharz: What you are basically doing is producing a better quality breast milk substitute protein source. I just have a comment and then a question. In presenting your nitrogen balance data I would suggest that you give the nitrogen balance as nitrogen percent or as a percent of nitrogen balance divided by nitrogen intake because that would show you have a better nitrogen percent utilization in this new product, the sweet whey protein-enhanced product. Now the question I have is in regard to amino acid balance. Threonine is a chronic problem, and it wasn't clear to me from your star graphs whether the threonine was still high or whether you brought it down. As we have shown the utilization of threonine from formula is different than human milk, the oxidation is not as good; not that hyperthreonemia is pathological but we do see that with whey protein. What did you see with this new product in terms of plasma threonine levels? Finally the other amino acid of interest to me is cysteine; have you looked at glutathione levels? We are not just talking about protein, we are also talking about other physiological issues like antioxidants in terms of glutathione; so threonine and cysteine and the various points I raised.

Dr. Macé: I will follow your advice for the metabolic study. The threonine levels are quite reduced in this formula. I am not sure about the cysteine level. I will have to check that.
Dr. Butte: It was fascinating to see that you had equal energy intake, equal weight gain, with the new formula vs. the breastfed group. So of course it would be very nice to measure the body composition in these children. Have you thought about measuring the sleeping metabolic rate? Another study we did a long time ago was sleep monitoring and in those studies we found that breastfed infants had higher rates of rapid eye movement vs. non-rapid eye movement compared to the formula-fed infants. It is also intriguing that you are now matching the plasma tryptophan levels to see the effects on the sleeping metabolic rate and doing sleep studies.

Dr. Dewey: I want to follow up on Dr. Butte's question because you mentioned the growth data but you didn't show them. I am very interested to know what the sample size was in your groups; whether the birth weights were similar between the breastfed and the modified formula group; if it was sex balanced; was the breastfed group exclusively breastfed, and lastly have you looked separately at birth to 4 months, or was it 1–4 months?

Dr. Haschke: May I clarify this? All infants were enrolled during the first week of life and were measured for the first time at 7 days of age. The data that were shown here were the weight gains between 30 and 120 days. All other questions can be answered by looking at the publication. Prof. Räihä was the first author and it was published 2 years ago in the Journal of Pediatric Gastroenterology and Nutrition [1]. The trial was done according to the FDA rules, and was FDA approved. All the questions you have related to sex, birth weight and breastfeeding are explained in that publication. We followed the rules.

Dr. Macé: There is no statistical difference in length gain but the size of the cohort was limited: 28 per group.

Dr. Rigo: Can you speculate on the results of the study on α-lactalbumin where it was shown that there is a reduction in blood urea nitrogen in boys only [2]? In your study you have two levels of protein intake so it is more difficult to see if there was an effect on blood urea nitrogen.

Dr. Macé: I have to admit that I put the two formulas in protein density which was not mentioned in the paper; it was grams per liter. The calorie density of the formulas was not also mentioned. In fact we don't know what the protein density of these formulas was, but at least with the information we got the difference was not so high and it is surprising that they saw a difference in urea excretion. But I am not a pediatrician; perhaps someone can answer that.

Dr. Haschke: The energy density of the two formulas was 67 kcal/100 ml.

Dr. Rigo: What is the bioactive role of glycomacropeptide?

Dr. Macé: This morning Dr. Butte also asked if cyclic guanosine monophosphate (cGMP) is released in human milk. I cannot answer this question; I don't know if anyone else has the answer. The κ-casein content of human milk is only 5% so I doubt that this small amount of cGMP, even if it is released during the ingestion of human milk, is going to have a biological impact on satiety for example. What has also been shown with cGMP, but again with high levels of cGMP, is that it increases the glucagon-like peptide-1 levels and cholecystokinin. So it seems that it can trigger the secretion of some of the ingredients involved in food intake, but this has been done in rats or even in vitro. Unfortunately I can't answer your question; it is not clear what the role of cGMP is in human milk.

Dr. Haschke: There is a very interesting effect of cGMP, in that it protects against caries. We discovered that approximately 10 years ago when we began to modify the formula protein level, and the cGMP fraction became available. Now the Colgate Company has bought our patent and they add it to toothpaste.

Dr. Lönnerdal: To follow up the question about cGMP; I think that there are two possible physiological effects and we should be a bit concerned about the concentration
dependency. Some of these bioactive components can be quite potent at low concentrations, but I will come back to that in my talk. cGMP provides oligosaccharides in bound form and oligosaccharides have prebiotic effects and also other potential antibacterial effects with regard to attachment, and potentially the cGMP-bound carbohydrates could be somewhat more stable in the gut. Further, cGMP is heavily sialylated so it is negatively charged and when we used a formula enriched in cGMP in infant rhesus monkeys, we found a positive effect on both iron and zinc absorption [3]. The concentration of cGMP may be too low in breast milk to have any significant effect but in formulas it potentially could have some significance with regard to absorption of these cations.

**Dr. Giovannini:** Do you have any data about the neural behavior of children with this formula? We studied long-chain polyunsaturated fatty acids and behavior some years ago [4]. Because tryptophan is the basis for serotonin metabolism, it is interesting to see how the children cry and sleep. I think these are very important parameters when there is more tryptophan in a formula with less protein vs. a traditional standard formula. It is important to recognize this problem because when we use different amino acids and different diet systems we may see differences in crying in the first period of life and also later in sleep.

**Dr. Macé:** I fully agree that tryptophan is important for the sleep pattern and things like that because of serotonin, but we haven’t found a deficit, it is like in human milk. So to answer your question, no we didn’t measure behavior in infants on these formulas. But I would not expect to see a difference with breastfed infants because the amino acids or the tryptophan levels are not higher or lower, they are close to human milk.

**References**

Recombinant Human Milk Proteins

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Abstract

Human milk provides proteins that benefit newborn infants. They not only provide amino acids, but also facilitate the absorption of nutrients, stimulate growth and development of the intestine, modulate immune function, and aid in the digestion of other nutrients. Breastfed infants have a lower prevalence of infections than formula-fed infants. Since many women in industrialized countries choose not to breastfeed, and an increasing proportion of women in developing countries are advised not to breastfeed because of the risk of HIV transmission, incorporation of recombinant human milk proteins into infant foods is likely to be beneficial. We are expressing human milk proteins known to have anti-infective activity in rice. Since rice is a normal constituent of the diet of infants and children, limited purification of the proteins is required. Lactoferrin has antimicrobial and iron-binding activities. Lysozyme is an enzyme that is bactericidal and also acts synergistically with lactoferrin. These recombinant proteins have biological activities identical to their native counterparts. They are equally resistant to heat processing, which is necessary for food applications, and to acid and proteolytic enzymes which are needed to maintain their biological activity in the gastrointestinal tract of infants. These recombinant human milk proteins may be incorporated into infant formulas, baby foods and complementary foods, and used with the goal to reduce infectious diseases.

Introduction

It is well recognized that there are several benefits associated with breastfeeding. Breast-fed infants have fewer infections [1], even in higher socioeconomic groups in affluent countries, and when they get sick, the illness is of shorter duration than in formula-fed infants [2]. This, of course, is even more accentuated in developing countries where sanitary conditions are poor and exposure to pathogens is high. The lower prevalence of infections is likely to be due to several factors, but many of these are unique milk proteins...
that have anti-infective properties. Breast-fed infants also have a different growth pattern, different nutritional status and different gut microflora than formula-fed infants. The high bioavailability of nutrients from breast milk is believed to be due in part to proteins in the milk that facilitate nutrient utilization [3]. While many modifications have been introduced to make infant formula more similar to breast milk, e.g. mixing vegetable oils, adding long-chain fatty acids, adding cow’s milk protein fractions, oligosaccharides and minerals/vitamins, the performance of formula-fed infants is still different than that of breast-fed infants [4]. Many biological activities have been associated with various proteins in breast milk, and it has still not been possible to mimic the protein composition of breast milk, as these proteins are species specific [5]. Among the protein are immunoglobulins, lactoferrin, α-lactalbumin, lysozyme, haptocorrin and α1-antitrypsin. Although bovine lactoferrin and α-lactalbumin are commercially available and have been added to infant formula, no biological activities have yet been correlated to these proteins in clinical studies, possibly because they are different in structure from their human milk counterparts. This may be important, as some proteins, like lactoferrin, have specific receptors in the small intestine that only recognize the species-specific protein [6].

By using genetic engineering, human milk proteins can now be produced in microorganisms, plants or milk from dairy animals [7]. Recombinant human milk proteins can thus be produced in large quantities and may be used in products such as infant formula. However, before this can be achieved, the bioactivities of these proteins need to be evaluated, their safety assessed and the attitude of the consumers carefully considered.

**Expression Systems**

*Expression of Human Milk Proteins in Microorganisms*

Microorganisms such as *Saccharomyces* and *Aspergillus* are being used for expression of human milk proteins. Human lactoferrin has been expressed in *Saccharomyces*, but is not commercially available, possibly due to low expression levels [8]. *Aspergillus*, however, is successfully used for the production of recombinant human lactoferrin [9], which is commercially available. Expression levels are very high, making it an attractive system; however, extensive purification is needed and the cost is most likely too high for use as a food additive. Consequently, pharmaceutical applications, being more cost tolerant, are being pursued for recombinant proteins produced in this expression system.

*Expression of Human Milk Proteins in Milk of Dairy Animals*

Some of the first commercial applications for recombinant proteins were obtained following their expression in the milk of transgenic animals.
Pharmaceutical recombinant proteins, such as clotting factors VIII and IX, were early expressed in the milk of sheep and goats. Recombinant human lactoferrin, a major protein component of human milk, is being expressed in transgenic cows [10] and is now commercially available. Purified recombinant human lactoferrin may, however, be too expensive to be used as a food additive or in infant formula. One possible application would be to use cow’s milk containing recombinant human milk proteins, such as lactoferrin, but it has not yet found a market, possibly due to logistics (transport, storage) and/or cost. Human lysozyme has been expressed in the milk of mice [11], and more recently in cows [E. Maga, personal commun.], but, again, there are no commercial applications yet.

Expression of Human Milk Proteins in Plants

Plant biologists have successfully been able to express recombinant proteins in various crops. By using strong promoters, high levels of expression can be achieved. It is also possible to direct the expression so that specific parts of the plant can be utilized, depending on the species. Fruit (bananas), seeds (rice, barley), leaves (tobacco) and tubers (potatoes) are all used for expression of various proteins.

Human lactoferrin has been produced in tobacco [12]. Expression levels were relatively low, however, and the protein needs to be purified extensively before it could be considered for any food applications, making it unlikely as a commercially viable product. There have been no tests of activity of this protein, and it is not yet commercially available. Human lactoferrin has also been expressed in potatoes [13], but it appears that expression levels so far are low. This system is attractive in that potatoes are a normal part of the diet of many people; however, it is uncertain if the protein will have any biological activity after the extensive boiling that is used for potatoes.

Rice has been used for expression of soybean ferritin to increase its iron content [14, 15] and is now being used for the expression of several human milk proteins, such as lactoferrin, lysozyme and α1-antitrypsin [16–21]. Very high expression levels can be achieved; e.g. 5 g of human lactoferrin/kg dehusked rice has been expressed in large scale field trials for several generations [17]. Rice has several advantages as an expression system: (1) rice does not contain any toxic compounds (potatoes contain solanin); (2) rice is one of the first ‘non-milk’ foods introduced to infants, which in part is due to its low allergenicity, and (3) expression can be directed so the protein is either expressed as a storage protein (in the seed) driven by the Gt1 promoter, or when driven by the amylase promoter, expressed only during germination, making malting another possibility. In this case, the crop (i.e. rice seeds) does not contain the recombinant proteins; the proteins are only synthesized when the seeds are put in contact with water. Thus, recombinant human milk proteins can be introduced into the diet as rice in itself and be combined with various other food components (e.g. rice cereal), or a protein
extract can be produced, yielding a product with higher protein and lower starch content.

**Expression of Human Milk Proteins in Rice**

We are using rice as an expression system to evaluate the biological activity of select human milk proteins. To date, we have expressed lactoferrin, lysozyme and \( \alpha_1 \)-antitrypsin at very high levels, and large-scale field trials for several generations show that the transgenic rice is stable, expression levels are similar through generations, and the proteins are only expressed in the seeds. The genes were synthesized using codon optimization [22], i.e. the GC (guanine-cytosine) content was increased by nucleotide substitution but without changing any amino acid residue. Sequencing of the recombinant proteins confirmed that the amino acid sequence was identical to that of the native proteins. The signal sequence coding for storage was used. The gene was inserted by the so-called ‘gene gun’ technique and calli were grown in culture. Positive plants, detected by extraction and Western blots, were grown in greenhouses to obtain mature seeds. Seeds positive for the recombinant protein were subsequently grown in fields according to USDA regulations. Purified recombinant human lactoferrin and \( \alpha_1 \)-antitrypsin were found to be glycosylated, but the carbohydrate content was less than that of their native counterparts. In other words, rice does recognize the signals for N-linked glycosylation and the specific sites were glycosylated, but the glycans are smaller than those in the native proteins. The carbohydrates in the rice glycans are similar to those in the human N-linked glycoproteins, i.e. mannose and fucose. The terminal residues, however, are usually mannose or xylose, while the native proteins usually have fucose or sialic acid. This difference in terminal monosaccharides in the recombinant proteins may affect stability (e.g. liver clearance) when used for intravenous applications, but is unlikely to have an effect on food applications or to affect allergenicity. In the case of lactoferrin, which has its own specific receptor in the small intestine [6], we have shown that de-glycosylated human lactoferrin as well as different recombinant forms of human lactoferrin (\textit{Aspergillus}, rice) bind equally well to the receptor, i.e. the glycan moiety of lactoferrin is not involved in the binding to the receptor. Thus, we conclude that the glycosylation of these human milk proteins when synthesized in rice is unlikely to affect their nutritional value.

**Biological Activity of Recombinant Human Milk Proteins**

We have verified that the recombinant human milk proteins have their intended biological activities in vitro (fig. 1). Recombinant human lactoferrin was shown to both bind and release iron at low pH in a manner similar to that of native human lactoferrin [16]. It also bound similarly to the human lactoferrin
receptor, which is present on the surface of the human intestinal cell line, Caco-2, grown in culture, and with an affinity similar to that of native lactoferrin. In addition, recombinant human lactoferrin was shown to inhibit the growth of enteropathogenic *Escherichia coli* (EPEC), one of the most common

**Fig. 1.** Comparison of functional and biological activities between nHLf and rHLf. (a) Iron-binding capacity, (b) pH dependent iron-release properties, (c) antimicrobial activity against enteropathogenic *E. coli* (EPEC): 1 μg/ml of either nHLf or rHLf were incubated with $10^6$ cfu/ml of EPEC in the synthetic broth and bacterial growth were measured at various time points by absorbance at 630 nm. Control does not contain any protein but $10^6$ cfu/ml EPEC. (d) binding of nHLf and rHLf to Caco-2 cells. (e) uptake of nHLf and rHLf by Caco-2 cells (from [16]).
causes of diarrhea in infants and children, and at a concentration similar to that of native human lactoferrin. When tested in the conventional assay for lysozyme activity, which is based on the lysis of *Micrococcus lysodeiktus*, the recombinant human lysozyme had an activity similar to that of native human lysozyme. The recombinant human lysozyme also inhibited the growth of EPEC at the same concentration as the native enzyme. Breast milk contains a significant concentration of α₁-antitrypsin [23], and we have hypothesized that this inhibitor of proteolytic activity in the small intestine contributes to the ‘survival’ of some human milk proteins, so that they can exert their physiological activities in the upper small intestine. The recombinant human α₁-antitrypsin inhibited trypsin and elastase to an extent similar to that of native human α₁-antitrypsin [21]. Thus, for these three human milk proteins we have demonstrated that they have activities similar to those of their native counterparts. For two of these proteins, lactoferrin and α₁-antitrypsin, this occurred in spite of some differences in glycosylation.

**Stability of Recombinant Human Milk Proteins**

To be active in the small intestine, the recombinant proteins need to be able to withstand exposure to low pH in the stomach. While the gastric pH of infants rarely is below pH 4–5 for the first 6 months of life, in some cases the pH may be as low as pH 2–3. We therefore exposed the recombinant human milk proteins to low pH, from pH 2 to 5, for 30 min and then adjusted the pH back to neutral. Activities of all three recombinant proteins, assessed as above, were similar to those of the native proteins [16, 18, 21]. If recombinant human milk proteins are to be added to infant formula or baby foods, some degree of processing may be involved. We therefore exposed the recombinant proteins, both in pure form in solution and as added to infant formula, to various heat treatments, ranging from 78 to 100°C for 8 s up to 30 min. Except for the most severe treatment, 100°C for 5 min, which partially inactivated both recombinant and native human milk proteins, these proteins maintained activities similar to those of the native proteins [16, 18, 21].

**Digestive Fate of Recombinant Human Milk Proteins**

Proteins are in general effectively digested by the combined proteolytic activities in the stomach and small intestine. Infants, however, are an exception to this. Infants have low pepsin secretion and the pH of the infant stomach is usually around pH 4–5 [24], a pH too high for significant pepsin activity. In addition, secretion of pancreatic enzymes is immature, limiting the proteolytic activity in the small intestine. Further, some human milk proteins have structures that make them relatively resistant against proteolytic enzymes.
As an illustration of this, we have found significant concentrations of lactoferrin, secretory IgA and $\alpha_1$-antitrypsin in the stool of breast-fed infants [23, 25] and this persists up to at least 4–6 months of age. The proportion of proteins surviving decreases with increasing age, most likely as digestive function matures. It is, of course, also possible that some milk proteins survive passage of the stomach and the duodenum and may exert actions in the upper gut, to become digested further down in the small intestine.

We have developed an in vitro digestive system [26] to evaluate the survival of recombinant human milk proteins. Briefly, the proteins, in pure form or added to infant formula, are exposed to low pH and pepsin for 30 min at 37°C. The pH is then adjusted to pH 7 and pancreatin (a mixture of pancreatic enzymes) is added. The solution is then incubated for 30 or 60 min, and the proteolytic enzymes are inactivated by brief (2–3 min) boiling. The molecular weights of the proteins are assessed by Western blotting, and their activities are assessed as described earlier. For all three proteins we studied, activities remained after treatment, and to an extent similar to that of the native human milk proteins. While some degradation did occur, the major part of the activity remained, and the extent of degradation was similar for recombinant and native proteins.

**Further Testing**

We have shown that recombinant human milk proteins can be produced at very high levels in rice and that they can withstand low pH and heat treatment, as well as proteolytic digestion in vitro. Further efficacy and safety trials in animals and humans will be needed next.

Safety studies in rats will be necessary prior to human trials. For efficacy studies, we have chosen to first use a rat pup model, in which we can assess the resistance against proteolytic activity as well as anti-infective properties (incubate with pathogens). We have also used infant rhesus monkeys. This non-human primate model of human infants has a high degree of validity as their gastrointestinal physiology is very similar to that of human infants, and rhesus milk is reasonably similar to human milk both in nutrient content and with regard to bioactive proteins (e.g. lactoferrin). Another advantage of this model is that they can be fed regular infant formulas, without any modification of nutrient content, exclusively for 4–6 months. The addition of recombinant human milk proteins to infant formula can therefore be evaluated under realistic conditions. This model also allows us to infect the infant monkey with a pathogen, such as EPEC, and evaluate the effect of the added protein on diarrhea prevalence, severity and duration [27]. Such studies, of course, are not possible in human infants.

Once efficacy has been shown in these models, human trials are needed both with regard to efficacy and safety. Recently, we have evaluated the effects of
adding recombinant human lactoferrin and lysozyme to the WHO oral rehydration solution (ORS) used to treat children with acute diarrhea in a prospective, double-blind, randomized controlled trial in Peru. Children receiving ORS with added recombinant human milk proteins had a significant decrease in the duration of diarrhea as compared to children receiving control ORS [Zavaleta and Lönnertal, in preparation]. These recombinant human milk proteins may be incorporated into infant formulas, baby foods and complementary foods and used in industrialized as well as developing countries with the goal of reducing infectious diseases. However, for infant formula efficacy trials are essential as infant formulas are regulated by federal law, the Infant Formula Act, which does not allow additions of novel components unless benefits have been demonstrated. Thus, addition of components primarily for marketing reasons, which occurs in some countries, will not be allowed.

Conclusions

Expression of recombinant human milk proteins in rice is realistic and a possibility for the addition of bioactive factors to infant formula and baby foods. We have been able to produce several such proteins at very high expression levels and have shown that the transmission is stable through several generations. Large-scale field trials have produced such rice in several tons, making it possible for evaluation of efficacy. In vitro studies have shown that these proteins have activities and stabilities similar to those of the native proteins and that they, to a considerable extent, withstand heat treatment. Further animal work and studies in human subjects are needed to document their efficacy and safety.

References

Recombinant Human Milk Proteins


Discussion

Dr. Hernell: I just wanted to add to the discussion that also recombinant human milk bile salt-stimulated lipase produced in transgenic sheep has been subjected to two small clinical trials to treat fat malabsorption in patients with cystic fibrosis. So the recombinant enzyme has been proven safe and has a clinical effect as expected. Then in your list of various expression systems, I missed mammalian cell lines, e.g. Chinese hamster ovary (CHO) cells. If for expression of glycosylated proteins you are concerned about the glycosylation pattern, mammalian cells would probably be a useful alternative because they are likely to yield a glycosylation pattern closer to the native human protein.

Dr. Lönnerdal: It is a good point. I am not sure how economically realistic it would be to add CHO-produced proteins to food products. I don’t know what kind of capac-
ity you have to produce them in quantities needed for foods. I am happy that you brought up the glycosylation issue because it is also possible to insert enzymes on the terminal carbohydrates of the human glycan in rice. Thus, you can produce not just the recombinant human milk protein but you can also put in recombinant enzymes that will start mimicking the glycan. That is something that, if needed, can be done, but for lactoferrin we just have not yet seen a need for it. When it comes to \(\alpha\)-casein, where glycan plays a much more fundamental role, something like that may be needed or the use of CHO cells that will provide a mammalian glycosylation pattern.

**Dr. Haschke:** Thank you for introducing the fascinating world of bioactive components. From the industrial standpoint as you have mentioned, there are so many variables to overcome to apply this concept. The first one is the safety issue, and safety must finally be demonstrated in infants, even if there is no theoretical reason that there might be a safety question. Phase-1 studies cost a lot of money and are very time-consuming. The other thing is the functional outcome study. You showed one study in which the duration of diarrhea was reduced. This is similar to what we can achieve with lactobacillus GG, which is now added to infant formulas. It is exactly the same outcome; a reduction in the duration of diarrhea from 5.4 to 4 days. So of course probiotics are not 100% safe either. But they are more accepted by the consumer and it is very difficult for the industry to make the consumer believe that there is really an advantage unless we can really show positive effects in favor of the infant receiving it. So we have a long way to go, I don’t know whether you would agree with this. In the end, if we are doing something wrong, we will be punished.

**Dr. Lönnerdal:** You are quite right and I have sympathy with you. I am primarily interested in the function of the proteins and not the commercial applications, but I am acutely aware of the fact that the attitudes are very different. In the US the probiotic concept is not an easy-sell at all because these are live bacteria and ‘you don’t eat that’. In Europe, including Sweden, this is a common part of the diet and there are no negative associations with probiotics, whereas in the US there is a much more adverse reaction. Then you have the opposite: in the US you actually have limited negative publicity and attitudes against recombinant proteins as such, so there is not at all the same pressure on the industry to refrain from this because they are in soy, in corn, and on the market already. So again the attitude will depend on what the market will be.

**Dr. Hernell:** Just a comment to follow that. Today I think no one would even dream of not using recombinant insulin and growth hormone. That shows how fast we are moving. Some 20 years ago these recombinant hormones were new on the market; now it is established routine to use them. No one talks about safety anymore.

**Dr. Klassen:** Can you speculate on what the next step will be to demonstrate the synergistic effect you mentioned? In your opinion what bioactive factors should be combined to achieve further benefit? In Dr. Dewey’s presentation we have also seen that there is a tremendous amount of bioactive components present in milk and hence combinations thereof.

**Dr. Lönnerdal:** I can speculate. I have always believed in Aristotle who said, ‘there is a reason behind everything in Nature’. Why would you have so much \(\alpha_1\)-antitrypsin in breast milk? In a way you can view \(\alpha_1\)-antitrypsin as a ‘break molecule’. We have done in vitro studies, and \(\alpha_1\)-antitrypsin will basically delay digestion by pancreatic enzymes to some extent [1]. The possibility would then exist for some of the human milk proteins to have an effect in the duodenum, and perhaps the upper part of the jejunum, and then, after that, they would start being digested. It is a possibility that we have a synergistic effect by which \(\alpha_1\)-antitrypsin would limit the digestion of some of these bioactive milk components in order to allow them to perform various functions.

**Dr. Telmesani:** There is active research towards animals that can produce organs compatible to humans for transplantation. Is there any possibility and/or are there any studies towards cloning the human mammary gland to produce human milk?
Dr. Lönnerdal: I am not sure how easy it would be. We dreamt about many of these things 20 years ago, and we are moving fast. When it comes to whole organs and tissues like that I am not sure. We have immortalized human cell lines so certainly the possibilities are there to get closer to organs, but we may not reach the stage where we would have ‘human dairy industries’.

Dr. Yun Cao: Does the breast milk of mothers with very premature babies also contain this kind of active protein?

Dr. Lönnerdal: That is a very good question. In fact, for many of these bioactive proteins, the richest source you can find is colostrum and the milk of women who deliver prematurely, because in a way premature milk can be viewed as almost a ‘super colostrum’. Previous research on colostrum and ‘premature milk’ has shown that concentrations of lactoferrin are incredibly high, those of secretory IgA are high, and many of these bioactive proteins are very high in concentration. It is very costly to care for premature infants, and premature milk fortifiers or preterm formula may be applications where you would start to see such proteins added, as it is so important to protect the preterm infant.

Dr. Roggero: Could conservation (human milk bank) or pasteurization alter these biological proteins or substances?

Dr. Lönnerdal: It varies. One of my former students, Dr. Madeleine Sigman, has actually studied banked human milk and found that the extent to which these proteins survive varies with the protein. Lactoferrin is relatively tough, secretory IgA is also relatively tough, whereas lysozyme may be more affected by heat. The problem we have had is that the technical qualities of the milk change with heat treatment, so purifying proteins from breast milk is much more difficult because they tend to aggregate more after pasteurization. But from the infant point of view, it still seems that most of the bioactive proteins are still active after pasteurization.

Dr. Hernell: I just want to add that if you compare fresh and pasteurized human milk with respect to functional effects, the most striking difference is decreased fat absorption from pasteurized milk. The reason being that the milk bile salt-stimulated lipase is rapidly activated at 62.5°C. This temperature, which is typical for the pasteurization of human milk, is critical for both lactoferrin and IgA. A slight increase in temperature will rapidly increase the inactivation of both.

Reference

I would like first to thank you all for sharing your experiences and exchanging knowledge; we all have benefited from it. We all shared the objectives and the goal that we wish to further the lot of children in the world. To that goal these exciting 3 days have enhanced our ability to carry out our objectives. It is appropriate that we thank the sponsors who have brought us together here, and that is the Nestlé Nutrition Institute, its director, Dr. Ferdinand Haschke and Dr. Denis Barclay who have organized this meeting, and we must also not forget Dr. Philippe Steenhout who conceived this conference and organized it initially before he turned things over to Dr. Barclay and we are grateful for all their efforts. We also express our gratitude to the local organizers, Pierre Schaufelberger; we thank Vipapan Panitantum and Montip Nagsevi and all the other staff of Nestlé Viet Nam for organizing this so splendidly and for feeding us so excellently.

Now it is Dr. Rigo’s and my job to summarize what has happened in the last 3 days and that is essentially impossible. We decided to steal a few of your slides and show them again, mainly to remind you of what you heard during the meeting. That is what we are going to do over the next 30 min and Dr. Rigo will start and I will finish up.

Ekhard Ziegler

First of all I want to thank you, Dr. Ziegler, for his collaboration in this meeting; it was so easy for me to work with him. If any of you are asked to organize a meeting, you need to do it with Dr. Ziegler.

We started the workshop with a beautiful overview by Berthold Koletzko on the early feeding and the programming effect up to adult age. His topic focused on the protein hypothesis, and all of us are now waiting for the results of this wonderful controlled randomized study.

After that we had a very elegant presentation by Allison Yates on the dietary reference intakes and the new concept for protein and energy requirements. She showed us that all the components of the dietary reference...
intake were used in the new reference for the different nutrients and macro- and micronutrients in the diet of pediatric patients and also adults. She showed us the different components of this dietary reference intake.

After that we had the opportunity to meet a few of the main workers in the new recommendation data, and it was very interesting to discuss the new protein and energy requirements for infants and children. First of all, Nancy Butte presented the energy requirements. These data were based on the increase in total energy expenditure which was evaluated with doubly labeled water and also heart rates. She presented the new energy requirements in infants. The new energy requirement is far from the previous one, and at the end of the first year of life the new energy requirement is quite decreased to about 15%. Then it was shown that in large infants there is also a significant difference between the new energy requirements when the children are 5–12 years old.

In the following presentation Peter Garlick showed us the protein requirements for infants and children which are mainly based on multiple nitrogen balance studies in order to evaluate the need for maintenance and the question of utilization for growth. We can base the requirements on maintenance and protein deposition according to the slope observed during multiple metabolic balance studies in infancy. The protein deposition was calculated using recent measurements of total body potassium content in infants and children between 4 and 18 years of life. If we now look at the new approach to protein requirements we see that there is a relative reduction in the protein requirement during the first 6 months of life which was also based on the human milk intake in those infants. There is very good agreement between the milk protein intake and the requirement calculated by the factorial approach. So the protein requirement seems also to be slightly lower for large infants. Amino acid requirements were also presented according to the same technology. It was a factorial approach because presently there are some insufficient results on the indicator of amino acid oxidation. Dr. Garlick presented data for infants between 1 and 4 years of age and the new recommendation represents between 60 and 95% of the previous one according to the different essential amino acids.

Following this presentation the question was to know what the potential toxic effect or deleterious effect of high protein supply could be, and this was presented by Irene Axelsson. From these data we can see that there are different indices of protein overload when we increase the protein supply in the newborn infant. Eventually we can speculate on some long-term effects, and she presented data on the increase in kidney growth in formula-fed versus breastfed infants, and we know that nephrogenic activity is completely closed at the time of birth.

After that the second discussion was on the growth of breastfed and formula-fed infants, and in his presentation Ekhard Ziegler tried to answer the question, can the energy and protein intake of breastfed infants be emulated in formula-fed infants. He showed that for energy intake, even with this perturbation in the energy density of formulas, it is only possible to affect the
energy intake and growth during the first 2 months of life but no later. In terms of protein intake he concluded that emulating the protein intake of breastfed infants in the first 4 months of life is possible, but with complex interventions. It can probably also be achieved with starter formula with 1.8 g protein/100 kcal and follow-up formula with 1.5 g/100 kcal. But at a borderline protein concentration it appears possible that infants compensate with a greater intake of formula thereby consuming an excess amount of energy.

So following the first 2 days the question arose, is it possible to translate the new recommendations into clinical practice. When I teach nutrition to students in Belgium I have the habit of discussing the evolution of energy and protein supply during the first year of life, and I use this example: body weight according to time. During the first month of life we provide close to 200 ml/kg, or 600 ml of milk. The babies receive only milk until about 5 months of age and at this time the milk intake decreases progressively to 150 ml/kg body weight/day. At 5 months we start with complementary feeding and provide some food mixtures. At 6 months we start with a little meal at lunch, and an evening meal at 9 months of age. Therefore from the feeding we can calculate what could be the energy and protein supply during the first year of life. Presently the recommendation for the mother is to use the lowest protein content formula during all the first year of life. If we then recommend decreasing the protein supply, not giving too much meat or protein during the evening meal, and then calculate the protein supply, we find that energy is relatively high during the first month of life, 130 kcal/kg/day. It decreases at 3 months but stays close to 100 kcal, which is about 20% more than the new recommendations. When we look at protein supply it is about 2.4 during the first months of life and after 6 months it is still about 2 g protein/kg body weight/day. So much more than the new recommendation. We need to think about what could be the consequences of the new recommendations. Recommendations should be translated into regulations. We have the Codex Alimentarius and the European Union directives needed to provide the new recommendations. I also think that there is an implication for industry; products need to be adapted to the recommendations. We also need prospective studies to evaluate the safety of the new recommendations in newborn infants.

Jacques Rigo

The first talk I will review is Jacques Rigo's talk about body composition and he obviously has spent a great deal of effort and has immense data on the body composition of growing premature and full-term infants. He produced graphs showing, for instance, that the various methods we have, including TOBEC, DEXA and the indirect methods that Nancy Butte used, all produce about the same pattern of change in fat-free body mass; the change in fat mass is expressed as percentage per kilogram. But Jacques Rigo showed that
premature infants who are fed formula do better in terms of gain in fat-free mass, but they also gain a little bit more in fat mass.

We come now to the very exciting talk by Hans van Goudoever. I gained several insights that furthered my knowledge. One was that the gut of young animals, and by extension probably also of young infants, undergoes tremendous growth disproportional to body weight and body growth. One of the implications is that most infants who are growing rapidly probably have abdominal discomfort most of the time because their gut is also growing so fast. But the real scientific insight for me was that premature infants who receive not unreasonable protein intakes in the first few days don’t oxidize any amino acids. This can only mean that they are not getting enough protein because oxidation is turned on only when there is a surfeit of amino acids. Lysine oxidation is close to 0.3 and in oxidation it is close to 0 in premature infants. I really admire him for these studies because they are not easy to do; they are easy to do in piglets but in human infants they are quite involved. Therefore we are grateful to him for having done these excellent studies. He concluded overall that both in humans and pigs high amounts of dietary amino acids are utilized first pass by the intestine, and one implication is that, when we estimate required dietary intakes of protein for premature infants, we make a correction for the inefficiency of intestinal absorption and we use factors like 10 or 15%. I think we probably have to apply a much higher correction factor because some of the limiting amino acids are retained by the gut and not made available systemically.

Now we come to the talk by Harvey Anderson that we heard over the phone this morning. It was quite a feat for Denis Barclay to arrange this and for the audiovisual people to pull it off; I have never been at a conference where we had a speaker more than 9,000 km away. You remember that as he spoke he mostly raised questions which is for the most part where the field is; the regulation of food intake consists of a large number of questions with very few answers. He showed us some very interesting findings, but we are far from any good understanding. One of the real problems is that when we say regulation we have to think of very short-term, short-term and long-term, and ultimately what counts in human nutrition or any nutrition is long-term regulation, week to week. After all what makes us obese is long-term excess of energy intake or energy retention.

Now next we come to Carlo Agostoni who told us that most studies that have looked at the protein intake of older infants and toddlers find that the protein intake is high in terms of requirements. We don’t know why toddlers and older infants are receiving such high protein intakes, perhaps tradition or custom, but we cannot conclude that the infant has a need for such high protein intake. So the infant must somehow select because at that age it has very limited selection, but it is not an impossibility. Of course the current hypothesis is that such high protein intakes have adverse effects on the later development of obesity, and this I have to remind you is a useful hypothesis,
nothing more. I think that being big is not necessarily going to be a disadvan-
tage because we all know that height confers very significant advantages in
life. So there may be a higher risk of diabetes, but everything being equal a big
child could succeed in life much more than their little sibling.

Kathryn Dewey gave us a wonderful talk about breastfeeding and why and
when we should introduce complementary foods. I found her arguments very
compelling, even though I feel that in industrialized countries it is not a big
issue and I think she agrees with that. But in developing countries it is a big
and important issue, and we are grateful to her. We all heard her conclusions
and I think they are well supported by the data. I especially like her findings
about iron deficiency because I find the same thing that breastfed babies are
at a considerable risk of becoming iron-deficient.

Then we heard from Jehan-François Desjeux about a new development in
the rehabilitation of malnourished infants and children. The food that he told us
about is the RUFT, ready-to-use therapeutic food, and I was quite impressed by
the benefits of this food. I know very little about malnutrition but I think this is
a very promising approach. The conclusion was that, in severely malnourished
children presenting with dehydration and secondary cholera, rigorous intra-
venous and oral rehydration is effective with full and rapid recovery.

Katherine Macé gave us a wonderful overview of the new developments in
dairy technology. A summary of plasma tryptophan levels in babies receiving
the various formulas shows that the tryptophan levels in α-lactalbumin-
enriched formula are similar to those in breastfed babies, and when casein gly-
comacropeptide is removed the threonine levels become more like those in
breastfed babies. Later we heard that the glycomacropeptide isn't perhaps all
that bad after all if we were able to get rid of the threonine it contains, which is
the problem. She gave us her overview of what she thinks technology is going to
go. She thinks that a further decrease in the protein quantity in infant formulas
is certainly feasible, but their added benefit of health in term infants is ques-
tionable, and that implies also for follow-on formulas, not for starter formulas.

Finally Bo Lönnerdal's wonderful presentation, which you must still have
in mind. He and his colleagues have expressed human recombinant human
milk proteins in rice and this is a very exciting development. The nice thing is
that this is an edible plant, no extensive purification is needed, essentially the
recombinant rice can be fed. The conclusions were that bioactive proteins
could be added to infant foods, hopefully that will happen in our lifetime, the
regulatory agencies and the public willing.

I wish you all a good and safe trip home. It was great to have you all here.

Ekhard Ziegler

A few words from the perspective of the Nestlé Nutrition Institute. This
workshop is coming to an end and I must say it was very attractive, compelling
and motivating because we learned a great deal, with a lot of new findings which we could discuss and to which we could observe your reactions. So for us at the Nestlé Nutrition Institute, it was a great opportunity being together with you here in Viet Nam.

I must say there were a number of key issues that became completely clear to me, among these being protein energy requirements, amino acid requirements and utilization. There are also a few issues which remain unclear, such as breast feeding and obesity, and the timing and way to introduce complementary feeding. The speakers addressed all aspects in a very fair and balanced way and I would like to thank them sincerely for their efforts; many were not easy to present. Then there also issues where considerable research is needed for clarification. For example, Dr. Axelsson mentioned that we are hunters and that we should feed meat to infants. This is something completely new, and I think you would all agree that we need research to clarify this.

Another question which surprised me is that cereals are not considered amongst the best choices for complementary feeding. I am surprised for two reasons. The first is that in 1867 the founder of our company developed a cereal-based milk to promote child survival. Scientific publications on cereals were already in German publications, dominating pediatric literature, 40 years before appearance of the first US infant nutrition publications. I know this because I had to review all the literature for Dr. Sam Fomon for his book on the history of pediatric nutrition. I am also surprised because cereals are an integral part of culture and feeding habits in many countries. Emigration of European populations to North America and elsewhere have propagated European complementary feeding practices in which cereals are not the primary source of nutrition. However in Latin America, Africa and especially Asia where 80% of the world’s babies are born, infants and children would not receive adequate nutrition nor even survive without cereal-based complementary foods. Finally, cereals are very convenient since they are well suited to fortification and can thus contribute to enhancing the nutrition of infants and children in many emerging as well as industrialized countries. So research is needed; we are listening to you and perhaps we will meet 5 years from now to discuss the next generation of findings.

I would like to close by thanking the two Chairpersons of this workshop, Ekhard Ziegler and Jacques Rigo, for putting together this fascinating scientific program. Thanks also to all the speakers and participants for their contributions, as well as the local Nestlé team headed by Ms. Montip Nagsevi and Ms. Vipapan Panitantum, for their most professional workshop organization and wonderful social events. Hopefully we will all meet somewhere else in the world at another Nestlé Nutrition Workshop.

Ferdinand Haschke
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