Aus der Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital der Ludwig-Maximilians-Universität München

Direktor: Univ.-Prof. Dr. Christoph Klein

# Reference values for micronutrient intake and their derivation from micronutrient requirements. The effect of iron supply on neurodevelopment and immunity in infants, children, and adolescents

Dissertation

zum Erwerb des Doktorgrades der Humanbiologie

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

vorgelegt von

María Hermoso Cristóbal

aus

Jaén

2011

Mit Genehmigung der Medizinischen Fakultät

der Universität München

| | |
|---|---|
| Berichterstatter: | Prof. Dr. Berthold Koletzko |
| Mitberichterstatter: | Prof. Dr. Simon Rothenfußer |
| | Prof. Dr. Johannes Georg Schöber |
| Mitbetreuung durch den promovierten Mitarbeiter: | |
| Dekan: | Prof. Dr. med. Dr. h. C. M. Reiser, FACR, FRCR |
| Tag der mündlichen Prüfung: | 23.11.2011 |

**Table of contents**

# 1 INTRODUCTION

Classically, nutrient requirements have been based mainly on the amounts of nutrients needed to both prevent deficiencies and to maintain body stores of the nutrients. Over the last decade attention has increasingly focussed on using the reduction in risk of chronic disease as the basis for establishing nutrient requirements. This requires insight into the causal link between micronutrient intakes and either disease or health outcomes of public health concern. At the same time, it is now well recognised that optimal nutrition during pregnancy, lactation and infancy has long-term effects on child health, well-being and performance, extending into adulthood and old age. Despite the importance of early nutrition, conducting studies in infants and children and in pregnant and lactating women has important ethical limitations, and few adequate studies have been performed in infants and children. Thus, the available scientific database on the nutritional requirements of these populations is limited. Their nutrient requirements are therefore often based on approaches which ignore the long-term effects of nutrition.

The physiological requirement of a nutrient is defined as the amount and chemical form of the nutrient needed systematically to maintain normal health and development, without disturbance of the metabolism of any other nutrient and without extreme homeostatic processes and excessive depletion or surplus of bodily reports (Aggett et al., 1997). The corresponding dietary requirement would be the intake sufficient to meet the physiological requirement taking into account aspects such as the absorption of the nutrient from the diet. The nutrient requirement differs between individuals, and the recommended intake (value of reference for intake of a certain nutrient) for a certain population aims to cover the nutrient requirement of almost all individuals in that population (Pijls et al., 2009). Thus, reference values for nutrient intake do not refer to individuals, but are set for populations defined by age, gender and in some cases additional characteristics that are relevant for nutrient needs. They are used for assessing the adequacy of dietary intakes of populations and for planning desirable dietary intakes, and they are part of the basis for food and nutrition policies such as the development of food based dietary guidelines, food fortification and enrichment programmes, food regulations and trade, and nutritional education. They are also used in nutrition labeling because the amount of a nutrient contained in a portion or in 100g is expressed as a percentage of a nutrient reference value (Ashwell et al., 2008).

Most European countries have set reference values for nutrient intake for several population groups: for instance, for age groups (infants, children and adolescents, adults, elderly) and for population in special life stages (pregnant women, women who are lactating). The Netherlands, the United Kingdom, France, Latvia, Lithuania, the Nordic countries (Norway, Sweden, Finland, Denmark and Island), and Germany, Austria and Switzerland (DACH countries), published their own standards. Other countries based their reference values on those from other countries and organizations (Doets et al., 2008). The European Union's Scientific Committee for Food produced its own report, Nutrient and Energy Intakes for the

European Community. Outside Europe, the United States and Canada issued their own values, as well as Australia and New Zealand. At a global scale, the World Health Organization together with the Food and Agriculture Organization has also set reference values for nutrient intake.

**Variability of reference values for micronutrient intake**

Current reference values for micronutrient intake vary considerably across Europe. For example, for children at 5 years of age, there is a twofold difference between the value given fpr orpm by the German, Austrian and Swiss Nutrition Societies (DACH) and the value set by the European Union's Scientific Committee for Food. Table 1 illustrates differences of reference values for iron intake established by four different committees for some population groups and life stages. Variability is caused by various factors, e.g. disparities that exist between underlying concepts, the terminologies used and the methodologies and assumptions that have been made in the process of defining micronutrient requirements and deriving reference values for intake (Doets et al., 2008). Committees and expert groups tasked with the setting of reference values have used for their purposes the scientific evidence available at a certain point of time. This also contributes to variation, because reference values have evolved with the progress in science. Also, values are set for different population groups, but the definition of population groups is not consistent across Europe. For instance, reference values for infants are set for two up to four age groups in the first year of age. When establishing reference values, committees have considered different population characteristics such as the life cycle, physical activity level, smoking and bioavailability. When scientific reports informing about the process are available, transparency is in general not very well accomplished. In case there is transparency, there are enormous differences in the type of evidence used, in the selection of endpoints selected, and in the selected criteria for adequacy. Often, experts' opinions are used. When no scientific data are available to establish the requirements of certain groups (e.g. young children), extrapolation and interpolation methods between age groups are used, and these methods often differ (Doets et al., 2008).

Table 1: Comparison of current reference values for iron intake (mg/day) established for different ages and life stages.
Source; Nutri-RecQuest (Cavelaars et al., 2010)
(http://www.serbianfood.info/eurreca/index.php)

| | Infants 3 months | Infants 9 months | Children 5 years | Children 10 years | Adolescents 15 years | Lactating women 35 years | NPNL* women 35 years |
|---|---|---|---|---|---|---|---|
| **EC** | - § | 6 | 4 | 6 | 19 | 10 | 15 |
| **Netherlands** | 5 | 7 | 7 | 7 | 12 | 20 | 15 |
| **DACH** | 0.5 | 8 | 8 | 15 | 15 | 20 | 15 |
| **WHO** | - § | 10.5 | 7 | 7 | 35 | 17 | 33 |

§ Reference value not established for infants younger than 6 moths
* Non-pregnant, non-lactating

The need for the harmonisation of reference values for micronutrient intake across Europe was recognised by the European Commission's Directorate-General Research in EC Call T5.4.2.1 entitled "Nutrient status and requirements of specific population groups". In response to this call, funding for the EURRECA Network of Excellence (NoE) was then started for the period 2007–2011. 'EURRECA' stands for EURopean RECommendations Aligned and its full name is aligning micronutrient recommendations across Europe with special focus on vulnerable groups and consumer understanding (Pijls et al., 2009) . EURRECA attempts to consolidate the basis for the definition of micronutrient requirements across Europe (Ashwell et al., 2008).

Some initiatives already exist to overcome this fragmentation. A large variety of terms are used to define reference values worldwide, because several committees have created their own terminology: Dietary reference values (DRVs) used by UK, nutrient reference values (NRVs) used by Australia and New Zealand, reference values for nutrient intake used by the DACH countries, and dietary reference intakes (DRIs) used by US and Canada. Although these terms describe very similar concepts, their meanings are not exactly the same, which still causes considerable confusion. A group of international experts convened by the United Nations University's Food and Nutrition Programme, in collaboration with the Food and Agricultural Organization (FAO), the World Health Organization (WHO), and the United Nations Children's Fund (UNICEF) has agreed on the term nutrient intake values (NIVs), which encompasses all nutrient-based dietary standards derived from primary data and provide estimates on appropriate dietary substrate supply for populations of healthy people. The UNU Working Group has defined the following NIVs: Average nutrient requirement

(ANR), individual nutrient level (INL) and upper nutrient level (UNL). ANR is the estimated average or median requirement of a specific nutrient in a population, derived from a statistical distribution of requirement criterion and for a particular age and sex specific group based on a specific biological endpoint or biochemical measure. It is assumed that individual requirements follow a statistical distribution (symmetrical bell-shaped curve). INL is the individual nutrient level and $INL_{97}$ is the nutrient intake considered adequate to meet the known nutrient needs of practically all healthy individuals in a particular age and sex-specific group. Equivalent terms are population reference intakes (PRI) and recommended dietary allowance (RDA). This value, set at a level of intake that meets the needs of the majority of the population (mean + 2SD), is generally used as the target for provision of essential nutrients to populations and as the reference point for nutrient labelling of foods. The exception is energy, for which the ANR is used because the use of $INL_{97}$ or equivalents would lead to overfeeding. UNL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals of a particular life-stage group (King and Garza, 2007).

An agreement on terms and definitions is an important step towards the harmonisation of reference values. Equally important is the consolidation of the scientific basis for the definition of micronutrient requirements. There are two ways of helping this consolidation: First, understanding the differences between established micronutrient requirements and derived values for recommended intakes (e.g. methodological differences). Second, updating the scientific basis on which the requirement for a micronutrient can be based by reviewing the current best evidence available. Systematic reviews of the evidence provide a transparent and rigorous evidence review process for the expert committees and facilitate the update of reference values as new data become available (Chung et al., 2010). EURRECA is conducting systematic reviews on those micronutrients that are deemed to be of major importance for certain population groups, following a standardised methodology.

The research reflected in the three publications included in this dissertation exemplifies the two approaches. This research was carried out in the frame of the EURRECA Network of Excellence. The first two manuscripts tackle issues relevant to the determination of micronutrient requirements of lactating women and of infants until the $12^{th}$ month of life. This includes physiological factors, definitions and specific terms as well as methodological approaches used to establish reference values for micronutrient intake in these population groups. The sources of variation between current established requirements and reference values for micronutrient intake are pointed out. The third manuscript reports a systematic review of randomised controlled trials assessing the effect of iron supply on two important functional outcomes of infants, children and adolescents: 1) neurodevelopment (including cognition and psychomotor development) and 2) immune function. This review represents an update of the available evidence on the effect of iron on those two important outcomes, and its results should help making decisions on the optimal level of iron required from birth to puberty.

## Considerations on the micronutrient requirements of lactating women and infants and derived reference values for micronutrient intake

*Lactating women*

There are difficulties and ethical limitations in conducting research in women during reproductive stages, so metabolic data on which nutritional requirements are often based are lacking for this physiologically important stage of life. Requirements of nutrients in lactating women are therefore established applying a factorial method: the nutrient need of a woman who is lactating is calculated by summing the nutrient need of a non-lactating woman of similar periconceptional age, and the amount of nutrient secreted into an average volume of breast milk, making some adjustments for bioavailability in some cases. Although committees apply similar extrapolation methods to calculate requirements, reference data applied for nutrient composition of milk disparate (Atkinson and Koletzko, 2007). There are a number of aspects which should be considered when defining the nutritional requirements of healthy women during the lactation period. The second publication of this dissertation addresses challenges for establishing reference values for micronutrient intake in this population group and factors which can have a modifying impact on reference values, such as the variability of breast milk volume and composition, stages of lactation, maternal age (adolescent pregnancy), birth spacing and the duration of the recuperative interval, diet, lifestyle, socioeconomic and cultural factors, and the effect of maternal nutritional status. A comparison of reference values for micronutrient intakes for lactating women used in European countries as well as the level of change compared to reference values for non-pregnant, non-lactating women is provided.

*Infants*

Infancy is characterized by growth and development, which impose unique nutritional needs upon the already high maintenance needs of infants. Few quality studies have been performed in this age group, and the nutritional requirements of infants have been established through indirect methods such as usual intakes of presumably healthy populations, factorial approaches that take into account the amount of nutrient needed for growth and the amount lost in the body, and balance techniques. The first paper of this dissertation summarises and critically evaluates the methodological approaches followed to establish the micronutrient requirements of healthy infants and to derive reference values for micronutrient intake from those requirements. A sample of seven countries/committees which set their own reference values (not based on the reference values set by other countries/committees) and for which background reports were available, was used for this analysis. The publication also gives an overview of the latest knowledge on nutritional requirements (macronutrients and micronutrients) of infants in early and late infancy.

**The effect of iron supply on the neurodevelopment and immune function of infants, children, and adolescents**

Iron is a critical substrate for infants, children and adolescents. This is due, on one side, to rapid growth, hemoglobin expansion and development experimented during those periods; and, on the other side, due to the increased need for iron. Especially infants are considered a vulnerable group because they have a relatively high requirement of nutrients per unit body weight during a sensitive period of rapid growth and development. Adolescent girls are another risk group due to menstrual losses. However, there are still open questions on the adequate iron intakes during these periods of life and subsequently, on the dietary intake of iron to recommend, and also for instance to put in infant formula.

Ideally, the definition of micronutrient requirements would take into account relationships between micronutrient intakes in the diet and health outcomes. In the absence of primary data from quality studies linked to functionally relevant outcomes, calculations of the physiological requirement for absorbed iron for infants, children and adolescents are currently based on factorial modelling. The components of iron requirement used as factors in the modelling include basal iron losses, menstrual losses, increased requirement during growth for the expansion of blood volume, and/or increased tissue and storage iron. This approach does not address the issue of iron intake supporting long-term health and optimal functional capacities rather than just avoiding acute deficiency states.

Observational studies have shown that iron deficiency and iron deficiency anemia can have a serious impact on infants' and children's health and later development: alteration of the immune status, adverse effects on morbidity, delayed behavioural and mental development, below average school achievements and growth retardation. The clearest evidence between diet and health is found when observational studies are confirmed by experimental studies. Although some studies have been performed using health outcome to assess iron requirement in infancy, childhood and adolescence, results have been inconclusive. Therefore, a systematic review was carried out to review and update the best evidence available from randomised controlled trials assessing the effects of iron intake on measures of neurodevelopment, including cognitive and psychomotor development, and on immunity in infants, children and adolescents. That is the object of the third manuscript included in this dissertation.

**List of publications included in this dissertation**

- Publication 1: **Hermoso, M**., G. Tabacchi, I. Iglesia-Altaba, S. Bel-Serrat, L. A. Moreno-Aznar, Y. García-Santos, M. d. R. García-Luzardo, B. Santana-Salguero, L. Peña-Quintana, L. Serra-Majem, V. H. Moran, F. Dykes, T. Decsi, V. Benetou, M. Plada, A. Trichopoulou, M. M. Raats, E. L. Doets, C. Berti, I. Cetin & B. Koletzko (2010) The nutritional requirements of infants. Towards EU alignment of reference values: the EURRECA network. *Maternal & Child Nutrition,* 6**,** 55-83.

- Publication 2: Hall Moran, V., N. Lowe, N. Crossland, C. Berti, I. Cetin, **M. Hermoso**, B. Koletzko & F. Dykes (2010) Nutritional requirements during lactation. Towards European alignment of reference values: the EURRECA network. *Maternal & Child Nutrition,* 6**,** 39-54.

- Manuscript 1*: **Hermoso, M**., V. Vuvic, C. Vollhardt, C., A. Arsic, B. Roman-Viñas, I. Iglesia-Altaba, M. Gurinovic. & B. Koletzko. Iron and neurodevelopment and immunity in infants, children and adolescents: a systematic review. (submitted in February 2011)

* Manuscript 1 has been submitted for publication and is still under revision.

Appendix A includes the search strategy used to carry out the search in electronic databases for the systematic review reported in Manuscript 1.

# 2 REFERENCES

Aggett, PJ, Bresson, J, Haschke, F, Hernell, O, Koletzko, B, Lafeber, HN, et al. (1997) Recommended Dietary Allowances (RDAs), Recommended Dietary Intakes (RDIs), Recommended Nutrient Intakes (RNIs), and Population Reference Intakes (PRIs) are not "recommended intakes". *Journal of Pediatric Gastroenterology and Nutrition* **25,** 236-241.

Ashwell, M, Lambert, JP, Alles, MS, Branca, F, Bucchini, L, Brzozowska, A, et al. (2008) How we will produce the evidence-based EURRECA toolkit to support nutrition and food policy. *European Journal of Nutrition* **47 Suppl 1,** 2-16.

Atkinson, SA & Koletzko, B (2007) Determining life-stage groups and extrapolating nutrient intake values (NIVs). *Food and Nutrition Bulletin* **28,** S61-S76.

Cavelaars, AE, Kadvan, A, Doets, EL, Tepsic, J, Novakovic, R, Dhonukshe-Rutten, R, et al. (2010) Nutri-RecQuest: a web-based search engine on current micronutrient recommendations. *European Journal of Clinical Nutrition* **64 Suppl 2,** S43-47.

Chung, M, Balk, EM, Ip, S, Lee, J, Terasawa, T, Raman, G, et al. (2010) Systematic review to support the development of nutrient reference intake values: challenges and solutions. *American Journal of Clinical Nutrition* **92,** 273-276.

Doets, EL, de Wit, LS, Dhonukshe-Rutten, RA, Cavelaars, AE, Raats, MM, Timotijevic, L, et al. (2008) Current micronutrient recommendations in Europe: towards understanding their differences and similarities. *European Journal of Nutrition* **47 Suppl 1,** 17-40.

King, JC & Garza, C (2007) Harmonization of nutrient intake values. *Food and Nutrition Bulletin* **28,** S3-12.

Pijls, L, Ashwell, M & Lambert, J (2009) EURRECA - A Network of Excellence to align European micronutrient recommendations. *Food Chemistry* **113,** 748-753.

# 3 AUTHOR'S CONTRIBUTION

The main contribution of the author to the three articles is described as follows.

- Publication 1: preparation of the manuscript, coordination of co-authors' input, revision of literature on the nutrient requirement of infants, description of approaches used to establish requirements and reference values, detailed comparison of methods used to establish requirements and reference values for vitamins by a sample of seven committees, conclusions and discussion, and revision of the manuscript and integration of reviewers' comments towards publication.

- Publication 2: comparison of current reference values for micronutrient for lactating women and comparison of values compared to non pregnant-non lactating women, revision of the methodological approaches to derive reference values for lactating women, and critical revision of the draft.

- Manuscript 1: selection of health outcomes in the infant population group for the systematic reviews conducted in the frame of EURRECA by means of a protocol (see article by Cavelaars et al in the list of publications). Conduction of the systematic review of RCTs evaluating the selected outcomes for the infant population group, Exploration of possibilities for meta-analysis, preparation of the draft manuscript, coordination of co-author's input, incorporation of part of the review concerning data on children and adolescents, conclusions and discussion .

# 4 LIST OF PUBLICATIONS

Since March 2008 to present, the author has contributed to the publications listed below. These publications are being produced in the frame of several research activities within the EURRECA Network of Excellence (see Introduction). The three publications included in this dissertation are marked with a star (*).

**Submitted/ in preparation:**

1. * Hermoso M, Vucic V, Vollhardt C, Arsic A, Gurinovic M and Koletzko B (2011). Iron and neurodevelopment and immunity in infants, children and adolescents: a systematic review. **Submitted.**

2. Hermoso M, Vollhardt C, and Koletzko B (2011). Effect of iron intake in biomarkers of iron status in infants: a systematic review with meta-analysis. In preparation.

**Published:**

3. Brown K, Timotijevic L, Barnett J, Ruprich J, Øehùøková I, **Hermoso M**, Frost-Andersen L, Lillegard I, Fernández-Celemín L, Larrañaga A, Srniæ-Lonèareviæ A and Raats MM (2011). Stakeholders' beliefs on consumer involvement in the development of dietary guidelines: A qualitative study in six European countries. *European Journal of Clinical Nutrition* **Epub ahead of print** Impact Factor 2009: 3.072.

4. Timotijevic L, Barnett J, Brown K, Shepherd R, Fernandez-Celemin L, Domolki L, Ruprich J, Dhonukshe-Rutten RA, Sonne AM, **Hermoso M**, Koletzko B, Frost-Andersen L, Timmer A and Raats MM (2010). The process of setting micronutrient recommendations: a cross-European comparison of nutrition-related scientific advisory bodies. *Public Health Nutrition* **14**(4):716-28. Impact Factor 2009: 2.749.

5. Cavelaars AEJM, Doets EL, Dhonukshe-Rutten RAM, **Hermoso M**, Fairweather-Tait SJ, Koletzko B, Gurinovic M, Moreno LA, Cetin I, Matthys C, van 't Veer P, Ashwell M and de Groot LCPG (2010). Prioritizing micronutrients for the purpose of reviewing their requirements: a protocol developed by EURRECA. *European Journal of Clinical Nutrition* **64 Suppl 2**, S19-S30. Impact Factor 2009: 3.072.

6. Timotijevic L, Raats MM, Barnett J, Brown K, Shepherd R, Fernandez L, Domolki L, Ruprich J, Sonne AM, **Hermoso M**, Koletzko B, Frost-Andersen L and Timmer A (2010). From micronutrient recommendations to policy: consumer and stakeholder involvement. *European Journal of Clinical Nutrition* **64 Suppl 2**, S31-S37. Impact Factor 2009: 3.072

7. Roman-Vinas B, Ortiz-Andrelluchi A, Mendez M, Sanchez-Villegas A, Peña-Quintana L, Moreno LA, **Hermoso M** and Serra-Majem L (2010). Is the food frequency questionnaire suitable to assess micronutrient intake adequacy for infants, children and adolescents? *Maternal and Child Nutrition* **6 S2**, 112-121. Impact Factor 2009: 1.741.

8. Berti C, Decsi Tamas, Dykes F, **Hermoso M**, Koletzko B, Massari M, Moreno LA, Serra-Majem L and Cetin I (2010). Critical issues in setting micronutrient recommendations for pregnant women: an insight. *Maternal and Child Nutrition* **6 S2**, 5-22. Impact Factor 2009: 1.741.

9. Fekete K, Berti C, Cetin I, **Hermoso M**, Koletzko B and Decsi Tamas (2010). Perinatal folate supply: relevance in health outcome parameters. *Maternal and Child Nutrition* **6 S2**, 23-38. Impact Factor 2009: 1.741.

10. * Hall Moran V, Lowe N, Crossland N, Berti C, Cetin I, **Hermoso M**, Koletzko B and Dykes F (2010). Nutritional requirements during lactation. Towards European alignment of reference values: the EURRECA network. *Maternal and Child Nutrition* **6 S2**, 39-54. Impact Factor 2009: 1.741.

11. Iglesia-Altaba I, Doets EL, Bel-Serrat S, Roman-Vinas B, **Hermoso M**, Pena-Quintana L, Garcia-Luzardo M, Santana-Salguero B, García-Santos Y, Vucic V, Frost-Andersen L, Perez-Rodriguo Carmen, Aranceta J, Cavelaars AE, Decsi Tamas, Serra-Majem L, Gurinovic M, Cetin I, Koletzko B and Moreno LA (2010). Physiological and public health basis for assessing micronutrient requirements in children and adolescents. The EURRECA network. *Maternal and Child Nutrition* **6 S2**, 84-99. Impact Factor 2009: 1.741.

12. Sanchez-Villegas A, Brito N, Doreste-Alonso J, Nissensohn M, Henriquez-Sanchez P, **Hermoso M**, Berti C and Serra-Majem L (2010). Methodological aspects of the study of dietary patterns during pregnancy and maternal and infant health outcomes. A systematic review. *Maternal and Child Nutrition* **6 S2**, 100-111. Impact Factor 2009: 1.741.

13. *** Hermoso M**, Tabacchi G, Iglesia-Altaba I, Bel-Serrat S, Moreno LA, García-Santos Y, del Rosario García-Luzardo M, Santana-Salguero B, Peña-Quintana L, Serra-Majem L, Hall Moran V, Dykes F, Decsi T, Benetou V, Plada M, Trichopoulou A, Raats MM, Doets EL, Berti C, Cetin I and Koletzko B (2010). The nutritional requirements of infants. Towards EU alignment of reference values: the EURRECA network. *Maternal and Child Nutrition* **6 S**, 55-83. Impact Factor 2009: 1.741.

**Oral communications at scientific meetings**

14. **Hermoso M**, Vucic V, Vollhardt C, Arsic A, Roman-Viñas B, Iglesia-Altaba I, Gurinovic M and Koletzko B (2011). Iron and neurodevelopment and immunity in infants, children and adolescents. A systematic review. *In 3rd International Symposium on Trace Elements & Health*. Murcia, Spain.

15. **Hermoso M** and Koletzko B for the EURRECA Network of Excellence (2009). Defining micronutrient requirements of infants as a basis for dietary recommendations in the first year of life. *In Nutrition and Nurture in Infancy and Childhood: Bio-Cultural Perspectives*. Grange-Over-Sands, Cumbria, UK.

16. **Hermoso M** and Koletzko B (2008). Alignment of European approaches for micronutrient recommendations in early childhood: the EURRECA project. *In Workshop "Transition from milk to food"*. Obergurgl, Austria

# 5 SUMMARY

Current reference values for micronutrient intake vary considerably across Europe. Variability is caused by several factors, including the disparities that exist between underlying concepts, the terminologies used, the methodologies and assumptions that have been made in the process of defining micronutrient requirements and deriving reference values for intake, and the time point at which references have been set. This causes confusion among decision makers, health professionals, and consumers. Updated, solid evidence on which micronutrient requirements can be based, and identification of factors leading to differences in reference values are important for informing future initiatives aimed at providing standardized approaches to overcome variability of micronutrient reference values across Europe. Ideally, the definition of micronutrient requirements takes into account relationships between micronutrient intakes in the diet and health outcomes. Sometimes, there are no sufficient primary data from quality studies linked to functionally relevant outcomes. This is the case of iron. Iron is a critical substrate for infants, children and adolescents due to the rapid growth and development experimented during those periods of life and the increased iron needs. However, there is controversy about the adequate level of iron they need.

The present work presents a review of current knowledge regarding the micronutrient requirements of specific population groups (lactating women, infants, children and adolescents). A particular focus is put on iron requirements.

Reference values for micronutrient intake used in European countries for infants and for lactating women were analysed to determine the methodological approaches followed to derive them. The main issues contributing to disparities in micronutrient reference values for infants and for lactating women were highlighted, as well as the physiological factors that play a role in the estimation of micronutrient requirements.

A systematic review was conducted to summarize the evidence currently available from randomized controlled trials (RCT) concerning the effect of iron supply of infants, children and adolescents until 18 years of age on measures of neurodevelopment, and on immunity. The Cochrane Library, MEDLINE and EMBASE were searched up to and including February 2010. Studies were also identified by checking the bibliographies of the articles retrieved. Studies were considered from inclusion in the systematic review if they complied with the following criteria: RCTs with an adequate control group; iron supply provided by natural food sources, supplements, formula, or fortified foods; studies carried out in infants, children and adolescents until the age of 18 years, and parameters relevant to the selected outcomes measured. No language restrictions were applied.

The selected articles were evaluated and data were extracted in a standardized form. The internal validity of the studies was assessed based on indicators specific to the RCT methodology. Based on these indicators, the overall risk of bias was determined and classified as high, moderate, or low.

The results and conclusions deduced from this work can be summarized as follows:

1. Following reasons were identified as main factors for the existing variety in established reference values for intake for infants: the conversion of requirements into reference values differently on the basis of body weight or of energy intake without explaining normative data used, the variety of extrapolation methods applied, and the establishment of different age groups in the first 12 months of life without a clear rationale other than the transition from milk to foods.

   Differences in the normative data used for extrapolating requirements for lactation is the main source of variation of reference values for intake for healthy lactating women.

   In general, there is a serious lack of transparency in the process of establishing micronutrient requirements and reference values for intake for both population groups

2. For the systematic review, 13 supplementation studies met the selection criteria for neurodevelopment and 7 for immunity. Most of the studies had a high or moderate risk of bias.

   Due to the variety of results reported, it is difficult to derive clear conclusions for the outcome neurodevelopment. Overall, the studies showed a modest positive effect of iron supplementation on cognition and psychomotor outcomes, especially after long-term supplementation periods (>2 months) and in anemic infants and children.

   A comparison of studies using immunity outcome was not possible due to the heterogeneity of the parameters tested in the different studies. Currently available evidence is too incomplete to make evidence-based conclusions on the effect of iron on immunity.

# 6 ZUSAMMENFASSUNG

Referenzwerte für die Mikronährstoffzufuhr unterscheiden sich sehr stark zwischen den einzelnen europäischen Staaten. Der Zeitraum in dem die nationalen Richtlinien überprüft werden, die verschieden verwendeten Methoden zur Bestimmung des Mikronährstoffbedarfes, die unterschiedlichen Konzepte zur Darstellung von Referenzwerten, tragen unter anderem dazu bei, dass eine große Variationsbreite zwischen den Referenzwerten zu Stande kommt. Daraus resultiert eine große Unübersichtlichkeit für politische Entscheidungsträger, Gesundheitsexperten und Verbraucher. Eine fundierte wissenschaftliche Grundlage, die die Einschätzung des Bedarfs der Mikronährstoffe ermöglicht, und die Identifizierung der Faktoren die zur Variation zwischen Referenzwerten führen, sind wichtig, um zukünftigen Initiativen zur Harmonisierung von Referenzwerten für die Mikronährstoffzufuhr zu dienen und Unterschiede innerhalb Europas zu überwinden. Idealerweise wird der menschliche Bedarf an Mikronährstoffen in Zusammenhang mit gesundheitlichen Zielparametern bestimmt. Oft ist aber die Datenlage aus klinischen Studien nicht ausreichend, um den Bedarf mittels klinischer Evidenz festzulegen. Ein Beispiel hierfür stellt das Eisen dar. Besonders während des Wachstums besteht ein erhöhter Eisenbedarf. Allerdings ist die Höhe des Eisenbedarfs von Säuglingen, Kindern, und Jugendlichen umstritten.

Gegenstand dieser Arbeit ist die Darstellung aktueller Kenntnisse bezüglich des Mikronährstoffbedarfs bestimmter Bevölkerungsgruppen (stillende Frauen, Säuglinge, Kinder und Jugendliche). Ein besonderer Fokus wird auf den Eisenbedarf gelegt.

Auf europäischer Ebene vorliegende Referenzwerte zur Mikronährstoffzufuhr für stillende Frauen und Säuglinge wurden daraufhin untersucht, welche methodischen Ansätze zur Entwicklung dieser Referenzwerte angewendet worden waren. Dabei wurde hervorgehoben, welche Faktoren zu Unterschieden führen, und welche physiologischen Faktoren bei der Bestimmung des Mikronährstoffbedarfs eine Rolle spielen.

Eine umfassende systematische Literaturübersicht wurde durchgeführt mit dem Ziel, alle randomisierten kontrollierten Studien (RCT) zu identifizieren, in denen die Wirkung der Eisenaufnahme von Säuglingen, Kindern und Jugendlichen bis zum 18. Lebensjahr in Bezug auf die Zielgrößen Neuroentwicklung und Immunität geprüft wurden. Die Cochrane Library, MEDLINE und EMBASE wurden bis einschließlich Februar 2010 durchsucht. Die Literaturverzeichnisse der ausgewählten Studien wurden auf weitere relevante Artikel geprüft. RCTs mit einer adäquaten Kontrollgruppe, die die Eisenversorgung aus Lebensmitteln, Supplementen, Säuglingsnahrung oder angereicherten Lebensmitteln bei Säuglingen, Kindern und Jugendlichen bis zum 18. Lebensjahr prüften, und die Bestimmung ausgewählter Zielparametern durchführten, wurden eingeschlossen. Die Sprache der Artikel stellte hierbei kein Auswahlkriterium dar.

Die jeweiligen Artikel wurden begutachtet und ihr Inhalt in standardisierter Form extrahiert. Die interne Validität einzelner Studien wurden anhand eines Fragenkataloges geprüft und dabei das Risiko von systematischen Fehlern nach einem dreistufigen Schema (hoch, moderat, niedrig) bewertet.

Es lassen sich folgende Ergebnisse und daraus resultierende Schlussfolgerungen zusammenfassen:

1. Folgende Gründe wurden für die Uneinheitlichkeit in vorliegenden Referenzwerten zur Nährstoffzufuhr für Säuglinge als Hauptfaktoren identifiziert: Der Mikronährstoffbedarf bei Säuglingen wird auf Körpergewicht oder Energieaufnahme basierend in Referenzwerte für die Mikronährstoffzufuhr umgewandelt. Allerdings sind oft weder die verwendeten normativen Daten, noch die angewandten Extrapolationsmethoden erklärt. Die Etablierung verschiedener Altersgruppen, und damit verbundene spezielle Referenzwerte innerhalb der ersten 12 Lebensmonate sind nicht begründet, mit Ausnahme des Übergangs von Milch zur Beikost.

   Die Vielfalt der verwendeten normativen Daten zur Extrapolierung von Referenzwerten ist die Hauptquelle der Schwankung zwischen Referenzwerten für stillende Frauen in den verschiedenen europäischen Ländern.

   Die unzureichenden erhältlichen Informationen bezüglich der Entwicklung von Referenzwerten für Säuglinge und stillende Frauen in den europäischen Ländern machen einer erheblichen Mangel an Transparenz deutlich.

2. Bei der systematischen Literaturübersicht entsprachen 13 Studien den Selektionskriterien der Zielgröße Neuroentwicklung und 7 der Zielgröße Immunität. Die meisten Studien hatten ein hohes oder moderates Risiko für systematische Fehler.

   Aufgrund verschiedenartiger Ergebnisse hinsichtlich der Zielgröße Neuroentwicklung war keine klare Tendenz ersichtlich. Insgesamt jedoch zeigten die Studien eine geringe positive Wirkung der Eisensupplementierung auf die kognitive und auch auf die psychomotorische Entwicklung, besonders nach langfristigen Supplementierungsperioden (> 2 Monate) und vor allem in anämischen Säuglingen und Kindern.

   Ein Vergleich von Studien, die die Zielgröße Immunität betrachten, war aufgrund der hohen Heterogenität der Zielparameter nicht möglich. Zurzeit ist die verfügbare Datenlage aus RCTs zu unvollständig, um begründete Aussagen über die Wirkung von Eisen auf die Immunität bei Säuglingen, Kindern und Jugendliche zu treffen.

# 7 PUBLICATION 1

Hermoso, M., G. Tabacchi, I. Iglesia-Altaba, S. Bel-Serrat, L. A. Moreno-Aznar, Y. García-Santos, M. d. R. García-Luzardo, B. Santana-Salguero, L. Peña-Quintana, L. Serra-Majem, V. H. Moran, F. Dykes, T. Decsi, V. Benetou, M. Plada, A. Trichopoulou, M. M. Raats, E. L. Doets, C. Berti, I. Cetin & B. Koletzko (2010) The nutritional requirements of infants. Towards EU alignment of reference values: the EURRECA network. *Maternal & Child Nutrition*, 6, 55-83.

Original Article

# The nutritional requirements of infants. Towards EU alignment of reference values: the EURRECA network

**Maria Hermoso*, Garden Tabacchi†, Iris Iglesia-Altaba‡, Silvia Bel-Serrat‡, Luis A. Moreno-Aznar‡, Yurena García-Santos§, Mª del Rosario García-Luzardo§, Beatriz Santana-Salguero§, Luis Peña-Quintana§¶, Lluis Serra-Majem¶, Victoria Hall Moran**, Fiona Dykes**, Tamás Decsi††, Vassiliki Benetou‡‡, Maria Plada‡‡, Antonia Trichopoulou‡‡, Monique M. Raats§§, Esmée L. Doets¶¶, Cristiana Berti***, Irene Cetin*** and Berthold Koletzko***

*Division of Metabolic Diseases and Nutritional Medicine. Dr von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Munich, Germany, †Institute of Physiology and Human Nutrition, University of Palermo, Palermo, Italy, ‡'Growth, Exercise, Nutrition and Development' (GENUD) Research Group, Escuela Universitaria de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain, §Unidad de Gastroenterología, Hepatología y Nutrición Pediátrica. Hospital Universitario Materno-Infantil de Canarias. Las Palmas de Gran Canaria, Spain, ¶Departamento de Ciencias Clínicas. Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, **Maternal and Infant Nutrition and Nurture Unit (MAINN), University of Central Lancashire, Preston, UK, ††University of Pécs, Pécs, Hungary, ‡‡Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, §§Department of Food, Consumer Behaviour and Health, University of Surrey, Guilford, UK, ¶¶Division of Human Nutrition, Wageningen University and Research Centre, Wageningen, the Netherlands, and ***Unit of Obstetrics and Gynecology, Department of Clinical Sciences Hospital 'L. Sacco', and Center for Fetal Research Giorgio Pardi, University of Milan, Milano, Italy*

**Abstract**

This paper presents a review of the current knowledge regarding the macro- and micronutrient requirements of infants and discusses issues related to these requirements during the first year of life. The paper also reviews the current reference values used in European countries and the methodological approaches used to derive them by a sample of seven European and international authoritative committees from which background scientific reports are available. Throughout the paper, the main issues contributing to disparities in micronutrient reference values for infants are highlighted. The identification of these issues in relation to the specific physiological aspects of infants is important for informing future initiatives aimed at providing standardized approaches to overcome variability of micronutrient reference values across Europe for this age group.

*Keywords:* nutrient requirements, nutrient recommendations, recommended intakes, nutrient intake values, infants, EURRECA.

Correspondence: Maria Hermoso, Division of Metabolic Diseases and Nutritional Medicine, Dr von Hauner Children's Hospital. Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, 80337 Munich, Germany. E-mail: maria.hermoso@med.uni-muenchen.de

## Background

Different countries and committees have set micronutrient reference values for infancy, i.e. up to 1 year of age. These values vary considerably across Europe. The variability is caused by various factors, e.g. disparities exist between underlying concepts, the terminologies used and the methodologies and assumptions that have been made in the definition of micronutrient requirements and derivation of reference values for intake (Doets *et al*. 2008). Infants are considered a vulnerable group because they have a relatively high requirement of nutrients per unit body weight during a sensitive period of rapid growth and development. The aim of the present paper is to review specific aspects of healthy term infants in relation to micronutrient requirements in early and late infancy, and to critically evaluate the approaches used to determine those requirements and derived reference values for nutrient intake.

## 1. Nutritional requirements of infants, methodological approaches used for establishing requirements and derived reference values

Infancy is characterized by rapid growth and development. Both are determined by genetic and environmental factors. An important environmental factor is nutrition, because an inadequate diet can compromise growth and the full utilization of an individual's genetic potential (Reyes-Posso 2008). Nutrition is important throughout childhood, but especially during the first 2 years of life, as the growth rate during this period is high and less dependent on growth hormones than in later periods of childhood. The rapid rates of growth and development of infants impose unique nutritional needs upon their already high maintenance needs (Heird 1996). The quantity and quality of nutrient supply during early life modulates the differentiation of tissues and organs and has short- and long-term consequences for health (Koletzko 2008).

The preferred form of nutrition for healthy infants is breastfeeding (Agostoni *et al*. 2009). Current global recommendations, derived from a technical expert committee report commissioned by the World Health Organization (WHO) (Kramer & Kakuma 2002), are that infants should be exclusively breastfed for the first 6 months of life with the introduction of complementary foods thereafter and continued breastfeeding for the first 2 years of the infant's life (WHO 2003b). This report concluded that infants who were exclusively breastfed for 6 months did not differ in growth from those exclusively breastfed for 4 months and experienced less morbidity from gastrointestinal infection. Recent expert committee reports in Europe and in the USA concluded that complementary feeding, in addition to continued breastfeeding, should be introduced not before 17 weeks and not later than 26 weeks of age (Agostoni *et al*. 2008; Greer *et al*. 2008). The comparative benefits of exclusive breastfeeding for six months compared with exclusive breastfeeding for between 4 and 6 months, particularly in infants living in industrialized countries, however, has been challenged (Fewtrell *et al*. 2007). In Kramer & Kakuma's paper (2002), only two of 20 the studies included in the review were randomized intervention trials of different exclusive breastfeeding recommendations, and both were conducted in non-industrialized countries (Cohen *et al*. 1994; Dewey *et al*. 1999). Due, in no small part, to clear ethical and feasibility issues of conducting such trials, the overriding majority of studies in the review were observational and therefore vulnerable to considerable residual confounding and bias. Indeed, other systematic reviews have suggested that breast milk may not meet the full requirements for energy and certain micronutrients of 6-month-old infants (Butte 2002; Reilly *et al*. 2005). Clearly, there is an urgent need for more work in this area.

Despite the multiple benefits of breastfeeding, a significant proportion of mothers of healthy term infants in many industrialized countries chose to formula feed their infants. There is a lack of guidance regarding the appropriate timing of the introduction of complementary foods to formula fed infants because there is virtually no data available to form evidence-based recommendations. It has been argued

**Key messages**

- Infancy is characterized by growth and development, which impose unique nutritional needs upon the already high maintenance needs of infants.
- Primary data from quality studies linked to functionally relevant outcomes are needed in infants in order to improve the current knowledge on nutritional requirements in this period of life.
- Great disparities exist in established nutrient intake values for infants in Europe. This is mainly due to the different methodological approaches used to define the nutritional requirements of infants. A lack of transparency in the documentation of the decision making by the committees in charge hampers understanding these differences.
- Solid, up-to-date, transparent scientific basis will help committees tasked with setting reference values for nutrients, defining requirements of infant formula, and defining feeding practice.

that, as formula-fed infants receive higher amounts of energy, protein, iron and zinc than breastfed infants, they may not require solid foods until a later age (Fewtrell *et al*. 2007). Despite this, across Europe, complementary feeding is introduced earlier in formula fed than in breastfed infants (Schiess *et al*. 2010). It has been hypothesized that an early introduction of complementary foods to formula fed infants might increase the risk of later overweight and obesity in children and adults. There is evidence to suggest that formula feeding is associated with a more rapid weight gain in early infancy (Dewey 2001; Baker *et al*. 2004) and with an increased risk for obesity in childhood and adolescence (Gillman *et al*. 2001; von Kries *et al*. 2001; Arenz *et al*. 2004; Koletzko *et al*. 2009b). Higher intakes of protein and energy with infant formula compared with breast milk may favour rapid weight gain, adipogenic development of adipocytes and the accumulation of fat (Koletzko *et al*. 2009a). This could lead to increased likelihood of later overweight, but it is as yet unclear whether an earlier introduction of complementary foods plays a relevant role.

### 1.1 Concepts and use of nutrient intake values

The European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence works similarly towards a general framework including harmonized approaches, methods and key terms to be used for the development of micronutrient reference values (Doets *et al*. 2008). The United Nations University (UNU), in collaboration with the Food and Agricultural Organisation (FAO), World Health Organisation (WHO) and the United Nations Children's Emergency Fund (UNICEF), has previously proposed an international harmonization of nutrient based dietary standards (King & Garza 2007). The term Nutrient Intake Values (NIVs), conceived by UNU, FAO, WHO and UNICEF, encompass all nutrient-based dietary standards derived from primary data and provide estimates on appropriate dietary substrate supply for populations of healthy people (King & Garza 2007). NIVs are used for assessing the adequacy of nutrient intakes and for planning diets of groups and individuals. They are also applied to a number of aspects of food and nutrition policy such as regulatory issues and trade, labelling, planning programmes for alleviating public health nutrition problems, food fortification and dietary guidance (Aggett *et al*. 1997). NIVs are based on physiological requirements, which are defined by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) as the amounts and chemical forms of nutrients needed systematically to maintain normal health and development, without disturbance of the metabolism of any other nutrient and without extreme homeostatic processes and excessive depletion or surplus of bodily reports (Aggett *et al*. 1997). The nutrient requirements of individuals vary markedly, and NIVs do not refer to individuals, but to populations that are defined by age, gender and in some cases additional characteristics relevant for nutrient needs. Existing NIVs for infants are shown and compared in section 2.

The UNU Working Group has defined the following NIVs: average nutrient requirement (ANR), individual nutrient level (INL) and upper nutrient level (UNL). ANR is the estimated average or median requirement of a specific nutrient in a population, derived from a statistical distribution of requirement criterion and for a particular age and sex specific group based on a specific biological endpoint or biochemical measure (King & Garza 2007). It is assumed that individual requirements follow a statistical distribution (symmetrical bell-shaped curve). INL is the individual nutrient level and $INL_{97}$ is the nutrient intake considered adequate to meet the known nutrient needs of practically all healthy individuals in a particular age and sex-specific group (King & Garza 2007). Equivalent terms are population reference intakes and recommended dietary allowance. This value, set at a level of intake that meets the needs of the majority of the population (mean + 2SD), is generally used as the target for provision of essential nutrients to populations and as the reference point for nutrient labelling of foods. The exception is energy, for which the ANR is used because the use of $INL_{97}$ or equivalents would lead to overfeeding. UNL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals of a particular life-stage group (King & Garza 2007).

### 1.2 Methods used to estimate the nutritional requirements of infants and derived NIVs

Indicators of function provide information to target requirements for health protection and not just to prevent clinical deficiencies. However, there are a limited number of functionally relevant outcome measures for infants that reflect the response to dietary intake (IOM 2000b). The first months of life pose particular challenges with regards to clinical research, and few quality studies have been performed in this age group. The paucity of available data for estimating nutrient requirements of infants hampers the establishment of adequate NIVs (Pombo *et al*. 2001). Usual intakes of presumably healthy populations, factorial approaches and balance techniques are the methods used most often to estimate nutrient needs of infants. None are fully satisfactory because they seldom adequately address the issue of nutrient intake supporting long-term health and optimal functional capacities rather than just avoiding acute deficiency states (Butte 2002).

NIVs for infants during the first 6 months of life are usually derived from estimated intakes of fully breastfed infants. For older infants (7–12 months), estimates of intakes from both human milk and complementary foods are used as a reference. However, the actual intakes of breastfed infants are difficult to determine due to the variability of milk volume and composition between women, as well as the changing composition of the milk during the course of lactation, during the day and even during a feeding. Moreover, the bioavailability of substrates and their metabolism differ between infants fed human milk, those fed infant formula and infants given complementary feeds. Therefore, the composition of human milk and the nutrient intake of breastfed infants do not always provide useful guidance for infants who are not exclusively breastfed (Aggett *et al*. 1997).

Factorial approaches are generally based on estimates of maintenance needs, nutrient accretion that accompanies growth, measures of digestibility and/or absorption (bioavailability), and utilization efficiency. The requirements for growth are derived from estimates of body composition at different ages, from which the nutrient accretion over time (increase in the total body content of that nutrient from one age to another) can be calculated, taking into account the metabolic cost of accretion. However, very limited direct analytical information on body composition is available, which cannot be reliably extrapolated between age groups. Maintenance needs are derived from estimates of losses related to cellular turnover and unavoidable metabolic inefficiency. Data on inevitable losses in newborns, toddlers and infants are scant. Given that in adults such information is best gained under circumstances of negligible intakes of the nutrient of interest when homeostatic conservation is maximal, it is unlikely that such data could be acquired ethically in infants (Aggett *et al*. 1997).

Balance studies at known intakes provide information about net whole retention and net intestinal absorption or secretion and whole-body retention of nutrients. These studies are difficult to perform in infants, although some studies have been performed in term and pre-term infants. One limitation is that they tend to overestimate net retention and thus underestimate requirements due to technical difficulties in sampling. To extrapolate nutrient requirements from balance studies, subjects should be in equilibrium at the intake of the nutrient in question, which is difficult to determine in periods of fast growth. The intake has to be manipulated so that it balances losses. The length of the study period also depends on the size of the body stores of the nutrient and the rate at which the stores are mobilized (Prentice *et al*. 2004). The interpretation of balance studies often relies heavily on estimates derived by factorial approaches, that is, the appropriateness of retained quantities of target nutrients is determined by comparison with expected retention based on estimates derived by factorial methods. Thus, estimates of growth velocity and tissue composition are key to interpreting balance results (Butte 2002). The use of isotopic labels of endogenous or exogenous (dietary) pools of nutrients can enable better characterization of the flux and pool sizes underlying homeostasis. Thus, the balance-study approach, with these refinements, is likely to remain a key method in investigating requirements until new methods become established (Aggett *et al*. 1997).

Extrapolation and interpolation methods are often employed to derive NIVs for infants, especially from 7

months of age. Values for nutrient needs are extrapolated from one life stage to another using weighting for body size, energy requirement and other metabolic differences. These approaches have several limitations, as outlined elsewhere (Atkinson & Koletzko 2007). The rationale for the method chosen for extrapolating NIVs should be completely transparent and described in detail for each nutrient (Atkinson & Koletzko 2007).

### 1.3 Nutrient requirements of infants for energy, macro- and micronutrients

#### *1.3.1 Energy and macronutrients requirements*

##### Energy

During infancy, energy requirements are defined as the amount of energy needed to balance total energy expenditure at a desirable level of physical activity, and to support optimal growth and development consistent with long-term health (FAO/WHO/UNU 2004). Energy requirements during growth and development can be partitioned into components of basal metabolism, thermogenesis, physical activity and energy cost of growth (Butte 2005). The energy needed for growth is estimated to be around 35% of the total energy requirement in the first month of life, and this requirement declines continuously to about 3% at 12 months, remaining low until the onset of the pubertal growth spurt (Butte 2005).

##### Protein

The protein requirement of infants can be defined as the minimum intake that will allow nitrogen equilibrium at an appropriate body composition during energy balance at moderate physical activity, plus the needs associated with the deposition of tissues consistent with good health (WHO/FAO/UNU 2007). The nine essential amino acids (leucine, isoleucine, valine, tryptophan, phenylalanine, methionine, threonine and histidine) need to be obtained from the diet. The conditionally essential amino acids (arginine, cysteine, glutamine, glycine, proline and tyrosine) are those that the infant in unable to produce in sufficient amounts and hence all or part of the daily needs for those amino acids must be provided through the diet (Pencharz & Elango 2008). The average protein content of human milk is 11.7 $g/L^{-1}$ (Pencharz & Elango 2008). Exclusive breastfeeding meets the protein and amino acid requirements during the first 4–6 months of life. During the second 6 months of life, solid foods contribute a significant amount of protein to the infant diet. The biological value of a protein refers to its ability, when it is the sole dietary source of protein, to support protein synthesis and therefore body maintenance and growth. On this scale, breast milk proteins and egg have the highest value (1.0). All animal proteins (with the exception of gelatin) are complete, that is they contain all the essential amino acids and are of high biological value. Most vegetable proteins, except soya, are incomplete because they offer an unbalanced assortment of amino acids that cannot alone satisfy the body's needs (Michaelsen *et al*. 2000).

##### Lipids

Fats are the main source of energy for infants, and n-6 and n-3 long-chain polyunsaturated fatty acids (LCPUFAs) are essential for normal growth and development and maturation of numerous organ systems, most importantly the brain and eye. Moreover, lipid-soluble vitamins (A, D, E, K) require dietary lipids for absorption (Mena & Uauy 2008). Exclusively breastfed infants receive a dietary fat supply usually in the range of 40–55% of total dietary energy intake (Michaelsen *et al*. 1990). The introduction of complementary feeding that is rich in carbohydrates may reduce fat intake to 30–40% of energy intake. For infant formula a fat content in the range of 40–60% of total energy content has been recommended (Scientific Committee on Food 2003). This corresponds to a fat content of 4 to 6 g/100 kcal as established by the European Commission Directive on Infant Formulae and Follow on Formulae (European Commission 2006). Human milk provides linoleic acid (LA), alpha-linoleic acid (ALA), docosahexaenoic acid (DHA), arachidonic acid (AA), and other LCPUFAs to breastfed infants. The level of AA is relatively constant on a worldwide basis whereas the level of DHA is more variable and depends on maternal diet and lifestyle (Koletzko *et al*. 2008). Recent consensus recommendations support that pregnant

and lactating women should achieve a DHA intake of at least 200 mg/d (Koletzko *et al*. 2007). The ESPGHAN recommends the following content of essential fatty acids in infant formula: 0.3–1.2 g/100 kcal (2.7–10.8% total energy) of LA and 0.5–2.4 g/100 kcal (0.54–2.59% total energy) of ALA, with a minimum LA/ALA ratio of 5:1 and a maximum of 15:1 (Koletzko *et al*. 2005). The authors of a recent review conclude that the available evidence supports the addition of DHA to infant formula: the addition of at least 0.2% of fatty acids as DHA appears necessary for achieving a benefit on functional endpoints, but DHA levels should not exceed 0.5% of fatty acids because systematic evaluation of higher levels of intake have not been published (Koletzko *et al*. 2008). Based on current knowledge, infant formula contents of AA should be at least those of added DHA, and eicosapentaenoic acid (EPA) should not exceed levels of DHA (Koletzko *et al*. 2008).

Examples of reference values used in European countries for average daily energy, protein, fat and essential fatty acids intakes in populations of healthy infants set by different European and international committees is presented for comparison in Table 1.

#### Digestible and indigestible carbohydrates

Lactose is the main digestible carbohydrate in human milk, providing about 40% of the energy content (Koletzko *et al*. 2005). In addition to lactose, mature breast milk contains a large variety of oligosaccharides in concentrations of approximately 5–10 g/$L^{-1}$ (Kunz *et al*. 2000). Human milk oligosaccharides may prevent bacterial adhesion by interfering with the docking of bacteria on the intestinal cell surface and with the expression of certain enzymes in the intestine required for bacterial adhesion (Bode 2006). The total digestible carbohydrate content of infant formula and follow-on formula is set between 9 and 14 g/kcal, which is based on the calculated glucose consumption of the central nervous system (Scientific Committee on Food 2003; European Commission 2006). Non-digestible carbohydrates such as fructooligosaccharides, galactooligosaccharides, inulin, soy polysaccharide, resistant starch, and gums are added to dietary products, enteral formulas and breast milk substitutes consumed by infants. Additionally, some resistant starches and non digestible carbohydrates are formed during processing (Aggett *et al*. 2003). The overall benefits to health of supplementing infant formula or infant solid foods with fibre, inulin (probiotics) or prebiotic oligosaccharides remain unclear (Agostoni *et al*. 2004; Kien 2008).

### *1.3.2 Micronutrient requirements*

#### Iron

During the first year of life, the body iron content increases markedly. In healthy term infants, iron stores at birth comprise most of the iron requirements during the first 4–6 months. From the 4th month, the requirement for dietary iron increases to an estimated 0.78 mg/day due to the stepwise depletion of endogenous stores and rapid growth with an expansion of blood volume and increased tissue and storage iron (AAP 1999; Fisher *et al*. 2000; IOM 2000b). Iron deficiency in humans is most prevalent in the late infancy period, which is characterized by peak hippocampal and cortical regional development, as well as myelogenesis, dendritogenesis and synaptogenesis in the brain where iron availability may play a role (Lozoff & Georgieff 2006). Although the iron content of breast milk is low, its bioavailability is high and needs of dietary iron are limited due to the large iron stores of newborn infants born at term, therefore iron deficiency at 6 months is uncommon in exclusively breastfed, term infants in industrialized countries (Yang *et al*. 2009). It has been shown that the same is true for healthy full-term born infants fed exclusively an infant formula with only 1.6 mg iron/$L^{-1}$ (Hernell & Lönnerdal 2002); despite this most modern infant formulae are fortified with iron. A recent study has shown that while iron supplementation of breastfed infants caused some preservation of the iron endowment, the effect was modest and did not extend beyond the period of supplementation (Ziegler *et al*. 2009). The study was underpowered to investigate any adverse affects of supplementation. However, other studies have reported adverse effects of iron supplementation in iron replete infants, for example on length growth (Domellof 2007). Due to the low prevalence of iron

**Table 1.** Comparison of reference values for average daily energy, protein, fat and essential fatty acids intakes by different countries and committees in populations of healthy infants

| | Age | | Energy | Protein | Fat | Essential fatty acids | |
|---|---|---|---|---|---|---|---|
| | | | Male/female | Male/female | Male/female | n-6 | n-3 |
| DACH[1] | Month | | kcal/d | g/d | % of energy | % of energy | |
| | 0–<4 | 0–1 | 500 / 450 | 12 | 45–50 | 4.0 | 0.5 |
| | | 1–2 | 700 / 700 | 10 | | | |
| | | 2–4 | | 10 | | | |
| | 4–<12 | 4–6 | 95 | 10 | 35–45 | 3.5 | 0.5 |
| | | 6–12 | | 10 | | | |
| NNR[2] | Month | | MJ/d | % of energy | % of energy | % of energy | |
| | <6 | 0–1 | 1.3 / 1.4 | – | – | – | – |
| | | 3 | 2.1 / 2.2 | | | | |
| | 6–11 | 6 | 2.6 / 2.7 | 7–15 | 30–45 | 4 | 1 |
| | | 12 | 3.4 / 3.7 | | | | |
| UK[3] | Month | | kcal/d | g/d | | | |
| | 0–<3 | | 545 / 515 | 12.5 | – | – | |
| | 4–<6 | | 690 / 645 | 12.7 | – | – | |
| | 7–<9 | | 825 / 765 | 13.7 | – | – | |
| | 10–<12 | | 920 / 865 | 14.9 | – | – | |
| USA/Canada[4] | Month | | kcal/d | g/d | g/d | g/d | |
| | 0-<6 | 1 | 472 / 438 | 9.1 | 31 | 4.4 | 0.5 |
| | | 2 | 567 / 500 | | | | |
| | | 3 | 572 / 521 | | | | |
| | | 4 | 548 / 508 | | | | |
| | | 5 | 596 / 553 | | | | |
| | | 6 | 645 / 593 | | | | |
| | 7-<12 | 7 | 668 / 608 | 11 | 30 | 4.6 | 0.5 |
| | | 8 | 710 / 643 | | | | |
| | | 9 | 746 / 678 | | | | |
| | | 10 | 793 / 717 | | | | |
| | | 11 | 817 / 742 | | | | |
| WHO/FAO/UNU[5a, 5b] | Month | | kcal/d | g/d | % of energy | | |
| | 3–6 | | 700 | 13 | | | |
| | 6–9 | | 810 | 14 | 30–40 | | |
| | 9–12 | | 950 | 14 | | | |
| The Netherlands[6] | Month | | MJ/[kg.d] | g/d | % of energy | g/day | |
| | 0–2 | | 0.39 | 9/8 | 45–50 | 0.64 | – |
| | 3–5 | | 0.35 | 10/9 | 45–50 | 0.64 | – |
| | 6–11 | | 0.35 | 10/10 | 40 | | 0.15–0.2 |

[1]German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition Research (SGE), Swiss Nutrition Association (SVE) (2000) Referenzwerte für die Nahrstoffzufuhr/Reference Values for Nutrient Intake, 1st edition, 3 vollständig durchgesehener und korrigierter Nachdruck 2008. Frankfurt am Main: Umschau/Braus.

[2]Nordic Council of Ministers (2004) Nordic Nutrition Recommendations 2004, 4th edition: Integrating nutrition and physical activity. Copenhagen.

[3]Department of Health (1991) Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. London: HMSO.

[4]Food and Nutrition Board, Institute of Medicine (2005) Dietary Reference Intakes for Energy, Carbohydrate, Fibre, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press: Washington DC.

[5a]Joint FAO/WHO/UNU Expert Consultation on Human Energy Requirements (2004) Human energy requirements: report of a Joint FAO/WHO/UNU Expert Consultation. (FAO Food and Nutrition Technical Report Series, no. 1) Rome.

[5b]Joint WHO/FAO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (2007) Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. (WHO technical report series; no. 935) Geneva.

[6]Health Council of the Netherlands (2001) Dietary Reference Intakes: energy, proteins, fats and digestible carbohydrates. The Hague: Health Council of the Netherlands; publication no. 2001/19R (corrected edition: June 2002).

deficiency in infants in affluent populations, universal iron supplementation appears unjustified. The ESPGHAN Committee on Nutrition recommends an iron content ranging from 0.3 to 1.3 mg/100 kcal in infant formulae (Koletzko *et al*. 2005). Substantial evidence indicates that some indictors of iron status, particularly ferritin and haemoglobin concentrations, are lower in boys than in girls (Domellof *et al*. 2002). It has been suggested that boys may have lower body iron stores at birth or higher intestinal iron losses than girls (Yang *et al*. 2009). Other possible explanations for the reported gender difference could be due to genetic or hormonal factors, but further research is warranted (Domellof *et al*. 2002).

### Zinc

Both iron and zinc are critical for normal growth, haematopoiesis, immune function and neurologic development during infancy. The young infant has a relatively high zinc requirement to support his very rapid growth. Zinc and protein intakes have been shown to be predictors of head growth (Krebs 2000, 2007). Some studies show that zinc intakes are lower than recommendations established for this age group, but it has been proposed that some current recommendations are higher than actual needs (García-Ramos Estarriol *et al*. 2000).

About 40% of the breast milk zinc content is absorbed, which is considered to be sufficient to meet the requirements of zinc in the first 6 months. To date there is no marker of zinc deficiency that is sensitive and specific. This explains why there is considerable variability in the requirements and recommendations of various international agencies. A committee of experts convened by the WHO proposed that the recommendations should be based on the metabolic requirements of each age, including a factor that represents the interference of phytates in the absorption. These recommendations (lower limits of consumption of zinc) were adjusted to diets with low bioavailability of zinc (phytate content >15 mg/day), median bioavailability (10–15 mg phytate/day) and high bioavailability (<15 mg phytate/day) (Gil Hernandez *et al*. 2006). It has been concluded that complementary feeding should provide 84–89% of the zinc requirements between 6 and 24 months of life (Dewey 2001). Animal products are the best source of zinc, both in their content and bioavailability.

### Calcium

Calcium is the most abundant mineral in the human body (Perez-Lopez 2007). Over 99% of total body calcium is found in teeth and bones. The remainder is present in blood, extracellular fluid, muscle and other tissues, where it plays a role in mediating vascular contraction and vasodilatation, muscle contraction, nerve transmission and glandular secretion. The calcium content of infant formulae is often greater than breast milk to account for a lower fractional calcium absorption in order to achieve a comparable calcium retention (IOM 1997).

### Iodine

Iodine is a trace element required for the production of the thyroid hormones, triiodothyronine and thyroxine. These hormones play a vital role in the early growth and development of most organs, especially the brain (Perez-Lopez 2007). Monitoring of iodine status during infancy is difficult as there are no established reference criteria for urinary iodine concentration for this age group (Zimmermann 2007). The iodine content of breast milk and the infant's small iodine pool in the thyroid turns over very rapidly and is highly sensitive to variations in dietary iodine intake. Studies from France, Germany, Belgium, Sweden, Spain, Italy, Denmark, Thailand and Zaire have shown that breast milk concentrations of iodine are lower than recommended levels, suggesting a less than optimal maternal and infant iodine nutrition in many countries (Azizi & Smyth 2009).

### Sodium

Infants are less efficient in excreting excess dietary sodium than adults (Michaelsen *et al*. 2000). High sodium intakes during infancy carry both short- and long-term risks. Short-term effects include hypernatraemia, tissue dehydration, renal dysfunction and increased blood pressure. A possible life threatening acute complication is severe hypernatriaemic dehydration, which occurs at high sodium intakes without

sufficiently rehydrating a child to compensate for past and ongoing losses, and can lead to renal insufficiency and failure to excrete sodium (Fomon 1993). The role of salt intake during complementary feeding has not been extensively explored. Investigations demonstrating that an excess of dietary sodium may raise blood pressure in newborns and young infants have suggested that infancy may be a period of greater salt sensitivity than later in life (Agostoni *et al*. 2008). There is evidence that early life factors are important determinants of adult blood pressure (Lawlor & Smith 2005). The long-term effects of high salt intake in infancy on blood pressure are possible, but so far not proven (Brion *et al*. 2007).

### Copper

Copper concentrations in human milk decrease during the course of lactation and appear to be unrelated to maternal serum levels (Dorea 2000). Based on the copper concentration of human milk, the Institute of Medicine (IOM) estimated that intakes of 200 mg/day were adequate for the first 6 months of life increasing to 220 mg/day for the second 6 months (IOM 2000b). For formula fed infants, the United States Food and Drugs Administration (FDA), Codex Alimentarius and the American Academy of Pediatrics (AAP) recommend a minimum level of an artificial formula for copper of 0.6 mg/kcal, whereas the ESPGHAN Committee on Nutrition recommends 35 to 80 mg/100 kcal (Koletzko *et al*. 2005). There has been concern about the potential risks of excess copper supply in infants fed powered infant formula mixed with tap water with a high copper content. Copper in drinking water contributes 0.1–1 mg/day in most situations, but water obtained from unprotected copper pipes or fittings can considerably increase total daily copper exposure (WHO 2003a). The WHO has proposed that a copper content of drinking water not exceeding 2 $mg/L^{-1}$ would provide an adequate margin of safety in populations with normal copper homeostasis (WHO 2003a); whereas it has been proposed that infants fed powdered formula prepared with tap water containing >2 $mg/L^{-1}$ copper may pose a risk for liver damage particularly in young infants (Uauy *et al*. 2008). A controlled study of 100 healthy infants consuming water with 2 mg of copper per litre from 3 months onwards did not show biochemical or clinical evidence of health problems (Olivares *et al*. 1998), but the intervention was only started after the age considered most sensitive for copper toxicity. An epidemiological survey in Germany did not demonstrate a link between high copper exposure from water and evidence of liver abnormalities in infants, but there was no systematic health assessment for markers of copper toxicity in the infant population studied (Zietz *et al*. 2003).

*Vitamin A* is a group of compounds, the precursor of which is *trans*-retinol. Vitamin A is essential for growth and differentiation of a number of cells and tissues. It is one of the most critical vitamins during the breastfeeding period because it has an important role in the healthy development of the newborn, with lung development and maturation being particularly important. Insufficient intake of vitamin A by infants may have serious consequences, especially regarding susceptibility to infections, the development and function of respiratory organs and the integrity of mucous membranes (Strobel *et al*. 2007). Vitamin A deficiencies are uncommon in breastfed infants in Europe, although retinol and retinyl esters content in breast milk varies widely depending on maternal intake. b-carotene is found in plants and is considered a provitamin of vitamin A because humans are able to convert it into retinol. However, its bioequivalence to retinol in infants is not known. For this reason, the Scientific Committee on Food of the European Commission advised that vitamin A activity in infant formula should be provided by retinol or retinyl esters, while any carotene content should not be included in the calculation and declaration of vitamin A activity (SCF 2003).

*Vitamin D* is required to maintain adequate calcium metabolism and bone health at all ages. Many countries recommend vitamin D supplementation during infancy to avoid rickets resulting from its low content in human milk (Molgaard & Michaelsen 2003). For prevention of rickets, the Committee on Nutrition of the AAP recommends supplementing breastfed infants, whether consuming formula or not, with 400 IU (10 mg) of vitamin D per day from birth (Wagner & Greer 2008). In addition to dietary supply, the infant's vitamin D status is also modified by sun-

light exposure and hence the season of the year and the geographical latitude. Dark-skinned children or children whose clothing obscures the skin may be at greatest risk of vitamin D deficiency. Ziegler *et al*. (2006) reported that 10% of breastfed infants living in Iowa (41 degrees N) were deficient in vitamin D as determined by low serum 25-OH-D levels; most were dark-skinned (Ziegler *et al*. 2006). Sunlight exposure is critical for vitamin D synthesis because cutaneous biosynthesis upon exposure of skin to ultraviolet B light is the major source of vitamin D for most people, which influences vitamin D status and calcium absorption. Thus, there is an increased reliance on dietary sources during winter months to help maintain adequate vitamin D status (Cashman 2007; Kimlin 2008).

### Vitamin C

The biological functions of vitamin C are based on its ability to provide reducing equivalents for a variety of biochemical reactions (IOM 2000a). Ascorbic acid is important in facilitating iron absorption and a good ascorbic acid status in the newborn is important for collagen synthesis and may contribute to protecting cells against oxidative insults (Jain *et al*. 2008). Breastfed infants of well nourished mothers do not require vitamin C supplementation.

### Vitamin E

This vitamin serves as a non-specific chain-breaking antioxidant and protects cell membranes in the retina and lungs against oxidant-induced injury (IOM 2000a). Breast milk concentrations of tocopherol (~3 IU/100 kcal) appear adequate for meeting infant requirements and particularly high levels are found in colostrum. A study in the USA observed that infants and children had low intakes of vitamin E compared with the requirements established by the IOM (IOM 2000a), but there is some concern that those reference values may be too high (Briefel *et al*. 2006).

*Folate and cobalamin* are necessary cofactors in the synthesis of RNA and DNA, and cobalamin is required for maintaining the nervous system. Both vitamins are therefore critical to the rapid growth and development during the early years of life (Hay *et al*. 2008). Reports of severe neurodevelopmental damage and long-term neurologic sequelae due to infantile cobalamin deficiency demonstrate the importance of adequate cobalamin status during the first months of life (von Schenck *et al*. 1997; Bjorke-Monsen *et al*. 2008). However, reference values for indices of cobalamin and folate status in infants are lacking (Hay *et al*. 2008).

Several factors may affect dietary intake of micronutrients such as the composition of foods (e.g. iron and calcium absorption depends on vitamin C and vitamin D intakes, respectively) (Sandstrom 2001; Cashman 2007) and the iodine concentration in the soil that is determined by geographical location (Aston & Brazier 1979).

Nutritional status of infants depends on micronutrient supply with human milk that is modified by maternal micronutrient intake and lifestyle, such us tobacco smoking, alcohol intake or drug consumption. Moreover, micronutrient content and bioavailability of infant formula and complementary foods and variability of nutrient absorption are of importance (IOM 2000a).

## 2. Current European nutrient intake values for infants

Previous EURRECA research activities collated and compared current micronutrient reference values from 35 countries and committees, by means of a questionnaire and background documents (Doets *et al*. 2008). There follows a summary of the specific characteristics that concern the infant population group, together with a comparison of reference values used in European countries and discussion of the methodological approaches to set them.

### 2.1 Characteristics of the infant population group in currently used micronutrient intake values

The definition of age groups within infancy, each considered as relatively homogenous with regard to nutrient requirement, differ between countries and committees that have set reference values for intake. This may be due to differences in reasoning in defining population groups, but in most publications these

arguments are not clearly described (Doets *et al*. 2008). Most European countries have set reference values for one or two age groups during infancy: 13 of the 30 countries investigated by Doets and colleagues and the WHO/FAO defined two age groups, while 12 countries and the European Commission (EC) defined only one age group. Four countries defined four different age groups, and one country, the Russian Federation, set three age groups. Serbia did not set reference values for infants under the age of 1 year. The five Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), Croatia, Estonia, Italy and the EC, do not give values for infants under 6 months. France did not set reference values for minerals under 1 year of age, but they did for vitamins. None of the countries or committees set different values for male and female infants.

Specific subpopulation groups within the infant group are specified for particular micronutrients by some countries. Groups recognized are bottle and formula fed infants, respectively (the Netherlands: calcium, phosphorus, zinc and vitamin B6; WHO/FAO: zinc and magnesium), and cows' milk fed infants (WHO/FAO: calcium).

The infant population group is one of the populations recognized as a 'vulnerable' group (defined as a population group in a healthy population having a higher nutrient requirement) by 8 of the 35 countries and committees questioned in the scoping exercise for collecting reference values (Doets *et al*. 2008). The most commonly cited micronutrients thought to be related to infant vulnerability were vitamin D (in three countries), vitamin A ($n = 2$), iodine ($n = 2$), copper ($n = 2$) and iron ($n = 2$).

For the purposes of EURRECA, a list of terms and definitions that are relevant to the infancy period and to infant feeding have been agreed for use within the network (Table 2).

## 2.2 Comparison of nutrient intake values for infants

Micronutrient reference values for infants vary considerably across Europe; for some micronutrients there is a great difference between the highest and the lowest values. Table 3a and b show the differences between reference values used in Europe that have been set by the different European countries and European and international committees for each micronutrient at 3 and 9 months of age (lowest and highest values for each micronutrient, mean value, 25th and 75th percentile). As reference values are expressed as single values, multiple values, ranges and so on, Doets and colleagues defined standardization procedures to enable comparison (Doets *et al*. 2008).

Table 4a–d give an overview of the level of reference values set by each country/committee for vitamins, minerals and trace elements at two points of time (3 and 9 months), defined as 'high level' when they exceed the 75th percentile or 'low level' when they fall below the 25th percentile.

Twenty-two countries, WHO/FAO and the EC have their own background reports on the setting of nutrient reference values; 13 countries based their reference values on those of other countries or organizations; Germany, Austria and Switzerland (DACH) and the Nordic countries cooperated in setting reference values. The Nordic countries, the DACH countries, France, Latvia, the Netherlands and the United Kingdom, WHO/FAO and the EC, defined their own reference values (Doets *et al*. 2008). Table 5 gives an overview of the European countries and international working groups with published micronutrient reference values used in Europe. From those countries and committees setting their own reference values, only a small number of documents provided detailed information of the evaluation of evidence supporting the reference values (United Kingdom 1991; EC 1993; DACH 2000; the Netherlands 1992, 2000 and 2003; France 2001; NNR 2004; WHO/FAO 2004). We will solely refer to these documents when examining the methodological approaches for setting their micronutrient reference values in detail (see tables in section 2.2.1 to 2.2.4).

### *2.2.1 Water soluble vitamins (Table 6)*

### *2.2.2 Fat soluble vitamins (Table 7)*

### *2.2.3 Minerals (Table 8)*

### *2.2.4 Trace elements (Table 9)*

**Table 2.** List of terms and definitions related to the infant population group and adopted by EURRECA Research Activity 'infant nutrition'

| Term | Definition |
|---|---|
| Infant | A person in the first year of life.<br>The first 12 months of life have been defined as an age group to set reference values by national and international committees. Within this age group, reference values are set for one to four different age subgroups. Most European countries defined one or two age subgroups.<br>For the purposes of EURRECA, the infant group is defined from birth up to 12 months of |
| age. Young infant | An infant up to the 6th month of life (completed 180 days). |
| Older infant | An infant from about the 6th month of life up to 1 year of age. |
| Newborn | An infant from birth to 28 completed days.<br>The term newborn includes premature infants, post-mature infants and full term newborns.[1] |
| Pre-term birth | Delivery before 37 completed weeks of gestation.[1] |
| Full-term | Infant born between 37 completed weeks of gestation and less than 42 weeks. |
| Post-mature neonate | Infant born after 42 weeks gestation. |
| Gestational age | Time elapsed between the first day of the last menstrual period and the day of delivery. Measured in completed weeks. [Note: If pregnancy was achieved using assisted reproductive technology, is calculated by adding 2 weeks to conceptional age (time elapsed after conception)].[2] |
| Chronological age | Time elapsed since birth. Measured in days, weeks, months or years.[2] |
| Post-menstrual age | Gestational age + chronological age. Measured in days, weeks, months or years.[2] |
| Corrected age (similar term: adjusted age) | Chronological age reduced by the number of weeks born before 40 weeks of gestation. Measured in weeks or months used for preterm children until 3 years old.[2] |
| Toddler | Young child who is of the age of learning to walk between infancy and childhood. Toddling usually begins between age 12 and 24 months |
| Low birthweight (LBW) infant | A neonate weighing less than 2500 g at birth (up to and including 2499 g), irrespective the gestational age.[1] |
| Very low birthweight (VLBW) infant | A neonate weighing less than 1500 g at birth.[1] |
| Extremely low birthweight (EXLW) | A neonate weighing less than 1000 g at birth.[1] |
| Small for gestational age (SGA) infant | Neonates with birthweight and/or length at least 2 standard deviations (SDs) below the mean for gestational age ( −2 SD) based on the data derived from a reference population.[3] |
| Exclusive breast-feeding | Exclusive breast-feeding implies that the infant receives only breast milk (including milk expressed or from a wet nurse) and no other liquids or solids except for Oral Rehydration Solution (ORS) drops or syrups consisting of vitamins, mineral supplements, or medicines.[4] |
| Predominant breastfeeding | Predominant breastfeeding implies that breast milk (including milk expressed or from a wet nurse) is the predominant source of nourishment, in combination with the supply of certain liquids (water and water-based drinks, fruit juice) ritual fluids and ORS, drops or syrups (vitamins, mineral, medicines).[4] |
| Full breastfeeding | Exclusive breastfeeding and predominant breastfeeding together.[5] |
| Breastfeeding | Breastfeeding implies that the infant receives breast milk (including milk expressed or from a wet nurse) and can also receive any food or liquid including non-human milk and formula.[4] |
| No breastfeeding | The infant receives no breast milk.[5] |
| Complementary feeding (similar terms: weaning, weaning foods, Beikost) | The term 'complementary feeding' should embrace all solid and liquid foods other than breast milk or infant formula and follow-on formula.[6] |
| Bottle-feeding | Any liquid (including breast milk) or semi-solid food from a bottle with nipple/teat.[4] |
| Infant formulae | Foodstuffs intended for particular nutritional use by infants during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding.[7] |
| Follow-on formulae | Foodstuffs intended for particular nutritional use by infants when appropriate complementary feeding is introduced and constituting the principal liquid element in a progressively diversified diet of such infants.[7] |
| Breast milk substitutes | Any food being marketed or otherwise presented as a partial or total replacement for breast milk, whether or not suitable for that purpose.[8] |

[1]WHOSIS (WHO statistical information system).
[2]American Academy of Pediatrics. Age Terminology During the Perinatal Period. Pediatrics 2004;114:1362–1364.
[3]International Small for Gestational Age Advisory Board consensus. Development conference statement: management of short children born small for gestational age, April 24–October 1, 2001. Pediatrics. 2003 June;111(6 Pt 1):1253–61.
[4]Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA. WHO 2008.
[5]Indicators for assessing breastfeeding practices. Report of an informal meeting in June 1991, Geneva. World Health Organization, Geneva.
[6]Agostoni *et al*. Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition Journal of Pediatric Gastroenterology and Nutrition 2008,
46:99–110.
[7]Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC.
[8]International Code of Marketing of Breast-milk Substitutes. World Health Organization Geneva, 1981.

**Table 3.** Comparison of micronutrient recommendations for infants at 3 and 9 months of age

| Micronutrient (Unit) | Lowest reference value | Highest reference value | Median | P25† | P75‡ |
|---|---|---|---|---|---|
| a. Comparison of micronutrient recommendations for infants at 3 months of age | | | | | |
| Thiamin (mg) | 0.2 | 0.5 | 0.3 | 0.2 | 0.3 |
| Rivoflavin (mg) | 0.3 | 0.6 | 0.4 | 0.4 | 0.4 |
| Niacin (mg) | 2 | 8 | 4 | 2.3 | 5 |
| Vitamin B12 (mg) | 0.3 | 0.5 | 0.4 | 0.3 | 0.5 |
| Folate (mg) | 24 | 80 | 50 | 40 | 64 |
| Vitamin C (mg) | 25 | 50 | 35 | 29 | 40 |
| Vitamin B6 (mg) | 0.1 | 0.5 | 0.3 | 0.2 | 0.3 |
| Vitamin A (mg) | 350 | 500 | 400 | 375 | 435 |
| Vitamin D (mg) | 5 | 22.5 | 10 | 6.3 | 10 |
| Vitamin E (mg) | 2.7 | 6 | 3 | 3 | 4 |
| Calcium (mg) | 210 | 800 | 400 | 360 | 500 |
| Phosphorus (mg) | 100 | 400 | 300 | 141 | 300 |
| Potassium (mg) | 233 | 800 | 600 | 400 | 800 |
| Sodium (mg) | 100 | 638 | 210 | 200 | 250 |
| Magnesium (mg) | 24 | 70 | 50 | 39 | 55 |
| Iodine (mg) | 15 | 110 | 40 | 40 | 50 |
| Iron (mg) | 0.3 | 12.5 | 5 | 1.7 | 7 |
| Zinc (mg) | 1 | 5 | 3,5 | 2 | 4 |
| Selenium (mg) | 6 | 25 | 10 | 10 | 10 |
| Copper (mg) | 0.2 | 1.5 | 0.4 | 0.2 | 0.5 |
| b. Comparison of micronutrient recommendations for infants at 9 months of age | | | | | |
| Thiamin (mg) | 0.2 | 0.6 | 0.4 | 0.3 | 0.4 |
| Rivoflavin (mg) | 0.4 | 0.7 | 0.4 | 0.4 | 0.5 |
| Niacin (mg) | 2 | 8 | 5 | 4 | 6 |
| Vitamin B12 (mg) | 0.3 | 1.5 | 0.5 | 0.5 | 0.5 |
| Folate (mg) | 32 | 80 | 50 | 50 | 66 |
| Vitamin C (mg) | 20 | 55 | 35 | 30 | 40 |
| Vitamin B6 (mg) | 0.2 | 0.6 | 0.4 | 0.3 | 0.6 |
| Vitamin A (mg) | 300 | 600 | 388 | 350 | 400 |
| Vitamin D (mg) | 5 | 22.5 | 10 | 7 | 10 |
| Vitamin E (mg) | 2.7 | 6 | 4 | 3 | 4 |
| Calcium (mg) | 270 | 800 | 540 | 438 | 600 |
| Phosphorus (mg) | 200 | 500 | 400 | 300 | 500 |
| Potassium (mg) | 425 | 1110 | 750 | 700 | 800 |
| Sodium (mg) | 180 | 850 | 370 | 320 | 500 |
| Magnesium (mg) | 48 | 100 | 70 | 60 | 75 |
| Iodine (mg) | 40 | 135 | 50 | 50 | 63 |
| Iron (mg) | 6 | 15 | 9 | 7.8 | 10 |
| Zinc (mg) | 2 | 5 | 4.5 | 3.8 | 5 |
| Selenium (mg) | 8 | 40 | 15 | 10 | 19.3 |
| Copper (mg) | 0.2 | 1.5 | 0.4 | 0.3 | 0.7 |

†25th percentile; ‡75th percentile.

**Table 4.** Overview of the level of reference values between countries for vitamins, minerals and trace elements

| Country | Year | Ref. | Vitamin C | | Vitamin B6 | | Vitamin A | | Vitamin D | | Vitamin E | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months |
| a. Overview of the level of reference values between countries for vitamins | | | | | | | | | | | | |
| Albania | 2005 | [1] | | High | | | | High | Low | Low | | High |
| EC | 1993 | [2] | – | Low | – | | – | | – | High | – | – |
| Croatia | 2004 | [3] | – | | – | | – | | – | | – | |
| the Netherlands | 1992 | [4a] | | | | | High | | Low | Low | – | – |
| | 2000 | [4b] | | | | | | | | | | |
| | 2003 | [4c] | | | | | | | | | | |
| | 2008# | [4d] | | | | | | | | | | |
| Ireland | 1999 | [5] | Low | Low | | | Low | | | | – | – |
| DACH | 2000 | [6] | High | High | | | High | High | | | | |
| Belgium | 2006 | [7] | | | High | | Low | | High | High | – | – |
| Romania | 1990 | [8] | | | High | | High | High | | | – | – |
| Latvia | 2001 | [9] | | | | | | | | | | |
| France | 2001 | [10] | High | High | | | Low | | High | High | | |
| The former Yugoslav Republic of Macedonia | 2001 | [11] | | | | | | | | | | |
| Slovakia | 1997 | [12] | High | High | | | | | | | High | High |
| Lithuania | 1999 | [13] | | | | | | | | | | |
| Bulgaria | 2005 | [14] | Low | | | | | | Low | Low | Low | Low |
| Nordic countries | 2005 | [15] | – | Low | – | | – | Low | – | | – | |
| United Kingdom | 1991 | [16] | Low | Low | | | Low | | | | – | – |
| Bosnia and Herzegovina | 2005 | [17] | Low | Low | | | | | Low | Low | Low | Low |
| Spain | 2007 | [18] | High | High | | | High | High | | | High | High |
| Serbia | 1994 | [19] | – | – | – | | – | – | – | – | – | – |
| Italy | 1996 | [20] | – | | – | | – | | – | High | – | – |
| Russian Federation | 1991 | [21] | | | High | | | | | | | |
| Estonia | 2006 | [22] | – | | – | | – | Low | – | | – | |
| WHO/FAO | 2004 | [23] | Low | | | | | | Low | Low | Low | Low |
| Poland | 1996 | [24] | | | High | | High | High | | | | |
| Iceland | 2006 | [25] | – | Low | – | | – | Low | – | | – | |
| Hungary | 2005 | [26] | | High | | | | | | | | High |

b. Overview of the level of recommendations between countries for vitamins

| | | | Thiamin | | Riboflavin | | Niacin | | Vitamin B12 | | Folate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months |
| Albania | 2005 | [1] | | | | | Low | | | | High | High |
| EC | 1993 | [2] | – | | – | | – | | – | | – | |
| Croatia | 2004 | [3] | – | | – | | – | | – | | – | Low |
| the Netherlands | 2000 | [4b] | | | | | Low | Low | | | | |
| | 2003 | [4c] | | | | | | | | | | |
| Ireland | 1999 | [5] | | | | | – | – | | | | |
| DACH | 2000 | [6] | | | | | Low | | | High | | High |
| Belgium | 2006 | [7] | | | | | High | High | | | | |
| Romania | 1990 | [8] | High | | High | | High | | – | – | – | – |
| Latvia | 2001 | [9] | | | | | | High | | High | Low | Low |
| France | 2001 | [10] | | | | | | Low | | | High | High |
| The former Yugoslav Republic of Macedonia | 2001 | [11] | | | | | High | | | | Low | Low |
| Slovakia | 1997 | [12] | | High | | High | | | | | | |
| Lithuania | 1999 | [13] | | High | | High | | High | | High | Low | Low |
| Bulgaria | 2005 | [14] | | | | | Low | | | | High | High |
| Nordic countries | 2005 | [15] | – | | – | | – | | – | | – | |
| United Kingdom | 1991 | [16] | | | | | | | | | | |
| Bosnia and Herzegovina | 2005 | [17] | | | | | | Low | | | High | High |
| Spain | progr | [18] | | | | High | | | | Low | | |
| Serbia | 1994 | [19] | – | – | | – | – | – | – | – | – | – |
| Italy | 1996 | [20] | – | | | | – | | – | | – | |
| Russian Federation | 1991 | [21] | | High | | High | | High | | | | |
| Estonia | 2006 | [22] | – | | – | | – | | – | | – | |
| WHO/FAO | 2004 | [23] | | | | | Low | | | High | High | High |
| Poland | 1996 | [24] | High | High | High | High | High | High | | | Low | |
| Iceland | 2006 | [25] | – | | – | | – | | – | | – | |
| Hungary | 2005 | [26] | | | | | | | | | | |

**Table 4.** Continued

| Country | Year | Ref. | Vitamin C | | Vitamin B6 | | Vitamin A | | Vitamin D | | Vitamin E | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months |
| c. Overview of the level of recommendations between countries for minerals | | | | | | | | | | | | |
| | | | Calcium | | Phosphorus | | Potassium | | Sodium | | Magnesium | |
| | | | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months |
| Albania | 2005 | [1] | Low | Low | Low | Low | | | Low | | Low | |
| EC | 1993 | [2] | – | Low | – | | – | | – | – | – | – |
| Croatia | 2004 | [3] | – | | – | | – | – | – | – | – | |
| the Netherlands | 1992 | [4a] | Low | | – | Low | – | – | – | – | | Low |
| | 2000 | [4b] | | | | | | | | | | |
| Ireland | 1999 | [5] | High | | – | – | | | | | | |
| DACH | 2000 | [6] | Low | Low | Low | | | Low | Low | Low | Low | |
| Belgium | 2006 | [7] | | | | | – | – | – | – | | |
| Romania | 1990 | [8] | High | High | High | | – | – | – | – | High | |
| Latvia | 2001 | [9] | | | | | | | | | | |
| France | 2001 | [10] | – | – | – | – | – | – | – | – | – | |
| The former Yugoslav Republic of Macedonia | 2001 | [11] | | High | High | | | | | | | |
| Slovakia | 1997 | [12] | | | | | – | – | – | – | | High |
| Lithuania | 1999 | [13] | | | | | – | – | – | – | | |
| Bulgaria | 2005 | [14] | Low | Low | Low | Low | – | – | – | – | Low | Low |
| Nordic countries | 2005 | [15] | – | | – | | – | High | – | – | – | High |
| United Kingdom | 1991 | [16] | High | | High | | | | | | | |
| Bosnia and Herzegovina | 2005 | [17] | | Low | | | – | – | High | | Low | Low |
| Spain | progr | [18] | | | Low | Low | | | – | – | High | High |
| Serbia | 1994 | [19] | – | – | – | – | – | – | | | – | – |
| Italy | 1996 | [20] | – | | | – | – | | | | – | – |
| Russian Federation | 1991 | [21] | | | | | – | – | | | | |
| Estonia | 2006 | [22] | – | | – | | – | High | | | – | High |
| WHO/FAO | 2004 | [23] | Low (cow milk) | Low | – | – | – | – | | | Low | Low |
| Poland | 1996 | [24] | High | High | | | Low | Low | High | High | | |
| Iceland | 2006 | [25] | – | | – | | – | High | | | – | High |
| Hungary | 2005 | [26] | | | | Low | | | | | | |

d. Overview of the level of recommendations between countries for trace elements

| | | | Iodine | | Iron | | Zinc | | Selenium | | Copper | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months | 3 months | 9 months |
| Albania | 2005 | [1] | High | High | Low | High | | Low | High | High | – | |
| EC | 1993 | [2] | – | | – | Low | – | | – | Low | – | |
| Croatia | 2004 | [3] | – | | – | | – | | – | | | |
| the Netherlands | 1992 | [4a] | – | – | | Low | | Low | High | | | |
| Ireland | 1999 | [5] | | | | | | | | | | |
| DACH | 2000 | [6] | | High (no Switz) | Low | | Low | Low | | High | | |
| Belgium | 2006 | [7] | High | High | | | – | | | | | |
| Romania | 1990 | [8] | | Low | | Low | | | – | – | – | – |
| Latvia | 2001 | [9] | | | | | High | | | | | |
| France | 2001 | [10] | – | – | – | – | – | – | – | – | – | – |
| The former Yugoslav Republic of Macedonia | 2001 | [11] | | | | | High | | High | High | High | High |
| Slovakia | 1997 | [12] | | | High | | High | | | | | |
| Lithuania | 1999 | [13] | | | | | | Low | – | – | – | – |
| Bulgaria | 2005 | [14] | High | High | Low | High | | Low | | | | |
| Nordic countries | 2005 | [15] | – | | – | | – | | – | | – | |
| United Kingdom | 1991 | [16] | | | | | | | | | | |
| Bosnia and Herzegovina | 2005 | [17] | Low | High | High | High | Low | Low | Low | | High | High |
| Spain | progr | [18] | Low | Low | | Low | | | | | – | – |
| Serbia | 1994 | [19] | – | – | – | – | – | – | – | – | – | – |
| Italy | 1996 | [20] | | | – | Low | – | | – | Low | – | |
| Russian Federation | 1991 | [21] | | | | | | | – | – | | |
| Estonia | 2006 | [22] | – | | – | | – | | – | | – | |
| WHO/FAO | 2004 | [23] | High | High | – | High | | | Low | | – | |
| Poland | 1996 | [24] | | | High | High | High | | | | | |
| Iceland | 2006 | [25] | – | | – | | – | | – | | | |
| Hungary | 2005 | [26] | | | | | | | | – | | |

'High' means reference value is above the 75th percentile; 'low' means reference value is below the 25th percentile. Blank cells indicate that the reference value is between the 25th and 75th percentile.

**Table 5.** List of European countries and international working groups with published reference values for vitamins and minerals.

| | |
|---|---|
| Albania 2005 [1] | Adopted from the literature (especially Linus Pauling |
| Institute) European Community (EC) 1993 [2]* | Own |
| Croatia 2004 [3] | Aligned with EU legislation |
| the Netherlands 1992, 2000, 2003 [4a] [4b] [4c] | Own |
| Ireland 1999 [5] | Adopted from EC and United Kingdom. Own recommendations for calcium, iron (and folate and vitamin C) |
| Germany, Austria, Switzerland 2000 [6]* | Own |
| Belgium 2006 [7]** | Based on WHO/FAO, EC and European countries culturally and geographically related to Belgium (United Kingdom, the Netherlands, France) |
| Romania 1990 [8] | Own |
| Latvia 2001 [9] | Own |
| France 2001 [10] | Own |
| The former Yugoslav Republic of Macedonia 2001 [11] | Based on WHO/FAO and United Kingdom |
| Slovakia 1997 [12] | Adopted from unknown source |
| Lithuania 1999 [13] | Own |
| Bulgaria 2005 [14] | Based on WHO/FAO and IOM |
| Denmark, Finland, Norway, Sweden 2005 [15]* | Own |
| United Kingdom 1991 [16]* | Own |
| Bosnia and Herzegovina, entity: Republic of Srpska 2005 [17] | Adopted from unknown source |
| Spain [18] | Own |
| Serbia 1994 [19] | Adopted from unknown source |
| Italy 1996 [20] | Based on EC and FNB |
| Russian Federation 1991 [21] | Own |
| Estonia 2006 [22] | Based on Nordic Council of Ministers |
| WHO/FAO 2004 [23]* | Own |
| Poland 1996 [24]** | Based on United Kingdom, EC and FNB |
| Iceland 2006 [25] | Shared with Nordic countries. Own recommendations on calcium and vitamin D |
| Hungary 2005 [26] | Based on EC and IOM |

*Background scientific report available in English language; **Polish and Belgian reference values have been revised in 2008. [1] Berisha A., Bader E., Deligia C and Claude Dop M. (2005) Nutrition country profile Republic of Albania.
[2] Commission of the European Communities (1993) Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. Thirty first series.
[3] Croatian National Official Gazzette 81 (2004).
[4a] Food and Nutrition Council (1992) Dutch dietary reference values 1989 (in Dutch: Nederlandse normen 1989), 2nd edn. The Hague.
[4b] Health Council of the Netherlands (2000) Dietary Reference Values: calcium, vitamin D, thiamine, riboflavin, niacin, pantothenic acid, and biotin. The Hague: Health
Council of the Netherlands; publication no. 2000/12.
[4c] Health Council of the Netherlands (2003) Dietary Reference Intakes: vitamin B6, folic acid, and vitamin B12. The Hague: Health Council of the Netherlands; publication no. 2003/04.
[4d] Health Council of the Netherlands (2008) Towards an adequate intake of vitamin D. The Hague: Health Council of the Netherlands; publication no. 2008/15E. [5] Food Safety Authority of Ireland (1999) Recommended Dietary Allowances for Ireland, Dublin.
[6] German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition Research (SGE), Swiss Nutrition Association (SVE) (2000) Referenzwerte für die Nahrstoffzufuhr/Reference Values for Nutrient Intake, 1st edition in German. Frankfurt/Main.
[7] Hoge Gezondheidsraad (2006) Voedingsaanbevelingen voor Belgie,
herziening. [8] Institute of Public Health (1990) Recommended amounts.
Romania.
[9] Latvian Food Center (2001) Leteicamas energijas un uzturvielu devas Latvijas iedzivotajiem/recommended intake of energy and nutrients for Latvians.
[10] Martin A. (Guest Editor-in-Chief) (2001). Nutritional recommendations for the Grenc population. Sciences des aliments. International Journal of Food Science and Technology 21, 309–438.
[11] Ministry of Health and Republic Institute of Health Protection (2001). Nutrient recommendations as addition to Guidelines for Healthy nutrition of the population in Republic of Macedonia.
[12] Jajaba I., Simoncic R., Ginter E., Ondrejka J., Trusková I., Kalác J., Bzdúch V. (1997) Odporúcané výzivové dávky pre obyvateltvo Slovenska (8. revízia OVD). Výz. Zdravie, 4, 2:25–29. Official Journal of Ministry of Health.28, part 7–8 Slovakia.
[13] Lithuanian Ministry of Health, National Nutrition Center (order no. 510) (1999). Recommended dietary allowances for Lithuanian population (energy, protein, fat, carbohydrate, vitamins and minerals). Valstybes zinios/Official Gezette, 1999, no. 102–2936.
[14] Ministry of Health (2005) Ordinance No23 from 19 July 2005 for physiological normative requirement (DRI) for nutrition of the population (State Gazette
63/02.08.2005). Bulgaria.
[15] Nordic Council of Ministers (2004) Nordic Nutrition Recommendations 2004: Integrating nutrition and physical activity, 4th edition.
[16] Panel on DRVs of the Committee on Medical Aspects of Food Policy (COMA) (1991). Dietary Reference Values (DRVs) for food energy and nutrients for the UK, Report on Health and Social Subjects 41.
[17] Public Health Institute of the Republic of Srpska (2005) Dietary guideline with reference values all micronutrients. Dragana Stojisavljevic, MD, Dusanka Danojevic, MD, Janja Bojanic, MD, Ljubica Handric, MD; publication 2005/No 1Bosnia and Herzegovina, entity: Republic of Srpska.
[18] Moreiras O., Carbajal A., Cabrera L., Cuadrado C. (2007) Tablas de composición de alimentos Ediciones Pirámide (Grupo Anaya, SA). 11ª edición revisada y ampliada (278 pp) (ISBN: 978-82-2133-8). Book chapter: Ingestas recomendadas de energía y nutrientes para la población española, pp 227–230.
[19] Službeni Glasnik Srbije (1994) Pravilnik o normativima drustvne ishrane dece u ustanovama za decu. 50/94 (State Gazette RS (1994) Recommendations for collective nutrition of children 50/94). Serbia.
[20] Società Italiana di Nutrizione Umana (1996). Livelli di Assunzione Raccomandati di Energia e Nutrienti per la Popolazione Italiana (LARN) Revision.
[21] The Ministry of Health of the USSR (1991) Russian Federation.
[22] Vaask, Sirje; Liebert, Tiiu; Maser, Mai; Pappel, Kaie; Pitsi, Tagli, Saava, Merileid; Sooba, Eve; Vihalemm, Tiiu, Villa, Inga (2006). Tervise Arengu Instituut. Eesti toitumis- ja toidusoovitused. Estonia.
[23] World Health Organization, Food and Agriculture Organization of the United Nations (2004) Vitamin and minerals requirements in human nutrition, Second edition. WHO:Geneva and FAO:Rome.
[24] Ziemlanski S., Bulhak-Jachymczyk B., Budzynska-Topolowska J., Panczenko-Kresowska B., Wartanowicz M. (1996). Recommended dietary allowances for the polish population (energy, protein, fat, vitamins and minerals).
[25] The Public Health Institute of Iceland (2006) Ráoleggingar um mataraeoi og naeringarefni fyrir fullorona og born frá tveggja ára aldri.
Reykjavík. [26] Antal M. (2005) Tápanyagtáblázat (New Food Composition Tables). Rodler I., Medicina Könyvkiadó Rt. Medicina Publishing Comp., Budapest.

**Table 6.** Comparison of reference values for water soluble vitamins for infants and methods used to estimate requirements

| | Range between countries | | Method on which the estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Thiamine | 0.2–0.5 mg/d | 0.2–0.6 mg/d | Adequate intake from human milk | DACH, WHO/FAO, United Kingdom, the Netherlands | Adequate intake from human milk | DACH, WHO/FAO, United Kingdom |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| | | | | | Derived from adult values on the basis of energy expenditure | EC, NNR |
| Riboflavin | 0.3–0.6 mg/d | 0.4–0.7 mg/d | Adequate intake from human milk | DACH, WHO/FAO, the Netherlands | Adequate intakes from human milk | DACH, WHO/FAO |
| | | | Intake related to a satisfactory riboflavin status using erythrocyte glutathione reductase activation coefficient according to a study on Gambian infants (Bates *et al*. 1982) | United Kingdom | Necessary intake to restore the erythrocyte glutathione reductase activation coefficient | EC, United Kingdom |
| | | | | | Derived from adult values on the basis of energy expenditure | NNR |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| Niacin | 2.0–8.0 mg/d | 2.0–8.0 mg/d | Adequate intake from human milk | DACH, WHO/FAO, the Netherlands | Derived from adult values on the basis of energy expenditure | United Kingdom, EC, NNR |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| VitB12 | 0.3–0.5 mg/d | 0.3–1.5 mg/d | Adequate intake from human milk | DACH, WHO/FAO, the Netherlands | Adequate intake from human milk | WHO/FAO |
| | | | Intake required to normalize methyl malonic acid excretion according to a study carried out in infants of vegan mothers (Specker *et al* 1990) | United Kingdom | Intake seen to correct biochemical deficiency as evidenced by methylmalonic acid excretion | EC, United Kingdom, NNR |
| | | | | | Interpolated from young infants on the basis of weight increase | DACH |
| | | | | | Or interpolated between values for young infants and for adults | the Netherlands |

**Table 6.** Continued

| | Range between countries | | Method on which the estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Folate | 24–80 mg/d | 32–80 mg/d | Adequate folate intakes from human milk | DACH, WHO/FAO, United Kingdom, the Netherlands | Adequate intake from human milk | WHO/FAO |
| | | | | | Interpolated from young infants on the basis of weight increase | DACH |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| | | | | | Based on an intake of 3.6 mcg/kg body weight appeared to maintain plasma levels and showed maintained growth, heamopoiesis and clinical well being of the 20 infants involved in a trial (Asfour *et al*. 1977) | NNR, EC |
| | | | | | Set a value for formula fed infants based on the adequate intake from human milk plus intake from formula that produced lower red cell folate levels than breast milk does but no differences in haemoglobin concentration, weight gain or growth rate according to a supplementation study in infants (Foged *et al*. 1989) | United Kingdom |
| Vitamin C | 25–50 mg/d | 20–55 mg/d | Based on vitamin C content of breast milk considering maternal intake | DACH, WHO/FAO, United Kingdom, the Netherlands, FR | Based on vitamin C content of breast milk considering maternal intake | DACH, EC and United Kingdom |
| | | | | | From the reference value estimated for young infants | WHO/FAO |
| | | | | | Extrapolated from adult requirements by assuming a growth factor of 1.3 | NNR |
| | | | | | From those for adults based on square heights | France |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| Vitamin B6 | 0.1–0.5 mg/d | 0.2–0.6 mg/d | Usual vitamin B6 intakes from human milk | DACH, WHO/FAO, United Kingdom, the Netherlands | Usual intakes from human milk | DACH, United Kingdom |
| | | | | | Derived from adult values on the basis of dietary protein intake, assuming that 15% of energy is provided from protein as for adult values and then using values set for energy expenditures | DACH, United Kingdom |
| | | | | | Extrapolated from values for young infants on the basis of metabolic size, weight and growth, considering vitamin B6 content of breast milk, with consideration of average maternal intakes and average plasma pyridoxal phosphate (PLP) of nursing infants | EC |
| | | | | | Interpolated between values for young infants and for adults | the Netherlands |
| | | | | | Extrapolated values for adults using an adjustment for body height | France |

**Table 7.** Comparison of reference values for fat soluble vitamins for infants and methods used to estimate requirements

| | Range between countries | | Method on which estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Vitamin A | 350–500 mcg/d | 300–600 mcg/d | Usual intakes from breast milk | DACH, WHO/FAO, the Netherlands | Based on usual intakes from breast milk | DACH |
| | | | Intakes considered sufficient to build and maintain sufficient liver stores | United Kingdom | Extrapolated from adult requirements by using metabolic body weight and growth factors | NNR |
| | | | | | Considered sufficient to build and maintain sufficient liver stores | WHO/FAO, United Kingdom, EC |
| Vitamin D | 5–22.5 mcg/d | 5–22.5 mcg/d | Vitamin D intakes sufficient to maintain plasma 25-OHD levels within a range considered to support adequate bone health | DACH, WHO/FAO, United Kingdom, the Netherlands | Vitamin D intakes sufficient to maintain plasma 25-OHD levels within a range considered to support adequate bone health | DACH, WHO/FAO, United Kingdom, EC, the Netherlands |
| | | | | | Took into account additional intakes showing to have maximal effect in linear growth according to supplementation studies in infants | NNR |
| Vitamin E | 2.7–6 mg/d | 2.7–6 mg/d | Based on vitamin E content of breast milk<br>Consider vitamin E intake in relation to PUFA intake | | Derived from adult values on an energy allowance basis | France |

**Table 8.** Comparison of reference values for minerals for infants and methods used to estimate requirements

| | Range between countries | | Method on which estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Calcium | 210–800 mg/d | 270–800 mg/d | Usual calcium intakes from breast milk | the Netherlands, DACH, France | Based on usual intake from formula or food | DACH |
| | | | Based on calcium retention (factorial method for calcium needed for growth, including skeleton, urinary excretion and insensible losses) followed by absorption studies | WHO/FAO, United Kingdom | Based on calcium retention | the Netherlands, WHO/FAO, NNR, EC, United Kingdom |
| Phosphorus | 100–400 mg/d | 200–500 mg/d | Based on breast milk content | DACH, France | Based on the infant formulae content | DACH |
| | | | On the basic principle of an equimolar relationship in the body between calcium and phosphorus | United Kingdom | On the equimolar relationship with calcium | the Netherlands, United Kingdom, EC, NNR |
| | | | The minimum level based on a relation of Ca/P of 1.7 molar, to lower the risk on tetany | the Netherlands | On the amount required for growth derived from the values for calcium with the Ca/P ratio for weight gain of about 1.7 molar | France |
| Potassium† | 233–800 mg/d | 425–1100 mg/d | Based on the need to maintain electrolyte homeostasis and for growth of cellular mass based on factorial estimation of the urinary excretion, the amount | DACH United Kingdom | Factorial estimation | United Kingdom, EC |
| | | | needed for growth and lean tissue synthesis, the integumental and faecal losses (although the latter may represent homeostatic excretion of excessive intakes or losses incurred in maintaining sodium homeostasis) | | Extrapolated values from adult requirements by using metabolic body weight and growth factors (the values of the adults are set by considering the effect on blood pressure) | NNR |
| Sodium‡ | 100–638 mg/d | 180–850 mg/d | Based on the requirement for maintenance and growth, according to balance studies and body analysis | DACH | Based on the requirement for maintenance and growth, according to balance studies and body analysis | DACH |
| | | | On breast milk content of sodium | United Kingdom | On the daily increments in total sodium body content allowing for the declining proportion with age of extracellular fluid in body mass with an allowance for dermal, faecal and urinary losses | United Kingdom |
| Magnesium§ | 24–70 mg/d | 48–100 mg/d | On the basis of magnesium usual intake from breast milk | DACH, WHO/FAO, United Kingdom, France | On the basis of magnesium usual intake from breast milk | DACH, WHO/FAO, NNR, United Kingdom, France |
| | | | | | Extrapolated from the19–21 year age group, whose values are estimated on the basis of balance studies and taking the body weight into account | the Netherlands |

†Out of 26 countries, only 14 have recommendations for potassium (Albania, EC, Ireland, DACH, Latvia, the former Yugoslav Republic of Macedonia, Nordic countries, United Kingdom, Spain, Italy, Estonia, Poland, Iceland, Hungary).
‡Out of 26 countries, only 9 (Albania, Ireland, DACH, Latvia, the former Yugoslav Republic of Macedonia, United Kingdom, Bosnia and Herzegovina, Poland, Hungary) have recommendations for sodium for infants.
§According to WHO/FAO, the magnesium in human milk is absorbed with substantially greater efficiency (about 80–90%) than that of formula milks (about 55–75%) or solid foods (about 50%).

**Table 9.** Comparison of reference values for trace elements for infants and methods used to estimate requirements

| | Range between countries | | Method on which estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Iodine | 15–110 mg/d | 40–135 mg/d | Based on the iodine concentration in breast milk, which depends on the mother's iodine intake | DACH, WHO/FAO, United Kingdom | Balance studies were used to estimate the adequate mother's intake, and these are valid also for old infants | WHO/FAO |
| | | | Balance studies were used to estimate the adequate mother's intake, and these are valid also for old infants | WHO/FAO | Extrapolated from adults aged 19–24 y, considering the iodine status in balance studies, the connection between iodine status and goitre frequency in epidemiological studies, the relation between long-term iodine intake and iodine content of the thyroid gland, and inactivation of active autonomous thyroid adaptation mechanisms once a certain level of iodine intake has been exceeded. | DACH† |
| | | | | | Derived values for older infants from adult values, making a rough estimate on the basis of energy requirements and body weight | EC |
| | | | | | Based on data on goitre prevalence and urinary iodine excretion in European children and extrapolation from adult values based on energy and growth requirement | NNR |
| | | | | | Extrapolated from adult values using EARs for energy, which being based on basal metabolic rate have a weight component | United Kingdom |
| Iron‡ | 0.3–12.5 mg/d | 6–15 mg/d | A requirement for dietary iron exists only from the 4th month due to the newborn's reserve of placental iron (Hb iron) | DACH, WHO/FAO | Based on iron requirements for growth and on iron basal losses | DACH, NNR, United Kingdom |
| | | | Extrapolation based on metabolic weight | the Netherlands | Extrapolation based on metabolic weight | the Netherlands |
| | | | | | Based on the amount of body stores of iron and by the properties of the diet (iron content and bioavailability) | WHO/FAO |
| | | | | | Considers body weight and energy expenditure | EC |
| Zinc | 1–5 mg/d | 2–5 mg/d | Based on zinc content in breast milk | DACH | Based on zinc content in breast milk | DACH |
| | | | Extrapolation from adult values of losses and need for growth estimated with factorial methods and balance studies | United Kingdom, the Netherlands, WHO/FAO | Extrapolation from adult values of losses and need for growth estimated with factorial methods and balance studies | United Kingdom, the Netherlands, WHO/FAO |
| | | | Based on metabolic weight | the Netherlands | Based on metabolic weight | the Netherlands |
| | | | Basal metabolic rates and considering 3 different bioavailabilities | WHO/FAO | Basal metabolic rates and considering 3 different bioavailabilities | WHO/FAO |
| | | | | | Factorial method | EC, NNR |

**Table 9.** Continued

| | Range between countries | | Method on which estimated requirements is based | | | |
|---|---|---|---|---|---|---|
| | 3 m | 9 m | Younger infants | | Older infants | |
| | | | Method | Country | Method | Country |
| Selenium | 6–25 mg/d | 8–40 mg/d | During the first months of life low selenium intake is sufficient as selenium has been stored in the liver before birth; then, recommendations for younger infants are based on breast milk content of selenium | the Netherlands, DACH | It valid also for older infants, with an allowance for growth taken into account | United Kingdom |
| | | | Interpolation of the estimates for 65 Kg adult males of average normative requirement + 2SD deviation assumed of 12.5%; nonetheless these values are compatible with estimates of the selenium intake from breast milk and formula | WHO/FAO | Interpolation of the estimates for 65 Kg adult males of average normative requirement + 2SD deviation assumed of 12.5%; nonetheless these values are compatible with estimates of the selenium intake from breast milk and formula | WHO/FAO |
| | | | | | Based on extrapolation from adult values of the values of plasma glutathione peroxidase activity on the basis of body weight | DACH, the Netherlands, EC, NNR, WHO/FAO |
| Copper | 0.2–1.5 mg/d | 0.2–1.5 mg/d | Based on the amount of copper in breast milk, due to fetal copper stores in the liver and to the high absorption rate | the Netherlands, DACH, United Kingdom, France | Extrapolated from adult levels based on body weight | the Netherlands, NNR |
| | | | | | Based on the copper tissue content and an adjustment for loss of endogenous copper, assuming an absorption of 50% | United Kingdom, EC |
| | | | | | Based on the liver stores | France |

[†]Germany/Austria and Switzerland adopted two different values: Switzerland adopted those from the former WHO/FAO.
[‡]Different bioavailabilities (EC, UK: 15%), WHO/FAO's value is the average of 4 different bioavailabilities, DACH does not take into account bioavailability.

## 3. Concluding remarks

The summary in section 2 reflects great disparities in the established current micronutrient reference values for infants between European countries. Environmental and life-style factors can justify national or regional differences only to a limited extent. In most cases, the large variation of reference values is the result of different methodological approaches used to establish requirements and derived NIVs. This is illustrated in section 2 by the comparison of approaches followed by seven committees to establish reference values based on their published scientific reports. Further discrepancies are found when converting requirements to intake reference values on the basis of body weight or of energy intake, for which the selected normative data used are often not explained. Similarly, different extrapolation methods used result in different reference values. Furthermore, up to four different age groups are defined within the first year of life within which reference values are set. Other than the transition from milk to complementary foods, no clear explanations are given for the establishment of reference values for different age groups in the first 12 months of life.

Thus, a major issue in understanding the existing disparities of reference values is the lack of transparency in the documentation of the decision making by the committees in charge. As an example, only 7 of 30 committees have published scientific reports that we could use for comparing the approaches used to establish reference values. Even in the available reports, clear explanations on assumptions, decision criteria and reference data are often lacking. Improving transparency in the process of setting micronutrient reference values has major implications for nutritional policy (e.g. development of nutritional guidelines for infant feeding and regulation of infant formula and follow-on formula composition), and feeding practice.

Reference values need to be translated in nutritional guidelines. When attempting to apply any future guidelines to the infant population group, attention should also be placed upon the social, cultural and structural determinants of infant feeding practices. Factors such as socio-economic and political contexts, gender relationships, food availability, local cultural practices, lifestyles, attitudes and beliefs are known to play a role in child health and nutrition (Pelto 1987). Poverty may be used as an example of such a factor,for example in some Western countries the lower the socio-economic group of the parents the less likely the woman is to breastfeed or give complementary foods at the appropriate time (e.g. Ponza *et al*. 2004; Heinig *et al*. 2006; Bolling *et al*. 2007; Schiess *et al*. 2010), for a wide range of socio-cultural reasons and embedded cultural norms (Sellen 2001; Dykes 2005a,b, 2006; Spiro 2006; Scavenius *et al*. 2007; Bhutta *et al*. 2008), placing the infant under considerable nutritional disadvantage. Low income mothers and their infants are also more likely to eat an 'unhealthy' diet (high in fat, sugar and salt and low in fruit and vegetables) than those on a higher income (Attree 2005). Despite various initiatives aimed at improving diet in poor households, evidence suggests they have limited effectiveness among lower socio-economic groups who are their prime target (Lynch *et al*. 2007). Instead interventions should be multi-faceted and include measures to improve families' socio-economic circumstances (Attree 2005). It is therefore essential to recognize any socio-cultural, political and economic constraints upon women and families in securing optimum nutritional standards for their infants.

## Acknowledgments

The authors are grateful to all EURRECA members and partners involved in data collation, entry, analyses of current recommendations Liesbeth S. de Wit, Rosalie A.M. Dhonukshe-Rutten, Adriënne E.J.M. Cavelaars, Anna Brzozowska Trudy M.A. Wijnhoven, Mirjana Pavlovic, Torunn Holm Totland, Lene F. Andersen, Jiri Ruprich, Lisette C.P.G.M. de Groot, Pieter van 't Veer.

## Source of funding

The work report herein has been carried out within the EURRECA Network of Excellence (http://www.eurreca.org), which is financially supported by the Commission of the European Communities, spe-

cific Research Technology and Development Programme Quality of Life and Management of Living Resources, within the Sixth Framework Programme, contract no. 036196. This report does not necessarily reflect the Commission's views or its future policy in this area. BK is the recipient of a Freedom to discover Award of the Bristol Myers Squibb Foundation, New York, NY, USA.

## Conflict of interest

The authors have declared no conflict of interest.

## References

Aggett P.J., Bresson J., Haschke F., Hernell O., Koletzko B., Lafeber H.N., *et al*. (1997) Recommended dietary allowances (RDAs), recommended dietary intakes (RDIs), recommended nutrient intakes (RNIs), and population reference intakes (PRIs) are not 'recommended intakes'. *Journal of Pediatric Gastroenterology and Nutrition* **25**, 236–241.

Aggett P.J., Agostoni A., Axelsson I., Edwards C.A., Goulet O., Hernell O. *et al*. (2003) Nondigestible carbohydrates in the diets of infants and young children: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition* **36**, 329–337.

Agostoni C., Axelsson I., Goulet O., Koletzko B., Michaelsen K.F., Puntil J.W.L., *et al*. (2004) Prebiotic oligosaccharides in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology* **39**, 465–473.

Agostoni C., Decsi T., Fewtrell M., Goulet O., Kolacek S., Koletzko B., *et al*. (2008) Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenteroly and Nutrition* **46**, 99–110.

Agostoni C., Braegger C., Decsi T., Kolacek S., Koletzko B., Michaelsen K.F., *et al*. (2009) Breast-feeding: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition* **49**, 112–125.

American Academy of Pediatrics, Committee on Nutrition (1999) Refuerzo con hierro de las fórmulas infantiles. *Pediatrics*, **48**, 54–59.

Arenz S., Ruckerl R., Koletzko B. & von Kries R. (2004) Breast-feeding and childhood obesity – a systematic review. *International Journal of Obesity and Related Metabolic Disorders* **28**, 1247–1256.

Asfour R.W.N., Waslien C.I., Guindi S. & Darby W.J. (1977) Folacin requirements of children III. Normal infants. *American Journal of Clinical Nutrition* **30**, 1098–1105.

Aston S.R. & Brazier P.H. (1979) Endemic goitre, the factors controlling iodine deficiency in soils. *Science of the Total Environment* **11**, 99–104.

Atkinson S.A. & Koletzko B. (2007) Determining life-stage groups and extrapolating nutrient intake values (NIVs). *Food Nutrition Bulletin* **28**, S61–S76.

Attree P. (2005) Low-income mothers, nutrition and health: a systematic review of qualitative evidence. *Maternal and Child Nutrition* **1**, 227–240.

Azizi F. & Smyth P. (2009) Breastfeeding and maternal and infant iodine nutrition. *Clinical Endocrinology* **70**, 803–809.

Baker J.L., Michaelsen M.K., Rasmussen K.M. & Sorensen T.I. (2004) Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. *American Journal of Clinical Nutrition* **80**, 1579–1588.

Bates C.J., Prentice A.M., Paul A.A., Prentice B.A., Sutcliffe B.A. & Whitehead R.G. (1982) Riboflavin status n infants born in rural Gambia, and the effect of a weaning food supplement. *Transactions of the royal society of tropical medicine and hygiene* **76**, 253–258.

Bhutta Z.A., Shekar M. & Ahmed T. (2008) Mainstreaming interventions in the health sector to address maternal and child undernutrition. *Maternal and Child Nutrition* **4**, 1–4.

Bjorke-Monsen A.L., Torsvik I., Saetran H., Markestad T. & Ueland P.M. (2008) Common metabolic profile in infants indicating impaired cobalamin status responds to cobalamin supplementation. *Pediatrics* **122**, 83–91.

Bode L. (2006) Recent advances on structure, metabolism, and function of human milk oligosaccharides. *Journal of Nutrition* **136**, 2127–2130.

Bolling K., Grant C., Hamlyn B. & Thornton A. (2007) *Infant Feeding Survey 2005*. NHS: London.

Briefel R., Hanson C., Fox M.K., Novak T. & Ziegler P. (2006) Feeding Infants and Toddlers Study: do vitamin and mineral supplements contribute to nutrient adequacy or excess among US infants and toddlers? *Journal of the American Dietetic Association.* **106**, S52–S65.

Brion M.J., Ness A.R., Davey Smith G., Emmet P., Rogers I., Whincup P., *et al*. (2007) Sodium intake in infancy and blood pressure at 7 years: findings from the Avon Longitudinal Study of Parents and Children. *European Journal of Clinical Nutrition* **62**, 1162–1169.

Butte N.F. (2002) *Nutrient Adequacy of Exclusive Breastfeeding for the Term Infant during the First Six Months of Life*. WHO: Geneva.

Butte N.F. (2005) Energy requirements of infants. *Public Health Nutrition* **8**, 953–967.

Cashman K.D. (2007) Calcium and vitamin D. *Novartis Foundation Symposium* **282**, 123–138; discussion 138–142, 212–128.

Cohen R., Brown K., Canahuati J., Rivera L. & Dewey K. (1994) Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomized intervention study in Honduras. *The Lancet* **344**, 288–293.

Dewey K.G. (2001) Nutrition, growth, and complementary feeding of the breastfed infant. *Pediatric Clinics of North America* **48**, 87–104.

Dewey K., Cohen R., Brown K. & Rivera L. (1999) Age of introduction of complementary foods and growth of term, low-birthweight, breast-fed infants: a randomized intervention study in Honduras. *American Journal of Clinical Nutrition* **69**, 679–686.

Doets E.L., de Wit L.S., Dhonukshe-Rutten R.A., Cavelaars A.E., Raats M.M., Timotijevic L. *et al*. (2008) Current micronutrient recommendations in Europe: towards understanding their differences and similarities. *European Journal of Nutrition* **47** (Suppl. 1), 17–40.

Domellof M. (2007) Iron requirements, absorption and metabolism in infancy and childhood. *Current Opinion in Clinical Nutrition and Metabolic Care* **10**, 329–335.

Domellof M., Lonnerdal B., Dewey K.G., Cohen R.J., Rivera L.L. & Hernell O. (2002) Sex differences in iron status during infancy. *Pediatrics* **110**, 545–552.

Dorea J.G. (2000) Iron and copper in human milk. *Nutrition* **16**, 209–220.

Dykes F. (2005a) Government funded breastfeeding per support projects: Implications for practice. *Maternal and Child Nutrition* **1**, 21–31.

Dykes F. (2005b) 'Supply' and 'demand': breastfeeding as labour. *Social Science and Medicine* **60**, 2283–2293.

Dykes F. (2006) *Breastfeeding in Hospital: Midwives, Mothers and the Production Line*. Routledge: London.

European Comission (2006) Commission Directive 2006/141/EC of 22 December 2006 on infant formula and follow-on formula and amending Directive1999/21/EC. Official Journal L 401, 30/12/2006 P. 0001–0033.

FAO/WHO/UNU Expert Consultation (2004) Human Energy Requirements: report of a Joint FAO/WHO/UNU Expert Consultation. FAO Food and Nutrition Technical Report Series, No. 1, Rome.

Fewtrell M.S., Morgan J.B., Duggan C., Gunnlaugsson G., Hibberd P.L., Lucas A., *et al*. (2007) Optimal duration of exclusive breastfeeding: what is the evidence to support current recommendations? *American Journal of Clinical Nutrition* **85**, 635s–638s.

Fisher J.O., Birch L.L., Smiciklas-Wright H. & Picciano M.F. (2000) Breast-feeding through the first year predicts maternal control in feeding and subsequent toddler energy intakes. *Journal of the American Dietetic Association* **100**, 641–646.

Foged N.L.K., Rolschau J. & Blaabjerg O. (1989) Effect of folic acid supplementation on small-for-gestational age infants born at term. *European Journal of Pediatrics* **149**, 65–67.

Fomon S.J. (1993) Sodium, chloride, and potassium. In *Nutrition of Normal Infants* (ed. S.J. Fomon), pp. 219–232. Mosby Publishers: St Louis, MO.

García-Ramos Estarriol L., Gonzalez Diaz J.P. & Duque Hernandez J. (2000) Hábitos alimentarios e ingesta dietetica en el primer año de vida. *Anales Espanoles de Pediatria* **52**, 523–529.

Gil Hernandez A., Uauy Dagach R. & Dalmau Serra J. (2006) Bases for adequate complementary feeding in infants and young children. *Anales de Pediatría* **65**, 481–495.

Gillman M.W., Rifas-Shiman S.L., Camargo C.A., Berkey C.S., Frazier A.L., Rockett H.R.H., *et al*. (2001) Risk of overweight among adolescents who were breastfed as infants. *JAMA* **285**, 2461–2467.

Greer F.R., Sicherer S.H. & Burks A.W. (2008) Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics* **121**, 183–191.

Hay G., Johnston C., Whitelaw A., Trygg K. & Refsum H. (2008) Folate and cobalamin status in relation to breastfeeding and weaning in healthy infants. *American Journal of Clinical Nutrition* **88**, 105–114.

Heinig M.J., Follett J.R., Ishii K.D., Kavanagh-Prochaska K., Cohen R. & Panchula J. (2006) Barriers to compliance with infant-feeding recommendations among low-income women. *Journal of Human Lactation* **22**, 27–38.

Heird W.C. (1996) Nutritional requirements during infancy. In *Present Knowledge in Nutrition* (eds E.E. Ziegler & L.J. Filer), 7th edn, pp. 396–403. ILSI Press: Washington, DC.

Hernell O. & Lönnerdal B. (2002) Iron status of infants fed low iron formula: no effect of added bovine lactoferrin or nucleotides. *American Journal of Clinical Nutrition* **76**, 858–864.

Institute of Medicine (IOM) (1997) *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy Press: Washington DC.

Institute of Medicine (IOM) (2000a) *Dietary Reference Intakes (DRIs) for Vitamin C, Vitamin E, Selenium, and Carotenoids*. National Academy Press: Washington DC.

Institute of Medicine (IOM) (2000b) *Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press: Washington DC.

Jain S.K., Wise R., Yanamandra K., Dhanireddy R. & Bocchini J.A., Jr (2008) The effect of maternal and cord-blood vitamin C, vitamin E and lipid peroxide levels on newborn birth weight. *Molecular and Cellular Biochemistry* **309**, 217–221.

Kien C.L. (2008) Digestible and indigestible carbohydrates. In *Pediatric Nutrition in Practice* (ed. B. Koletzko), pp. 42–46. Karger: Basel.

Kimlin M.G. (2008) Geographic location and vitamin D synthesis. *Molecular Aspects of Medicine* **29**, 453–461.

King J.C. & Garza C. (2007) Harmonization of nutrient intake values. *Food and Nutrition Bulletin.* **28**, S3–12.

Koletzko B. (2008) Basic concepts in nutrition: Nutritional needs of children and adolescents. e-SPEN. *the European e-Journal of Clinical Nutrition and Metabolism* **3**, e179–e184.

Koletzko B., Baker S., Cleghorn G., Neto U.F., Gopalan S., Hernell O., *et al*. (2005) Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *Journal of pediatric gastroenterology and nutrition* **41**, 584–599.

Koletzko B., Cetin I. & Brenna J. for the Perinatal Lipid Intake Working Group (2007) Dietary fat intakes for pregnant and lactating women. *British Journal of Nutrition* **98**, 873–877.

Koletzko B., Lien E., Agostoni C., Böhles H., Campoy C., Cetin I., *et al*. (2008) The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *Journal of Perinatal Medicine* **36**, 5–14.

Koletzko B., von Kries R., Closa R., Escribano J., Scaglioni S., Giovannini M., *et al*. (2009a) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *American Journal of Clinical Nutrition* **89**, 1836–1845.

Koletzko B., von Kries R., Monasterolo R.C., Subias J.E., Scaglioni S., Giovannini M., *et al*. (2009b) Can infant feeding choices modulate later obesity risk? *American Journal of Clinical Nutrition* **89**, S1502–S1508.

Kramer M.S. & Kakuma R. (2002) *The Optimal Duration of Exclusive Breastfeeding: A Systematic Review*. WHO: Geneva.

Krebs N.F. (2000) Dietary zinc and iron sources, physical growth and cognitive development of breastfed infants. *Journal of Nutrition* **130**, 358S–360S.

Krebs N.F. (2007) Food choices to meet nutritional needs of breast-fed infants and toddlers on mixed diets. *Journal of Nutrition* **137**, 511S–517S.

von Kries R., Koletzko B., Sauerwald T., von Mutius E., Barnert D., Grunert V. & von Voss H. (2001) Breast feeding and obesity: cross sectional study. *British Medical Journal* **319**, 147–150.

Kunz C., Rudloff S., Baier W., Klein N. & Strobel S. (2000) Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annual Review of Nutrition* **20**, 699–722.

Lawlor D.A. & Smith G.D. (2005) Early life determinants of adult blood pressure. *Current Opinion in Nephrology and Hypertension* **14**, 259–264.

Lozoff B. & Georgieff M.K. (2006) Iron deficiency and brain development. *Seminars in Pediatric Neurology* **13**, 158–165.

Lynch S., Stoltzfus R. & Rawat R. (2007) Critical review of strategies to prevent and control iron deficiency in children. *Food and Nutrition Bulletin* **28**, S610–S620.

Mena P. & Uauy R. (2008) Fats. In *Pediatric Nutrition in Practice* (ed. B. Koletzko), pp. 47–51. Karger: Basel.

Michaelsen K.F., Skafte L., Badsberg J.H. & Jorgensen M. (1990) Variation in macronutrients in human bank milk: influencing factors and implications for human milk banking. *Journal of Pediatric Gastroenterology and Nutrition* **11**, 229–239.

Michaelsen K.F., Weaver L., Branca F. & Robertson A. (2000) Feeding and nutrition of infants and young children: guidelines for the WHO European Region, with emphasis on the former Soviet countries. WHO Regional Publications, European Series, No. 87.

Molgaard C. & Michaelsen K.F. (2003) Vitamin D and bone health in early life. *Proceedings of the Nutrition Society* **62**, 823–828.

Olivares M., Pizarro F., Speisky H., Lonnerdal B. & Uauy R. (1998) Copper in infant nutrition: Safety of world health organization provisional guideline value for copper content of drinking water. *Journal of Pediatric Gastroenterology and Nutrition* **26**, 251–257.

Pelto G.H. (1987) Cultural issues in maternal and child health and nutrition. *Social Science and Medicine* **25**, 553–559.

Pencharz P. & Elango R. (2008) Protein. In *Pediatric Nutrition in Practice* (ed. B. Koletzko), pp. 37–41. Karger: Basel.

Perez-Lopez F.R. (2007) Iodine and thyroid hormones during pregnancy and postpartum. *Gynecological Endocrinology* **23**, 414–428.

Pombo M., Castro L. & Barreiro J. (2001) El crecimiento, el desarrollo y los elementos traza. *Anales Espanoles de Pediatria* **54**, 63–71.

Ponza M., Devaney B., Ziegler P., Reidy K. & Squatrito C. (2004) Nutrient intakes and food choices of infants and toddlers participating in WIC. *Journal of the American Dietetic Association* **104**, S71–S79.

Prentice A., Branca F., Decsi T., Michaelsen K.F., Fletcher R.J., Guesry P., *et al*. (2004) Energy and nutrient dietary reference values for children in Europe: methodological approaches and current nutritional

recommendations. *British Journal of Nutrition* **92** (Suppl. 2), S83–S146.

Reilly J.J., Ashworth S. & Wells J.C.K. (2005) Metabolisable energy consumption in the exclusively breast-fed infant aged 3–6 months from the developed world: a systematic review. *British Journal of Nutrition* **94**, 56–63.

Reyes-Posso M. (2008) Alimentación complementaria en los niños menores de dos años. *Revista Ecuatoriana de Pediatría* **9**, 63–67.

Sandstrom B. (2001) Micronutrient interactions: effects on absorption and bioavailability. *British Journal of Nutrition* **85** (Suppl. 2), S181–S185.

Scavenius M., van Hulsel L., Meijer J., Wendte H. & Gurgel R. (2007) In practice, the theory is different: a processual analysis of breastfeeding in north east Brazil. *Social Science and Medicine* **64**, 676–688.

von Schenck U., Bender-Gotze C. & Koletzko B. (1997) Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. *Archives of Disease in Childhood* **77**, 137–139.

Schiess S., Grote V., Scaglioni S., Luque V., Martin F., Stolarczyk A., *et al*. (2010) Introduction of complementary feeding in 5 European countries. *Journal of Pediatric Gastroenterology and Nutrition* **50**, 92–98.

Scientific Committee on Food of the European Commission SCF (2003) *Report of the Scientific 1–8 Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae*. EC: Brussels.

Sellen D.W. (2001) Weaning, complementary feeding, and maternal decision making in a rural East African pastoral population. *Journal of Human Lactation* **17**, 233–244.

Specker B.L., Black A., Allen L. & Morrow F. (1990) Vitamin-B12 – low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. *American Journal of Clinical Nutrition* **52**, 1073–1076.

Spiro A. (2006) Gujarati women and infant feeding decisions. In *Maternal and Child Nutrition & Nurture: Controversies and challenges* (eds F. Dykes & V. Hall-Moran), pp. 232–249. London: Quay books.

Strobel M., Tinz J. & Biesalski H.K. (2007) The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *European Journal of Nutrition* **46** (Suppl. 1), 1–20.

Uauy R., Maass A. & Araya M. (2008) Estimating risk from copper excess in human populations. *American Journal of Clinical Nutrition* **88**, 867S–871S.

Wagner C.L. & Greer F.R. (2008) Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* **122**, 1142–1152.

WHO/FAO/UNU (2007) *Protein and Amino Acid Requirements in Human Nutrition*. Report of a Joint WHO/FAO/UNU Expert Consultation. In: Technical Report Series, No 935. WHO: Geneva, Switzerland.

World Health Organization (2003a) *Copper in drinking-water. Background document for preparation of WHO guidelines for drinking-water quality*. WHO: Geneva, Switzerland.

World Health Organization (2003b) *Global strategy for infant and young child feeding*. WHO: Geneva, Switzerland.

Yang Z., Lonnerdal B., Adu-Afarwuah S., Brown K.H., Chaparro C.M., Cohen R.J., *et al*. (2009) Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies. *American Journal of Clinical Nutrition* **89**, 1433–1440.

Ziegler E.E., Hollis B.W., Nelson S.E. & Jeter J.M. (2006) Vitamin D deficiency in breastfed infants in Iowa. *Pediatrics* **118**, 603–610.

Ziegler E.E., Nelson S.E. & Jeter J.M. (2009) Iron supplementation of breastfed infants from an early age. *American Journal of Clinical Nutrition* **89**, 525–532.

Zietz B.P., Dieter H.H., Lakomek M., Schneider H., Kessler-Gaedtke B. & Dunkelberg H. (2003) Epidemiological investigation on chronic copper toxicity to children exposed via the public drinking water supply. *Science of the Total Environment* **302**, 127–144.

Zimmermann M.B. (2007) The impact of iodised salt or iodine supplements on iodine status during pregnancy, lactation and infancy. *Public Health Nutrition* **10**, 1584–1595.

# 8 PUBLICATION 2

Hall Moran, V., N. Lowe, N. Crossland, C. Berti, I. Cetin, M. Hermoso, B. Koletzko & F. Dykes (2010) Nutritional requirements during lactation. Towards European alignment of reference values: the EURRECA network. *Maternal & Child Nutrition,* 6**,** 39-54

Original Article

# Nutritional requirements during lactation. Towards European alignment of reference values: the EURRECA network

**Victoria Hall Moran*, Nicola Lowe†, Nicola Crossland*, Cristiana Berti‡[1], Irene Cetin‡, Maria Hermoso§, Berthold Koletzko§ and Fiona Dykes***

**Maternal & Infant Nutrition and Nurture Unit (MAINN) and †Centre for Applied Sport and Exercise Sciences, University of Central Lancashire, Preston, PR1 2HE, UK, ‡Unit of Obstetrics and Gynecology, Department of Clinical Sciences Hospital 'L. Sacco', and Center for Fetal Research Giorgio Pardi, University of Milan, Milano, Italy, and §Division of Metabolic Diseases and Nutritional Medicine, Dr von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Munich, Germany*

**Abstract**

There is considerable variation in reference values for micronutrient intake during lactation across Europe. The European Micronutrients Recommendations Aligned project aims to harmonize dietary recommendations throughout Europe. Recommended nutrient intakes during lactation are based on limited data and are often extrapolated from known secretion of the nutrient in milk with adjustments for bioavailability, so that differences between values can be partly ascribed to differences in methodological approaches and how these approaches were applied. Few studies have considered the impact of lactation on the mother's nutritional status. Rather, focus has been placed on the influence of maternal nutritional status on the composition of her breast milk. Most common nutritional deficits in breast milk are the result of maternal deficiencies of the water-soluble vitamins, thiamine, riboflavin and vitamins B6 and B12. Other than maternal vitamin A status, which to some extent is reflected in breast milk, concentrations of fat-soluble vitamins and most minerals in breast milk are less affected by maternal status. Factors relating to suboptimal maternal nutritional status during lactation include maternal age, diet and lifestyle factors and spacing of consecutive births. Recent research is providing new knowledge on the micronutrient requirements of lactating women. Identifying needs for research and improving understanding of the differences in values that have been derived by various committees and groups across Europe will enhance transparency and facilitate the application of dietary recommendations in policy-making decision and their translation into recommendations for lactating women. Given the wide variation in breastfeeding practices across Europe, making nutritional recommendations for lactating women is complex and challenging. Thus, it is crucial to first examine the cultural practices within and across European populations and to assess its relevance before making recommendations.

*Keywords:* lactation, breastfeeding, micronutrient, reference value, nutritional requirement, EURRECA.

Correspondence: Victoria Hall Moran, Maternal & Infant Nutrition and Nurture Unit, University of Central Lancashire, Preston PR1 2HE, UK. E-mail: vlmoran@uclan.ac.uk

[1]Supported by the Invernizzi Foundation Fellowship.

## Introduction

There is considerable variation in reference values for micronutrient intake across Europe. The disparity is caused by various factors such as differences in underlying concepts, in the terminology used and in the methodologies and assumptions made to define micronutrient requirements and to derive reference values of intake (Doets *et al*. 2008).

The need for harmonization of dietary recommendations throughout Europe was recognized by the European Commission in 2005 (Ashwell *et al*. 2008).

The aim of harmonization is to improve understanding of the differences in values that have been derived by various committees and groups across Europe and to enhance their transparency to facilitate their application in policy making decision. The European Micronutrients Recommendations Aligned (EURRECA) Network of Excellence has four phases of activity. Research activity (RA) 1 evaluated best practice for assessing intake and status methods and collated current recommendations. RA 2 will apply best practice developed in RA 1 to population groups within a healthy population that may be vulnerable to micronutrient deficiencies. RAs 3 and 4 will develop and evaluate toolkits for dissemination of recommendations. Women who are pregnant or lactating have been identified by EURRECA as a vulnerable population group. While the nutrient requirements during pregnancy are discussed in this supplement by Berti and colleagues, the aim of this paper is to review specific aspects of healthy lactating women in relation to their micronutrient requirements.

## Sources of disparity in nutrient recommendations across Europe

Great disparities exist between reference values for lactating women established in European countries (Tables 1,2). Some countries have published their own guidelines, some have harmonized their recommendations with other countries [e.g. the German-speaking countries (DACH) and the Nordic countries] and some have adopted recommendations suggested by others (e.g. Slovenia has adopted DACH recommendations). These disparities have arisen from the use of different concepts and sometimes different data and because the expert committees who set the recommendations often base their decisions on judgements concerning the quality of the available research (Pijls *et al*. 2009). As national reference values are reviewed at different time points, decisions may also be based on different scientific data (Doets *et al*. 2008). There is also discrepancy in the terminology used to describe reference values, creating difficulties with making comparisons across countries. For example, the UK's 'dietary reference values' are constructed around the estimated average requirement (EAR). The reference nutrient intake (RNI) is the value 2 standard deviations above the EAR. The RNI is equivalent to the population reference intake (PRI) used by the European Union (EU), and the recommended daily allowance (RDA) is used in other countries. Such divergent terminology and concepts can lead to confused messages that may have a serious impact on policy and significant health consequences (Pavlovic *et al*. 2007).

In relation to lactation specifically, as metabolic data upon which estimates of requirements are based are often lacking for physiological states such as lactation because of practical difficulties or ethical limitations in conducting research in women during these reproductive stages, differences between values can also be partly ascribed to differences in methodological approaches and how these approaches are applied (Atkinson & Koletzko 2007). For example, Atkinson and Koletzko (2007) compared the methods of extrapolation used to determine recommended intakes for vitamins A, C and E during lactation in the USA/Canada, the Caribbean, the EU, the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), the German-speaking countries (Austria, Germany and Switzerland) and the UK. They found that, although the general method of extrapolation was similar across reports, the reference values applied for nutrient composition of milk was often disparate,

**Key messages**

- The EURECCA network aims to harmonize the micronutrient recommendations for lactating women across Europe.
- The micronutrient requirements of lactating women are influenced by a range of factors including the intensity of lactation, maternal age, diet and lifestyle factors, and birth spacing.
- When making nutritional recommendations for lactating women attention must be paid to the social, cultural and economic factors that influence what women may eat.

**Table 1.** Recommended intake level for vitamins for lactating women (values are inclusive of any increment for lactation)

| Country | Year | Reference | Reference value type | Vitamin A | Vitamin B6 | Vitamin C | Vitamin D | Vitamin E | Thiamine | Riboflavin | Niacin | Vitamin B12 | Folate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Units | | | | mg RE | mg | mg | mg | Mg | mg | mg | mg NE | mg | mg DFE |
| Albania | 2005 | [1] | RDA | 1.3 (1.2)† | 2 | 120 (115) | 5 | 19 | 1.4 | 1.6 | 17 | 2.8 | 500 |
| Belgium | 2009 | [2] | RDI | 0.85 | 2 | 130 | 20 | 15 | 1.6 | 1.8 | 16 | 1.7 | 350 |
| Bosnia and Herzegovina | 2005 | [3] | RDA | 1.3 (1–6 m)<br>1.2 (7–12 m)‡ | 2.1 | 95 (1–6 m)<br>90 (7–12 m) | 10 | 12 (1–6 m)<br>11 (7–12 m) | 1.6 | 1.8 (1–6 m)<br>1.7 (7–12 m) | 20 | 2.6 | 280 |
| Bulgaria | 2005 | [4] | RI | 1.2 (1.1) | 2 | * | 5 | 19 | 1.4 | 1.6 | 17 | 2.8 | 500 |
| Croatia | 2004 | [5] | RI | 1.3 | 2 | 120 | 10 | 19 | 1.4 | 1.6 | 17 | 2.8 | 500 |
| DACH countries§ | 2004 | [6] | RNI or AI (vit E) | 1.5 | 1.9 | 150 | 5 | 17 | 1.4 | 1.6 | 17 | 4 | 600 |
| Estonia | 2006 | [7] | RI | 1.1 | 1.6 | 100 | 10 | 11 | 1.7 | 1.7 | 20 | 2.6 | 500 |
| France | 2001 | [8] | PRI or AI (folate, riboflavin, thiamine, vitamin D, vitamin E) | 0.9 | 2 | 130 | 10 | 12 | 1.8 | 1.8 | 15 | 2.8 | 400 |
| Greece | 1993 | [9] | PRI | 0.9 | 1.4 | 70 | 10 | * | 1.1 | 1.7 | 16 | 1.9 | 350 |
| Hungary | 2005 | [10] | Safe intake | 1.2 | 2 | 120 | 10 | 19 | 1 | 1.7 | 16 | 2.6 | 425 |
| Iceland | 2006 | [11] | RI | 1.1 | 1.6 | 100 | 10 | 11 | 1.6 | 1.7 | 20 | 2.6 | 500 |
| Ireland | 1999 | [12] | RDA | 0.95 | 1.4 | 80 | 10 | * | 1.1 | 1.7 | 16 | 1.9 | 400 |
| Italy | 1996 | [13] | RDA | 0.95 | 1.4 | 90 | 10 | * | 1.1 | 1.7 | 16 | 1.4 | 350 |
| Latvia | 2001 | [14] | Recommended average daily intake | 1.3 | 2.2 | 150 | 10 | 12 | 1.6 | 2 | 20 | 3 | 300 |
| Lithuania | 1999 | [15] | RDA | 1.2 | 2 | 100 | 10 | 14 | 1.4 | 1.9 | 16 | 4 | 480 |
| Macedonia – Former YR | 2001 | [16] | RDA | 1.2 | 2 | 110 | 10 | 12 | 1.6 | 0.7 | 17.6 | 2.6 | 300 |
| The Netherlands | 1992 | [17] | AI or RI (riboflavin, thiamine, B6, B12) | 1.25 | 1.9 | 110 | 7.5 | * | 1.7 | 1.7 | 20 | 3.8 | 400 |
| Nordic countries¶ | 2005 | [18] | RI | 1.1 | 1.6 | 100 | 10 | 11 | 1.6 | 1.7 | 20 | 2.6 | 500 |
| Poland | 2008 | [19] | RDI or AI (vitamin D, E) | 1.3 (1.2) | 2 | 120 (115) | 5 | 8 | 1.5 | 1.6 | 17 | 2.8 | 500 |
| Portugal | 2005 | [20] | RNI or safe intake (vitamin A) | 0.85 | 2 | 70 | 5 | * | 1.5 | 1.6 | 17 | 2.8 | 500 |
| Russian Federation | 1991 | [21] | Recommended level of intake | 1.2 | 2.3 | 115 | 12.5 | 12 | 1.8 | 1.97 | 16.8 | 3 | 300 |
| Slovakia | 1997 | [22] | RDA | 1.2 | 2.3 | 130 | 10 | 18 | 1.4 | 1.8 | 20 | 2.6 | 300 |
| Slovenia | 2004 | [23] | RNI or AI (vitamin E) | 1.5 | 1.9 | 150 | 5 | 17 | 1.4 | 1.6 | 17 | 4 | 600 |
| Spain | 2007 | [24] | RI | 1.3 | 2 | 85 | 10 | 17 | 1.1 | 1.7 | 18 | 2.6 | 500 |
| UK | 1991 | [25] | RNI | 0.95 | 1.2 | 70 | 10 | * | 1 | 1.6 | 15 | 2 | 260 |
| WHO/FAO | 2004 | [26] | RNI | 0.85 | 2 | 70 | 5 | * | 1.5 | 1.6 | 17 | 2.8 | 500 |

Values obtained from the web-based tool Nutri-RecQuest (described by Cavelaars *et al*. 2010). mg RE, mg retinol equivalent; mg NE, mg niacin equivalents; mg DFE, mg dietary folate equivalents; AI, estimated value for adequate intake; AR, adequate area of intake; PRI, population recommended intake; RDA, recommended daily allowance; RI, recommended intake; RNI, recommended nutrient intake; LL, lower limit; UL, upper limit. *Reference value is available but cannot be converted to the standard unit without making assumptions. †Values in parentheses indicate reference values for 18 years. ‡Indicating reference values for different stages of lactation. §DACH refers to reference intakes for Germany, Austria and Switzerland. ¶'Nordic countries' refers to Denmark, Finland, Norway and Sweden. Iceland has adapted these so is referred to separately. References: [1] Berisha A., Bader E., Deligia C and Claude Dop M, 2005. Albania. [2] Hoge Gezondheidsraad, Voedingsaanbevelingen voor België. Herziening 2009, HGR dossiernummer: 8309. [3] Public Health Institute of the Republic of Srpska, 2005. Bosnia and Herzegovina, entity: Republic of Srpska. [4] Ministry of Health, 2005. Bulgaria. [5] Croatian National Official Gazzette, 2004. Croatia. [6] German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition research (SGE), Swiss Nutrition Association (SVE) 2004. Germany, Austria, Switzerland. [7] Vaask, Sirje; Liebert, Tiiu; Maser, Mai; Pappel, Kaie; Pitsi, Tagli, Saava, Merileid; Sooba, Eve; Vihalemm, Tiiu, Villa, Inga, 2006. Estonia. [8] Martin A, Guest Editor-in-Chief, 2001. France. [9] Commission of the European Communities 1993 Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. [10] Antal M. 2005. Hungary. [11] The Public Health Institute of Iceland, 2006. Iceland. [12] Food Safety Authority of Ireland, 1999. Ireland. [13] LARN (1996) Livelli di Assunzione Raccomandati di Energia e Nutrienti per la Popolazione Italiana. Revision. Societá Italiana di Nutrizione Umana (SINU). [14] Latvian Food Center, 2001. Latvia. [15] Ministry of Health, 1999. Lithuania. [16] Ministry of Health and Republic Institute of Health Protection, 2001. The former Yugoslav Republic of Macedonia. [17] Food and Nutrition Council, 1992. Netherlands. [18] Nordic Council of Ministers, 2005. Denmark, Finland, Norway, Sweden. [19] National Food and Nutrition Institute, Wydawnictwo Lekarsie PZWL, Warsaw 2008. [20] World Health Organization (WHO), Food and Agriculture Organization of the United Nations 2004. WHO/FAO. [21] The Ministry of Health of the USSR, 1991. Russian Federation. [22] Ministry of Health, 1997. Slovakia. [23] German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition research (SGE), Swiss Nutrition Association (SVE) 2004. Germany, Austria, Switzerland. [24] Moreiras O, Carbajal AL, Cabera C 2007. Tablas de composicion de alimentos. Edicones Piramide 11a edicion revisada y ampliada. [25] Panel on DRVs of the Committee on Medical Aspects of Food Policy (COMA), 1991. United Kingdom. [26] World Health Organization (WHO), Food and Agriculture Organization of the United Nations 2004. WHO/FAO.

**Table 2.** Recommended intake level for minerals for lactating women (values are inclusive of any increment for lactation)

| Country | Year | Reference | Reference value type | Calcium mg | Phosphorus mg | Potassium mg | Sodium mg | Magnesium mg | Iodine mg | Iron mg | Zinc mg | Selenium mg | Copper mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Albania | 2005 | [1] | RDA | 1000 (1300) | 700 (1250) | 5100 | 1500 | 320 (360) | 290 | 9 (10) | 12 (13) | 70 | 1.3 |
| Belgium | 2009 | [2] | RDI or Acceptable daily intake (K, Na) | 1200 | 1000 | 3000 LL, 4000 UL | 600 LL, 2000 UL | 400 | 250 | 10 | 14 | 75 | 1.5 |
| Bosnia and Herzegovina | 2005 | [3] | RDA | 1200 | 1200 | ¶ | ¶ | ¶ | ¶ | ¶ | ¶ | ¶ | ¶ |
| Bulgaria | 2005 | [4] | RI or AI (Ca, Na) | 1000 (1300) | 700 (1250) | 5100 | 1500 | 320 | 290 | 9 (10) | 12 (13) | 70 | 1.3 |
| Croatia | 2004 | [5] | RI | 1000 | 700 | * | * | 310 | 290 | 9 | 12 | 70 | 1.3 |
| DACH countries | 2005 | [6] | RNI or AI (Cu) | 1000 | 900 | * | * | 390 | 260 | 20 | 10 | 30 LL, 70 UL | 1 LL, 1.5 UL |
| Estonia | 2006 | [7] | RI | 900 | 900 | 3100 | * | 280 | 200 | 15 | 11 | 55 | 1.3 |
| France | 2001 | [8] | PRI or AI (Se) | 1000 | 850 | * | * | 390 | 200 | 10 | 19 | 60 | 2 |
| Greece | 1993 | [9] | PRI | 1200 | 950 | 3100 | * | * | 160 | 10 | 12 | 70 | 1.35 |
| Hungary | 2005 | [10] | Safe intake or suggested maximum intake (Na) | 1200 | 930 | 3500 | 2000 | 450 | 200 | 15 | 13 | 75 | 1.4 |
| Iceland | 2006 | [11] | RI | 1000 | 750 | 3100 | * | 280 | 200 | 15 | 11 | 55 | 1.3 |
| Ireland | 1999 | [12] | RDA | 1200 | 950 | 3100 | * | * | 160 | 15 | 12 | 75 | 1.4 |
| Italy | 1996 | [13] | RDA | 1200 | 1200 | 3100 | * | * | 200 | 18 | 12 | 70 | 1.5 |
| Latvia | 2001 | [14] | Recommended average daily intake | 1200 | 1200 | 4000 | 3300 | 340 | 200 | 18 | 19 | 75 | 3 |
| Lithuania | 1999 | [15] | RDA | 1200 | 1200 | 2500 | 1500 | 380 | 200 | 20 | 15 | * | * |
| Macedonia – former YR | 2001 | [16] | RDA | 1200 | 1200 | 2000 | 1500 | 360 | 200 | 20 | 22 | 125 | 2.5 |
| The Netherlands | 1992 | [17] | AR or AI (Ca, Fe) | 1000 | 900 LL, 1800 UL | * | * | 300 LL, 400 UL | * | 20 | 18 | 75 LL, 150 UL | 2 LL, 3.5 UL |
| Nordic countries | 2005 | [18] | RI | 900 | 900 | 3100 | * | 280 | 200 | 15 | 11 | 55 | 1.3 |
| Poland | 2008 | [19] | RDI or AI (Ca, K, Na) | 1000 (1300) | 700 (1250) | 5100 | 1500 | 320 (360) | 220 (290) | 18 | 12 (13) | 70 | 1.3 |
| Portugal | 2005 | [20] | RNI | 1000 | * | * | * | 270 | 200 | § | § | 35 (0–3 m) 35 (4–6 m) 42 (7–9 m) | * |
| Russian Federation | 1991 | [21] | Recommended level of intake | 1200 | 1800 | * | * | 450 | 150 | 33 | 25 | * | * |
| Slovakia | 1997 | [22] | RDA | 1500 | 1500 | * | * | 390 | 300 | 20 | 16 | 70 | 2.5 |
| Slovenia | 2004 | [23] | RNI or AI (Cu, Se) | 1000 | 900 | * | * | 390 | 260 | 20 | 10 | 30 LL, 70 UL | 1 LL, 1.5 UL |
| Spain | 2007 | [24] | RI | 1500 | 700 | 3500 | * | 450 | 155 | 18 | 25 | 75 | * |
| UK | 1991 | [25] | RNI or AI (Cu) | 1250 | 990 | 3500 | 1600 | 320 | 140 | 14.8 | 13 (<4 m) 9 (>4 m) | 75 | 1.5 |
| WHO/FAO | 2004 | [26] | RNI | 1000 | * | * | * | 270 | 200 | § | § | 35 (0–3 m) 35 (4–6 m) 42 (7–9 m) | * |

Values obtained from the web-based tool Nutri-RecQuest (described by Cavelaars *et al*. 2010). mg RE, mg retinol equivalent; mg NE, mg niacin equivalents; mg DFE, mg dietary folate equivalents; AI, estimated value for adequate intake; AR, adequate area of intake; PRI, population recommended intake; RDA, recommended daily allowance; RI, recommended intake; RNI, recommended nutrient intake; LL, lower limit; UL, upper limit. *Reference value is available but cannot be converted to the standard unit without making assumptions. †Values in parentheses indicate reference values for 18 years. ‡Indicating reference values for different stages of lactation. §Reference value varies according to bioavailability. ¶Reference values are not referred to in the country-specific guidelines. References: [1] Berisha A., Bader E., Deligia C and Claude Dop M, 2005. Albania. [2] Hoge Gezondheidsraad, Voedingsaanbevelingen voor België. Herziening 2009, HGR dossiernummer: 8309. [3] Public Health Institute of the Republic of Srpska, 2005. Bosnia and Herzegovina, entity: Republic of Srpska. [4] Ministry of Health, 2005. Bulgaria. [5] Croatian National Official Gazzette, 2004. Croatia. [6] German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition research (SGE), Swiss Nutrition Association (SVE) 2004. Germany, Austria, Switzerland. [7] Vaask, Sirje; Liebert, Tiiu; Maser, Mai; Pappel, Kaie; Pitsi, Tagli, Saava, Merileid; Sooba, Eve; Vihalemm, Tiiu, Villa, Inga, 2006. Estonia. [8] Martin A, Guest Editor-in-Chief, 2001. France. [9] Commission of the European Communities 1993 Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. [10] Antal M. 2005. Hungary. [11] The Public Health Institute of Iceland, 2006. Iceland. [12] Food Safety Authority of Ireland, 1999. Ireland. [13] LARN (1996) Livelli di Assunzione Raccomandati di Energia e Nutrienti per la Popolazione Italiana. Revision. Societá Italiana di Nutrizione Umana (SINU). [14] Latvian Food Center, 2001. Latvia. [15] Ministry of Health, 1999. Lithuania. [16] Ministry of Health and Republic Institute of Health Protection, 2001. The former Yugoslav Republic of Macedonia. [17] Food and Nutrition Council, 1992. Netherlands. [18] Nordic Council of Ministers, 2005. Denmark, Finland, Norway, Sweden. [19] National Food and Nutrition Institute, Wydawnictwo Lekarsie PZWL, Warsaw 2008. [20] World Health Organization-(WHO), Food and Agriculture Organization of the United Nations 2004. WHO/FAO. [21] The Ministry of Health of the USSR, 1991. Russian Federation. [22] Ministry of Health, 1997. Slovakia. [23] German Nutrition Society (DGE), Austrian Nutrition Society (OGE), Swiss Society for Nutrition research (SGE), Swiss Nutrition Association (SVE) 2004. Germany, Austria, Switzerland. [24] Moreiras O, Carbajal AL, Cabera C 2007. Tablas de composicion de alimentos. Edicones Piramide 11a edicion revisada y ampliada. [25] Panel on DRVs of the Committee on Medical Aspects of Food Policy (COMA), 1991. United Kingdom. [26] World Health Organization (WHO), Food and Agriculture Organization of the United Nations 2004. WHO/FAO.

creating differences between reports in their recommended intakes.

It has been argued that the limited systematic physiological difference in populations and climate across Europe (with the possible exception of difference in sunlight exposure and consequent vitamin D recommendations) does not justify the existing disparities in nutrient recommendations. The EURRECA network aims to develop a common framework that uses consistent terminology in order to develop and maintain nutrient recommendations based on the best current evidence (Pijls *et al*. 2009).

## Challenges to establishing nutritional recommendations for lactating women

Lactation is a highly demanding state for the mother with a nutritive burden considerably greater than that of pregnancy. The energy required to produce 1 L of milk is estimated to be approximately 700 kcal, and the milk secreted in 4 months of lactation represents an amount of energy roughly equivalent to the total energy cost of pregnancy (Cervera & Ngo 2001; Picciano 2003). Although it is well acknowledged that some of this requirement originates from the nutrients stored by the mother during pregnancy, there is a need for lactating mothers to increase their food intake in order to meet the elevated energy and micronutrient requirements (Cervera & Ngo 2001). Table 3 illustrates the percentage change in reference values for lactation from non-pregnant, non-lactating (NPNL) levels across Europe.

The duration and intensity of lactation (whether the infant is breastfed exclusively or only partially) may have an impact on a mother's nutritional status. For example, an exclusively breastfeeding woman has much greater energy and nutrient needs (with the exception of iron attributed to the potential protective effect of lactational amenorrhoea) than a woman who is only partially breastfeeding (Dewey 2004). These aspects of breastfeeding, however, have rarely been considered in studies of the nutritional impact of lactation in women. While the World Health Organization (WHO) recommends that infants should be exclusively breastfed for the first 6 months of life with breastfeeding continuing for up to 2 years of age or beyond (WHO 2003), in reality, there are wide deviations from this recommended norm both in terms of duration and intensity, especially in industrialized countries, challenging the meaningfulness of setting standard recommendations of nutrient intakes for lactation.

## Factors that could have a modifying impact on nutritional recommendations and reference values in lactating women

### Variability of breast milk

The calculation of recommended intakes during lactation often employs a factorial approach that simply sum the nutrient needs for a woman of similar periconceptional age who is not lactating with the amount of nutrient delivered into an average volume of breast milk, with adjustments for bioavailability in some cases (Atkinson & Koletzko 2007). While the composition of breast milk is thought to be relatively stable in well-nourished mothers, the infants' average daily intake of breast milk has been reported to vary between studies (WHO 1998). The volume of milk consumed by the infant at each breastfeeding has been shown to be dependent upon a number of factors, such as whether the breast that was being suckled was the more or less productive breast, whether the breastfeeding was unpaired, the time of day and whether the infant breastfed during the night or not (Kent *et al*. 2006).

### Stage of lactation

Some countries/organizations investigated (Bosnia and Herzegovina, Portugal, the UK, WHO) have set reference values for different stages of lactation for some nutrients (see Tables 1,2). It is argued that the lactational period should not to be divided into different stages such as early and late lactation for the purpose of setting recommendations because evidence supports that, as during pregnancy, physiological adjustments in nutrient utilization occur during this period that generally compensate for the shifts

**Table 3.** Percentage change in micronutrient reference values during lactation compared to reference values for non-pregnant, non-lactating (NPNL) women (unless otherwise indicated the figure represent an increase from NPNL levels)

| Country | Vitamin A | Vitamin B6 | Vitamin C | Vitamin D | Vitamin E | Thiamine | Riboflavin | Niacin | Vitamin B12 | Folate | Calcium | Phosphorus | Potassium | Sodium | Magnesium | Iodine | Iron | Zinc | Selenium | Copper |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Albania | 86 (71) | 54 | 33 (28) | * | 27 | 27 | 45 | 21 | 17 | 25 | 0 (30) | 0 (79) | 9 | 0 | 3 (16) | 93 | −50 (−44) | 50 (63) | 27 | 44 |
| Belgium | 70 | 11 | 18 | 60 | 0 | 45 | 50 | 14 | 21 | 75 | 33 | 25 | 0 | 0 | 11 | 25 | −26 | 75 | 25 | 25 |
| Bosnia and Herzegovina | 63 (50) | 31 | 58 (50) | 100 | 50 (38) | 45 | 38 (31) | 33 | 30 | 56 | 50 | 50 | * | * | * | * | * | * | * | * |
| Bulgaria | 71 (57) | 54 | * | 0 | 27 | * | 45 | 21 | 17 | 25 | 0 (30) | 0 (79) | 9 | 0 | 14 | 93 | −50 (−44) | 50 (63) | 27 | 44 |
| Croatia | 63 | 0 | 100 | 100 | 90 | 0 | 0 | −6 | 180 | 150 | 25 | * | * | * | 3 | 93 | −36 | * | 40 | 13 |
| DACH countries | 88 | 58 | 50 | 0 | 42 | 40 | 33 | 31 | 33 | 50 | 0 | 29 | * | * | 30 | 30 | 33 | 43 | 0 | 0 |
| Estonia | 57 | 23 | 33 | 33 | 38 | 55 | 31 | 33 | 30 | 67 | 13 | 50 | 0 | * | 0 | 33 | 0 | 57 | 38 | 44 |
| France | 50 | 33 | 18 | 100 | 0 | 64 | 20 | 36 | 17 | 33 | 11 | 13 | * | * | 8 | 33 | −38 | 90 | 20 | 33 |
| Greece | 50 | 27 | 56 | 100 | * | 22 | 31 | 14 | 36 | 75 | * | 73 | 0 | * | * | 23 | −32 | 71 | 40 | 23 |
| Hungary | 50 | 54 | 33 | 100 | 29 | 11 | 31 | 14 | 30 | 42 | 50 | 50 | 0 | 0 | 50 | 33 | 0 | 44 | 25 | 27 |
| Iceland | 57 | 23 | 33 | 0 | 38 | 45 | 31 | 33 | 30 | 25 | 25 | 25 | 0 | * | 0 | 33 | 0 | 57 | 38 | 44 |
| Ireland | 58 | 27 | 33 | 100 | * | 22 | 31 | 14 | 36 | 33 | 50 | 73 | 0 | * | * | 23 | −7 | 71 | 36 | 27 |
| Italy | 58 | 27 | 50 | 100 | * | 22 | 31 | 14 | 27 | 75 | 20 | 20 | 0 | * | * | 33 | 0 | 71 | 27 | 25 |
| Latvia | 30 | 10 | 50 | 100 | 0 | 33 | 25 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | −3 | 0 | 0 | 36 | 25 | 0 |
| Lithuania | 50 | 104 | 67 | 100 | 40 | * | 36 | −6 | 33 | 60 | 20 | 33 | 0 | 0 | 27 | 33 | 33 | 25 | * | * |
| Macedonia – Former YR | 50 | 33 | 57 | 100 | 50 | 45 | 250 | 40 | 30 | 50 | 50 | 50 | * | 0 | 29 | 33 | 33 | 83 | 0 | 0 |
| The Netherlands | 56 | 27 | 57 | 200 | * | 55 | 55 | 54 | 36 | 33 | 0 | * | * | * | 27 | * | 33 | 140 | 13 | 10 |
| Nordic countries | 57 | 23 | 33 | 33 | 38 | 45 | 31 | 33 | 30 | 25 | 13 | 50 | 0 | * | 0 | 33 | 0 | 57 | 38 | 44 |
| Poland | 86 (71) | 54 | 60 (53) | 0 | 0 | 36 | 45 | 21 | 17 | 25 | 0 (30) | 0 (79) | 9 | 0 | 3 (16) | 47 (93) | −44 | 60 (63) | 27 | 4 |
| Portugal | 70 | 54 | 56 | 0 | * | 36 | 45 | 21 | 17 | 25 | 0 | * | * | * | 23 | 33 | * | * | * | * |
| Russian Federation | 33 | 28 | 53 | 400 | 50 | 48 | 34 | 4 | 0 | 50 | 50 | 50 | * | * | 13 | 0 | * | 67 | * | * |
| Slovakia | 41 | 26 | 59 | 33 | 29 | 27 | 15 | 18 | 30 | 50 | 61 | 25 | * | * | 11 | 81 | 20 | 45 | 35 | 25 |
| Slovenia | 88 | 58 | 50 | 0 | 42 | 40 | 33 | 31 | 33 | 50 | 0 | 29 | * | * | 30 | 30 | 33 | 43 | 0 | 0 |
| Spain | 63 | 25 | 42 | 100 | 42 | 22 | 21 | 20 | 30 | 25 | 88 | 0 | 0 | * | 36 | 41 | 0 | 67 | 36 | * |
| UK | 58 | 0 | 75 | * | * | 25 | 45 | 15 | 33 | 30 | 79 | 80 | 0 | 0 | 19 | 0 | 0 | 86 (36) | 25 | 25 |
| WHO/FAO | 70 | 54 | 56 | 0 | * | 36 | 45 | 21 | 17 | 25 | 0 | * | * | * | 23 | 33 | * | * | * | – |

Values in parentheses indicate percentage change for lactating women aged 18 years (Albania, Bulgaria and Poland), or values at different stages of lactation (Bosnia and Herzegovina and the UK). *Unable to calculate percentage increase as either reference value is available but cannot be converted to the standard unit without making assumptions; reference value varies according to bioavailability; reference value is not referred to in the country specific guidelines.

in nutrient requirement with stages of lactation. Atkinson & Koletzko (2007) assert that the grading reference values do not appear to be based in strong science and having more than one reference value for lactation is impractical to implement.

### Maternal age

Several countries have set separate reference values for some micronutrients (typically vitamins A and C, calcium, phosphorus, magnesium, iron and zinc) for adolescent and adult lactating mothers (see Tables 1,2). It has be suggested that, as adolescence is a period of rapid growth (approximately 50% of adult body weight and 15% of final adult height is attained during this stage of the life cycle; Rogol *et al*. 2000), the additional nutritional demands of pregnancy and lactation may have a significant impact on the nutritional status of adolescent mothers and their breast milk, particularly those who are undernourished (Hall Moran 2007). In studies of adolescents in Bangladesh, for example, linear growth ceased during pregnancy and lactation, and lean body mass and percent body fat declined by 6 months post partum (Rah *et al*. 2008). Lower ponderal and body compositional measures were exhibited in those adolescents whose infants survived through the neonatal period compared with those who experienced a fetal loss or neonatal death, suggesting a depletion of maternal energy and nutrient reserves to meet the demands of both pregnancy and lactation (Rah *et al*. 2010). In contrast, Scholl *et al*. (1990, 1993) demonstrated that still-growing US adolescents continued to grow in stature and accrue fat mass during pregnancy and lactation. However, these young mothers had infants with lower birthweight, particularly when the mother continued to accrue higher amounts of arm or subscapular fat late in gestation. Scholl *et al*. (1993) suggest that, instead of mobilizing fat reserves late in pregnancy to enhance fetal growth, adolescent mothers appeared to be reserving them for their own continued development.

Other research has found that breastfeeding during adolescence may have a protective role on bone mass acquisition. While bone accretion rates peak during pubertal growth because of associated hormonal changes and adolescents experience bone mineral density (BMD) loss during lactation, this effect seems to be transient with subsequent repletion of BMD once breastfeeding is ceased (Bezerra *et al*. 2004). Chantry *et al*. (2004) reported that adjusted BMD between ages 20 and 25 were 5–10% higher in women who had breastfed as adolescents compared with those who had not. As higher peak BMD achieved during adolescence protects against postmenopausal osteoporosis (Rozen *et al*. 2003), breastfeeding during adolescence may have a protective role against this disease. It has been suggested that the mechanisms of BMD repletion (thought to be attributed to the reversal of hormonal changes that caused bone loss during lactation; Kalkwarf 1999) may have a larger physiologic effect when bone mineral is still accruing (Chantry *et al*. 2004).

It has been suggested that adolescents who are still growing have an increased demand for zinc during lactation (Institute of Medicine 2001). Meeting zinc demands during lactation will depend on the dietary supply, bioavailability and the capacity for adaptation of zinc metabolism. While recent evidence suggests that the biochemical responses of zinc to lactation are similar in adolescents and adults, significant correlations have been found between the activity of zinc-dependent enzymes and plasma zinc in adolescents, which may suggest a limiting action of poor maternal zinc status on the metabolic adaptation capacity of this population (Maia *et al*. 2007).

It is clear that, while there is some evidence to suggest that the nutritional demands of lactation may have a differential impact on the nutritional status of adolescent women, further research is needed to clarify this.

### Birth spacing and the recuperative interval

Reference values for lactation do not account for the influence of birth spacing on maternal nutritional status. There is a large nutritional burden associated with closely spaced consecutive births, particularly when lactation overlaps with pregnancy (Adair 1993). The duration and intensity of lactation influences the ability of the mother to replete her nutrient reserves

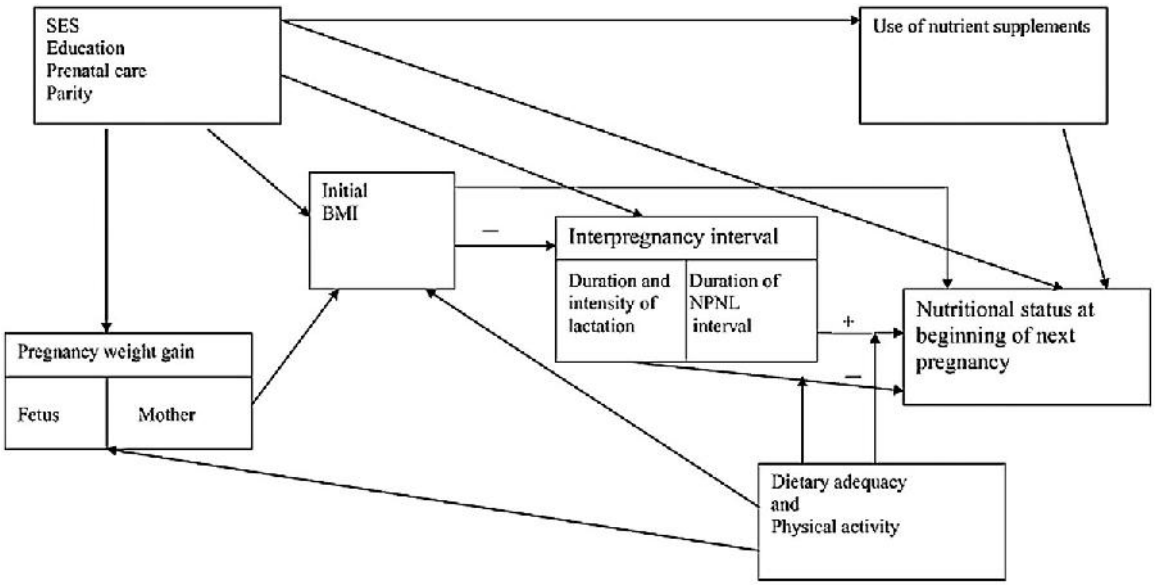


**Fig. 1.** Conceptual model for the relationship between interpregnancy interval and maternal nutritional status. BMI, body mass index; NPNL, non-pregnant, non-lactating; SES, socio-economic status (reproduced with permission from Dewey & Cohen 2007).

during the interval between pregnancies (Dewey & Cohen 2007). Pregnancies with short 'recuperative intervals' (defined as the amount of time that the woman was not lactating prior to the next conception) therefore are particularly vulnerable to nutrient depletion. The complex interaction between the factors that affect maternal nutritional status during short recuperative intervals is shown in Fig. 1 (Dewey & Cohen 2007). The nutritional consequences of a short interpregnancy interval (whether combined with duration and intensity of lactation or not) are influenced by many factors, such as mother's BMI, dietary adequacy, physical activity level, socio-economic status, education level, access prenatal care, parity and morbidity. It is likely that the relationship is context-specific, with positive associations perhaps more evident in undernourished populations (for a more detailed discussion, refer to Dewey & Cohen 2007).

The majority of studies on recuperative interval have investigated its impact on the infant, with longer birth intervals associated with a lower risk of malnutrition and stunting in some populations. The more limited data on maternal outcome have yielded inconclusive results. A recent systematic review found only three studies that considered the effect of recuperative interval on maternal anthropometric status; none looked at the influence on maternal anaemia or micronutrient status (Dewey & Cohen 2007). Recuperative interval was found to be positively associated with maternal thigh skinfold measurements (Merchant *et al*. 1990) and pregnancy weight gain (Pebley & DaVanzo 1993) in studies conducted in developing countries. Although weight gain between pregnancies in undernourished populations may be a positive maternal outcome, it may not be true in developed countries where pre-pregnancy non-obese weight has been positively associated with increased risk of perinatal complication (Cnattingius *et al*. 1998, Villamor & Cnattingius 2006).

### Factors based on diet and lifestyle

Aspects of dietary patterns and lifestyle, such as quality of foods, their combination with other foods and their processing and storage, can produce differences in nutrient absorption and metabolism, and therefore have an impact on nutritional recommendations and reference values. Little research has been

conducted on the wide variety of dietary factors that could have a specific impact on lactating women.

There is some case study evidence that has demonstrated severe vitamin B12 deficiency in exclusively breastfed infants of vegetarian and vegan mothers (Weiss *et al*. 2004; Baatenburg de Jong *et al*. 2005; Wagnon *et al*. 2005). Infants of vegetarian and vegan mothers have low vitamin B12 levels at birth, and this appears to be perpetuated if the vitamin B12-deficient mother breastfeeds her infant (Specker *et al*. 1990). There is little information regarding the long-term neurological effects of such deficiency, but these may include intellectual impairment, severe disturbance of gait and epilepsy (Graham *et al*. 1992). Vegetarianism is particularly common in adolescent girls, with a prevalence of between 8% and 37% (Worsley & Skrzypiec 1998). In addition, vegetarian teenagers are also more likely to exhibit health-compromising dietary behaviours that could further compound the nutritional challenge of lactation in this particularly vulnerable population, such as frequent dieting, binging, purging and laxative use for weight control (Neumark-Sztainer *et al*. 1997; Perry *et al*. 2001; Bas *et al*. 2005) and alcohol use (Greene-Finestone *et al*. 2008). Studies have yet to be conducted on the particular influence of vegetarian diets on the nutritional status of lactating adolescents.

The negative influence of maternal smoking on breastfeeding duration has been well described in the literature (Horta *et al*. 2001) and has been shown even after adjusting for socio-economic group and education level (Hopkinson *et al*. 1992). Some studies have suggested that smoking reduces daily milk output by approximately 250–300 mL (Vio *et al*. 1991; Hopkinson *et al*. 1992), possibly related to suppressed prolactin production in smokers (Anderson & Schioler 1982; Widstrom *et al*. 1991). While it is well documented that smoking has a negative impact on the smokers' nutritional status, particularly antioxidant nutrients (Gibson 2005), little is known about the influence of smoking on milk composition. It has been suggested, however, that smoking is associated with significant reductions in milk fat concentration (Vio *et al*. 1991; Hopkinson *et al*. 1992). The reduction in milk volume and fat content has been given as an explanation as to why women who smoke cease to breastfeed earlier than those who do not smoke (Hopkinson *et al*. 1992). However, it is not yet clear whether social and behavioural differences between smokers and non-smokers play a greater role in early cessation of breastfeeding than physiological factors. In their review, Amir & Donath (2002) claimed that, as women who smoke are less likely to intend to breastfeed and less likely to seek help with breastfeeding problems than non-smoking mothers, it cannot be assumed that the relationship between smoking and breastfeeding duration is a wholly physiological one. Recent qualitative research emphasized the importance of the role of psychosocial factors reporting that the reasons given by women who ceased to breastfeed earlier related to their perceptions that smoking while breastfeeding constituted a strong risk of harming their baby (Goldade *et al*. 2008).

### Socio-economic and cultural factors

It is recognized that lactation is not simply a matter of transmitting nutrition from mother to infant but a crucial relational process between mother and child (Dykes & Hall Moran 2006). Thus, when assessing nutritional requirements for lactating women, it is important to consider the socio-cultural, political and economic constraints upon women in securing optimum nutritional standards for themselves and their children. This is a crucial perspective as maternal dietary and infant feeding practices relate significantly to cultural norms and associated constraints (Sellen 2001; Dykes 2005; Spiro 2006; Scavenius *et al*. 2007; Bhutta *et al*. 2008); this explains the inevitable wide variations in practice across the industrialized world, with Europe being no exception.

Some cultural practices affect micronutrient status through routes other than access to foods and dietary intake. For example, South Asian Muslim women living in Europe may adopt a dress code of complete or partial covering of the body. They are thus exposed to little or no sunlight, affecting their vitamin D status, which can have serious consequences during pregnancy and lactation. Some cultural groups prefer to avoid giving colostrum to the baby as it is seen as old milk, a waste product, contaminating and even poisonous (Wambach & Riordan 2010). Weaning prac-

tices (timing and spacing) of complementary foods vary enormously across cultural groups, and these practices inevitably affect the course, style and duration of lactation. In many European communities, solid foods and soups are introduced well before the recommended 6 months (WHO 2000).

There is a range of deeply embedded cultural practices that will influence what the mother actually selects to eat during lactation. For example, Hispanic cultures, Chinese and some South Asian groups adhere to a set of implicit rules around 'hot' and 'cold' foods (Davis 2001; Wambach & Riordan 2010). This does not relate to temperature but rather to a set of foods that are considered to affect the body in particular ways. The practice of giving herbs and galactogogues (foods thought to enhance the quality and/or quantity of milk) to the mother is a widespread cultural practice that varies from community to community. These practices are commonly harmless but in some cases may be problematic, for example, taking some alcoholic drinks to stimulate lactation.

Socio-economic considerations at both macro- and micro-levels influence the dietary intake of women so for example, mothers living in conditions of poverty may have little opportunity to obtain foods known to be important during pregnancy and lactation (Coufopoulos & Hackett 2009; Stapleton & Keenan 2009). These factors create challenges for such individuals in their achievement of the nutrient reference value recommendations.

## Influence of maternal nutritional status on breast milk composition

Few studies have considered the impact of lactation on maternal nutritional status. Rather, focus has been placed on the influence of maternal nutritional status on the composition of her breast milk. The micronutrient content of breast milk is relatively constant. Although it is generally believed that its nutritional composition is preserved by homeostatic and nutrient transport mechanisms that can compensate for increases and moderate decreases in maternal nutrient supply, for many micronutrients, there is point at which maternal dietary insufficiency will have consequences in terms of maternal status and supply of nutrients to the infant via breast milk. There has been very little research conducted to help our understanding of the situations in which maternal milk mineral concentration is low; however, it is likely that maternal malnutrition may impair mammary gland function and the normal transport processes involved in the transfer of micronutrients into the milk (Lonnerdal 2000).

### *Minerals*

For many minerals, the infant is well protected by maternal homeostatic processes such that moderate deficiency or excessive dietary intake does not significantly alter the levels of these micronutrients in the mother's milk (Domellof *et al*. 2004). Many minerals are transferred into milk by active transfer rather than passive diffusion, and this process compensates for variations in maternal mineral status. One notable exception to this is selenium for which breast milk selenium concentration correlates well with maternal selenium status (Lonnerdal 2000).

Many European countries recommend an increase of at least 33% in iodine intake from NPNL levels (Table 3). This increase is required to accommodate the changes in maternal thyroid metabolism to support lactation, to supply sufficient iodine for milk to meet the needs for growth and development of the infant and to ensure that pregnant and lactating women do not suffer from iodine deficiency post partum (WHO 2007). However, Zimmermann & Delange (2004) report that national surveys from European countries reveal the median dietary intake of iodine in women of child bearing age is around half of the recommended levels. The iodine content of milk is dependent upon the mother's iodine intake, although there are compensatory mechanisms that enhance iodine uptake by the mammary gland of iodine deficient mothers. Optimally, the iodine content of mature milk should be in the range of 100–150 mg $dL^{-1}$; above 75 mg $L^{-1}$ is sufficient, but may fall to values below 30 mg $L^{-1}$ in areas with endemic goitre (Azizi & Smyth 2009). When maternal iodine status is inadequate, the uptake of iodine by the mammary gland increases during lactation, creating a deterioration of the maternal iodine status by the sequestration of some of the maternal iodine pool to

the breast milk (Delange *et al*. 1988). In women with marginal iodine status, the demands of lactation can precipitate clinical and biochemical symptoms, including increased thyroid volume, altered thyroid hormone levels and impaired mental function (Eltom *et al*. 2000; Dorea 2002). Despite these effects, there is currently insufficient evidence to substantiate the benefits of zinc supplementation in lactating women (Hess & King 2009).

An adequate supply of zinc is essential for the normal growth and development of the fetus and infant post partum. The high level of zinc in colostrum, which is 17 times higher than that in blood, illustrates the importance of zinc in the development of a newborn (Almeida *et al*. 2008). Most European countries recommend that zinc intake is increased by at least 50% from NPNL levels (Table 3). Current WHO recommendations for zinc intake during pregnancy and lactation range from 4.3 to 19 mg per day, depending upon months post partum and the bioavailability of zinc from the diet (WHO/Food and Agriculture Organization of the United Nations (FAO) 2004). In a randomized double-blind, placebo-controlled supplementation study of healthy women, Krebs *et al*. (1995) reported that zinc concentration of milk declines rapidly in the first 3 months post partum from 59.4 to 16.7 mmol $L^{-1}$, and this was not influenced by dietary supplementation with 15 mg Zn per day. It appears that an intake of 13 mg Zn per day during lactation is adequate, and increasing intakes beyond this does not resulted in increased milk zinc concentration (Moser-Veillon & Reynolds 1990; Hambidge & Krebs 2007). A number of studies of lactating women with marginal zinc status have revealed that homeostatic mechanisms can compensate for low maternal dietary zinc intakes. The proportion of dietary zinc absorbed in such women has been shown to increase by over 70% compared with non-lactating women or pre-conception values (Jackson *et al*. 1988; Sian *et al*. 2002). Evidence for a homeostatic response to low dietary zinc intake was further illustrated in a study that reported that despite having dietary zinc intakes that were 42% of the reference value, the zinc status of lactating women (assessed using plasma zinc concentrations) was not significantly lower than those of non-lactating women (Moser & Reynolds 1983).While such mechanisms act to enhance maternal zinc availability for fetal growth and milk zinc excretion, a consequence is that maternal reproductive function may be compromised (Hess & King 2009).

The transfer of calcium to breast milk is a physiologic response to lactation and facilitated by the upregulation of calcium absorption by the mother, conservation of excretion via kidney and mobilization of calcium from the bone (Ritchie *et al*. 1998). As a consequence, bone mineral density loss has been shown to occur during lactation (Karlsson *et al*. 2001), but this effect seems to be transient and seems to be unrelated to calcium intake. Reference values for calcium intake during lactation throughout Europe range from no additional intake to an increase of over 80% from NPNL levels (Table 3). In those countries that have set differential recommendations for lactating adolescents, reference values for calcium exceed those of adult lactating women. There is little evidence, however, to suggest that there exists a significant association between the mother's calcium intake (whether supplemented with calcium or not) and levels in breast milk. A randomized controlled trial of pregnant and lactating women in Gambia revealed that, despite having low dietary intakes of calcium (300–400 mg $d^{-1}$) and low breast milk concentrations, calcium supplements had no significant benefit in terms of breast milk concentration (Jarjou *et al*. 2006). Nor is there evidence to suggest that supplements prevent bone loss during lactation or the recovery of calcium status when breastfeeding is ceased (Kalkwarf & Specker 2002).

Many European countries recommend that iron intake is reduced during lactation compared with NPNL levels, albeit slightly less so for adolescent mothers (Table 3). This is because women who exclusively breastfeed are usually amenorrhoeic for at least 6 months, thereby conserving iron otherwise lost in menses (approximately 0.5 mg Fe $day^{-1}$). As iron is secreted in relatively low amounts in breast milk (approximately 0.24 mg Fe $day^{-1}$), net iron loss may be lower than in non-lactating women (Dewey 2004). Thus, breastfeeding may be protective against maternal iron deficiency. In addition, lactating women have been found to have greater serum ferritin concentra-

tions than non-lactating women, indicating elevated iron stores in those who breastfed (Kalkwarf & Harrast 1998). This difference was not, however, observed in haemoglobin status.

*Vitamins*

The concentration of many vitamins in breast milk is dependent upon the vitamin status of the mother, with maternal deficiencies leading to deficiencies in the breastfeeding infant. Vitamins of particular concern in this respect include thiamine, riboflavin, vitamins B6 and B12 and vitamin A (Allen 1998; Allen 2005; Langley-Evans 2009). As a consequence, most European countries recommend an increased intake of all vitamins during lactation from NPNL levels (Table 3). Of the B vitamins, B12 has been the focus of the most research. Studies in B12-deficient lactating women demonstrate an association between the B12 status of the mother and her infant at 3 months post partum (Casterline *et al*. 1997). Vitamin A deficiency is a major public health problem in developing countries, and strategies such as high-dose supplementation of women post partum with vitamin A is an effective way of ensuring adequate supplies to the infant through breast milk (Sommer *et al*. 2002) and preventing deficiency. Vitamin D deficiency has gained a lot of interest in recent years, with the resurgence in the prevalence of rickets, particularly among South Asian immigrants to western Europe (Alfaham *et al*. 1995; Gillie 2004). Reduced sunlight exposure attributed to the prevalent use of sunscreen creams, sunlight avoidance or the wearing of traditional Muslim dress can contribute to vitamin D deficiency in pregnant and lactating mothers, which can lead to lowered breast milk concentrations (Seth *et al*. 2009). The transfer of maternal vitamin D to breast milk is poor; therefore, supplementation of the mother to raise breast milk vitamin D levels is inefficient. Direct supplementation of the infant can be an effective approach in the prevention of infantile rickets (Kovacs 2008).

## Conclusion

Recommended intakes during lactation are often extrapolated from known secretion of the nutrient in milk with adjustments for bioavailability, so that differences between values can be partly ascribed to differences in methodological approaches and how these approaches are applied (Atkinson & Koletzko 2007). Recent research is providing new knowledge on the micronutrient requirements of lactating women. Identifying needs for research and improving understanding of the differences in values that have been derived by various committees and groups across Europe will enhance transparency and facilitate the application of reference values in policy-making decision and their translation into recommendations for lactating women. Given the wide variation in breastfeeding practices across Europe, making nutritional recommendations for lactating women is complex and challenging. It is crucial to first examine the cultural practices within and across European populations and to assess its relevance before making recommendations.

Care should be taken to avoid assumptions that providing the 'correct' information on nutritional requirements during lactation will lead women to make the 'right choices' in terms of their own nutrition and the patterns and practices of breastfeeding. This consumerist concept of decision making (knowledge in – behaviour out) is based on an illusion of linearity, and it ignores the complexities of decision making. In reality, decisions will be made based on macro-level (structural) factors such as socio-economic and political contexts, gender relationships and food availability along with micro-level factors such as local cultural practices, norms, lifestyles, attitudes and beliefs (Pelto 1987; Bilson & Dykes 2009).

When attempting to apply any future nutritional guidelines to this population group, attention should also be made to the social, cultural and economic factors that play a role in the eating behaviour and subsequent nutritional status of adolescents, particularly those who are pregnant or breastfeeding. In many western countries, adolescent childbearing is more prevalent among those with low levels of income and education than among their better-off peers (Singh *et al*. 2001). Poverty has been shown to be a significant factor that limits the ability of some childbearing adolescents to eat a healthy diet, even in those who aspire to it (Burchett & Seeley 2003).

Other socio-economic factors often experienced by such groups, including unemployment, poor housing, suboptimal mental and physical health, limited access to a wide variety of reasonably priced foods (Symon & Wrieden 2003), and an increased likelihood to smoke during and following pregnancy (Hamlyn *et al*. 2002) all contribute to difficulties in tackling behavioural change. Thus, achieving dietary change in this particularly vulnerable section of the population presents a major public health challenge.

## Source of funding

The work report herein has been carried out within the EURRECA Network of Excellence (http://www.eurreca.org), which is financially supported by the Commission of the European Communities, specific Research Technology and Development (RTD) Programme Quality of Life and Management of Living Resources, within the Sixth Framework Programme (contract no. 036196).

## Conflict of interest

This report does not necessarily reflect the Commission's views or its future policy in this area. The authors declare no conflict of interest.

## References

Adair S.-R. (1993) Biological determinants of pregnancy weight gain: a longitudinal study of Filipino women. *American Journal of Clinical Nutrition* **57**, 365–372.

Alfaham M., Woodhead S., Pask G. & Davies D. (1995) Vitamin D deficiency: a concern in pregnant Asian women. *British Journal of Nutrition* **73**, 881–887.

Allen L.H. (1998) Zinc and micronutrient supplements for children. *American Journal of Clinical Nutrition* **68**, (Suppl.), 495S–498S.

Allen L.H. (2005) Multiple micronutrients in pregnancy and lactation: an overview. *American Journal of Clinical Nutrition* **81**, 1206S–1212S.

Almeida A.A., Lopes C.M., Silva A.M. & Barrado E. (2008) Trace elements in human milk: correlation with blood levels, inter-element correlations and changes in concentration during the first month of lactation. *Journal of Trace Elements in Medicine & Biology* **22**, 196–205.

Amir L.H. & Donath S.M. (2002) Does maternal smoking have a negative physiological effect on breastfeeding? The epidemiological evidence. *Birth* **29**, 112–123.

Anderson A.N. & Schioler V. (1982) Influence of breast-feeding pattern on pituitary–ovarian axis of women in an industrialized community. *American Journal of Obstetrics and Gynecology* **143**, 673–677.

Ashwell M., Lambert J.P., Alles M.S., Branca F., Bucchini L., Brzozowska A. *et al*. (2008) How we will produce the evidence-based EURRECA toolkit to support nutrition and food policy. *European Journal of Nutrition* **47** (Suppl 1), 2–16.

Atkinson S.A. & Koletzko B. (2007) Determining life-stage groups and extrapolating nutrient intake values (NIV). *Food and Nutrition Bulletin* **28** (Suppl.), 61–76.

Azizi F. & Smyth P. (2009) Breastfeeding and maternal and infant iodine nutrition. *Clinical Endocrinology* **70**, 803–809.

Baatenburg de Jong R., Bekhof J., Roorda R. & Zwart P. (2005) Severe nutritional vitamin deficiency in a breast-fed infant of a vegan mother. *European Journal of Pediatrics* **164**, 259–260.

Bas M., Karabudak E. & Kiziltan G. (2005) Vegetarianism and eating disorders: association between eating attitudes and other psychological factors among Turkish adolescents. *Appetite* **44**, 309–315.

Bezerra F.F., Mendonca L.M.C., Lobato E.C., O'Brien K.O. & Donangelo C.M. (2004) Bone mass is recovered from lactation to postweaning in adolescent mothers with low calcium intakes. *American Journal of Clinical Nutrition* **80**, 1322–1326.

Bhutta Z.A., Shekar M. & Ahmed T. (2008) Mainstreaming interventions in the health sector to address maternal and child under-nutrition. *Maternal and Child Nutrition* **4**, 1–4.

Bilson A. & Dykes F. (2009) A bio-cultural basis for protecting, promoting and supporting breastfeeding. In *Infant and Young Child Nutrition: Challenges to Implementing a Global Strategy* (eds F. Dykes & V. Hall Moran), pp. 32–42. Wiley-Blackwell: Oxford.

Burchett H. & Seeley A. (2003) *Good Enough to Eat? The Diet of Pregnant Teenagers*. Maternity Alliance/Food Commission: London.

Casterline J.E., Allen L.H. & Ruel M.T. (1997) Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months post-partum. *Journal of Nutrition* **127**, 1966–1972.

Cavelaars A.E.J.M., Kadvan A., Doets E.L., Tepsic J., Novakovic R., Dhonukshe-Rutten R. *et al*. (2010) NutriRecQuest: a web-based search engine on current micronutrient recommendations. *European Journal of Clinical Nutrition* **64**, S43–S47.

Cervera P. & Ngo J. (2001) Dietary guidelines for the breast-feeding woman. *Public Health Nutrition* **4**, 1357–1362.

Chantry C.J., Auinger P. & Byrd R.S. (2004) Lactation among adolescent mothers and subsequent bone mineral density. *Archives of Pediatric Adolescent Medicine* **158**, 650–656.

Cnattingius S., Bergström R., Lipworth L. & Kramer M.S. (1998) Prepregnancy weight and the risk of adverse pregnancy outcomes. *New England Journal of Medicine* **338**, 147–152.

Coufopoulos A.M. & Hackett A.F. (2009) Homeless mothers and their children: two generations at nutritional risk. In *Infant and Young Child Nutrition: Challenges to Implementing a Global Strategy* (eds F. Dykes & V. Hall Moran), pp. 146–162. Wiley-Blackwell: Oxford.

Davis R.E. (2001) The postpartum experience for Southeast Asian women in the United States. *Maternal and Child Nursing* **26**, 208–213.

Dewey K.G. (2004) Impact of breastfeeding on maternal nutritional status. *Advances in Experimental Medicine & Biology* **554**, 91–100.

Dewey K.G. & Cohen R.J. (2007) Does birth spacing affect maternal or child nutritional status? A systematic literature review. *Maternal & Child Nutrition* **3**, 151–173.

Delange F., Chanoine J.P., Abrassart C. & Bourdoux P. (1988) Topical iodine, breastfeeding, and neonatal hypothyroidism. *Archives of Disease in Childhood* **63**, 106–107.

Doets E.L., de Wit L.S., Dhonukshe-Rutten R.A., Cavelaars A.E., Raats M.M., Timotijevic L. *et al*. (2008) Current micronutrient recommendations in Europe: towards understanding their differences and similarities. *European Journal of Nutrition* **47** (Suppl 1), 17–40.

Domellof M., Lonnerdal B., Dewey K.G., Cohen R.J. & Hernell O. (2004) Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *American Journal of Clinical Nutrition* **79**, 111–115.

Dorea J.G. (2002) Iodine nutrition and breast feeding. *Journal of Trace Elements in Medicine & Biology* **16**, 207–220.

Dykes F. (2005) 'Supply' and 'Demand': breastfeeding as labour. *Social Science & Medicine* **60**, 2283–2293.

Dykes F. & Hall Moran V. (2006) Transmitted nutritional deprivation from mother to child: a socio-biological perspective. In *Maternal and Infant Nutrition & Nurture: Controversies and Challenges* (eds V. Hall Moran & F. Dykes), pp. 6–39. Quay Books: London.

Eltom A., Eltom M., Elnagar B., Elbagir M. & Gebre-Medhin M. (2000) Changes in iodine metabolism during late pregnancy and lactation: a longitudinal study among Sudanese women. *European Journal of Clinical Nutrition* **54**, 429–433.

Gibson R.S. (2005) *Principles of Nutritional Assessment*, 2nd edn, Oxford University Press: Oxford.

Gillie O. (2004) *Sunlight Robbery*. Health Research Forum Occasional Reports: No. 1 p. 20. Available at: http://www.healthresearchforum.org.uk

Goldade K., Nichter M., Nichter M., Adrian S., Tesler L. & Muramoto M. (2008) Breastfeeding and smoking among low-income women: results of a longitudinal qualitative study. *Birth* **35**, 230–240.

Graham S.M., Arvela O.M. & Wise G.A. (1992) Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. *Journal of Pediatrics* **121**, 710–714.

Greene-Finestone L.S., Campbell M.K., Evers S.E. & Gutmanis I.E. (2008) Attitudes and health behaviours of young adolescent omnivores and vegetarians: a school-based study. *Appetite* **51**, 104–110.

Hall Moran V. (2007) Nutritional status in pregnant adolescents: a systematic review of biochemical markers. *Maternal & Child Nutrition* **3**, 74–93.

Hambidge K.M. & Krebs N.F. (2007) Zinc deficiency: a special challenge. *Journal of Nutrition* **137**, 1101–1105.

Hamlyn B., Brooker S., Oleinikova K. & Wands S. (2002) *Infant Feeding 2000*. The Stationery Office: London.

Hess S.Y. & King J.C. (2009) Effects of maternal zinc supplementation on pregnancy and lactation outcomes. *Food & Nutrition Bulletin* **30**, S60–S78.

Hopkinson J.M., Schanler R.J., Fraley J.K. & Garza C. (1992) Milk production by mothers of premature infants: influence of cigarette smoking. *Pediatrics* **90**, 934–938.

Horta B.L., Kramer M.S. & Platt R.W. (2001) Maternal smoking and the risk of early weaning: a meta-analysis. *American Journal of Public Health* **91**, 304–307.

Institute of Medicine (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. NAP: Washington DC.

Jackson M.J., Giugliano R., Giugliano L.G., Oliveira E.F., Shrimpton R. & Swainbank I.G. (1988) Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley. *British Journal of Nutrition* **59**, 193–203.

Jarjou L.M., Prentice A., Sawo Y., Laskey M.A., Bennett J., Goldberg G.R. *et al*. (2006) Randomized, placebo-controlled, calcium supplementation study in pregnant Gambian women: effects on breast-milk calcium concentrations and infant birth weight, growth, and bone mineral accretion in the first year of life. *American Journal of Clinical Nutrition* **83**, 657–666.

Kalkwarf H.J. (1999) Hormonal and dietary regulation of changes in bone mineral density during lactation and after weaning in women. *Journal of Mammary Gland Biology & Neoplasia* **4**, 319–329.

Kalkwarf H.J. & Harrast S.D. (1998) Effects of calcium supplementation and lactation on iron status. *American Journal of Clinical Nutrition* **67**, 1244–1249.

Kalkwarf H.J. & Specker B.L. (2002) Bone mineral changes during pregnancy and lactation. *Endocrine* **17**, 49–53.

Karlsson C., Obrant K.J. & Karlsson M. (2001) Pregnancy and lactation confer reversible bone loss in humans. *Osteoporosis International* **12**, 828–834.

Kent J.C., Mitoulas L.R., Cregan M.D., Ramsay D.T., Doherty D.A. & Hartmann P.E. (2006) Volume and frequency of breastfeedings and fat content of breast milk throughout the day. *Pediatrics* **117**, e387–e395.

Kovacs C.S. (2008) Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. *American Journal of Clinical Nutrition* **88**, 520S–528S.

Krebs N.F., Reidinger C.J., Hartley S., Robertson A.D. & Hambidge K.M. (1995) Zinc supplementation during lactation: effects on maternal status and milk zinc concentrations. *American Journal of Clinical Nutrition* **61**, 1030–1036.

Langley-Evans S. (2009) *Nutrition: A Lifespan Approach*. Wiley-Blackwell: Chichester.

Lonnerdal B. (2000) Regulation of mineral and trace elements in human milk: exogenous and endogenous factors. *Nutrition Reviews* **58**, 223–229.

Maia P.A., Figueiredo R.C.B., Anastácio A.S., Porto da Silveira C.L. & Donangelo C.M. (2007) Zinc and copper metabolism in pregnancy and lactation of adolescent women. *Nutrition* **23**, 248–253.

Merchant K., Martorell R. & Haas J. (1990) Maternal and fetal responses to the stresses of lactation concurrent with pregnancy and of short recuperative intervals. *American Journal of Clinical Nutrition* **52**, 280–288.

Moser P.B. & Reynolds R.D. (1983) Dietary zinc intake and zinc concentrations of plasma, erythrocytes, and breast milk in antepartum and postpartum lactating and nonlactating women: a longitudinal study. *American Journal of Clinical Nutrition* **38**, 101–108.

Moser-Veillon P.B. & Reynolds R.D. (1990) A longitudinal study of pyridoxine and zinc supplementation of lactating women. *American Journal of Clinical Nutrition* **52**, 135–141.

Neumark-Sztainer D., Story M., Resnick M.D. & Blum R.W. (1997) Adolescent vegetarians, a behavioural profile of a school-based population in Minnesota. *Archives of Pediatrics and Adolescent Medicine* **151**, 833–838.

Pavlovic M., Prentice A., Thorsdottir I., Wolfram G. & Branca F. (2007) Challenges in harmonising energy and nutrient recommendations in Europe. *Annals of Nutrition and Metabolism* **51**, 108–114.

Pebley A.R. & DaVanzo J. (1993) *Maternal Depletion and Child Survival in Guatemala and Malaysia*. RAND Health: Santa Monica, CA.

Pelto G. (1987) Cultural issues in maternal and child health and nutrition. *Social Science and Medicine* **25**, 553–559.

Perry C.L., McGuire M.T., Neumark-Sztainer D. & Story M. (2001) Characteristics of vegetarian adolescents in a multiethnic urban population. *Journal Adolescent Health* **29**, 406–416.

Picciano M.F. (2003) Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. *Journal of Nutrition* **133**, 1997S–2002S.

Pijls L., Ashwell M. & Lambert J. (2009) EURRECA – a network of excellence to align European micronutrient recommendations. *Food Chemistry* **113**, 748–753.

Rah J.H., Christian P., Shamim A.A., Arju U.T., Labrique A.B. & Rashid M. (2008) Pregnancy and lactation hinder growth and nutritional status of adolescent girls in rural Bangladesh. *Journal of Nutrition* **138**, 1505–1511.

Rah J.H., Shamim A.A., Arju U.T., Labrique A.B., Klemm R.D.W., Rashid M. & Christian P. (2010) Difference in ponderal growth and body composition among pregnant vs. never-pregnant adolescents varies by birth outcomes. *Maternal & Child Nutrition*.

Ritchie L.D., Fung E.B., Halloran B.P., Turnlund J.R., Van L., Cann C.E. & King J.C. (1998) A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. *American Journal of Clinical Nutrition* **67**, 693–701.

Rogol A.D., Clark P.A. & Roemmich J.N. (2000) Growth and pubertal development in children and adolescents: effects of diet and physical activity. *American Journal of Clinical Nutrition* **72**, 521S–528S.

Rozen G.S., Rennert G., Dodiuk-Gad R.P., Rennert H.S., Ish-Shalom N., Diab G. *et al*. (2003) Calcium supplementation provides an extended window of opportunity for bone mass accretion after menarche. *American Journal of Clinical Nutrition* **78**, 993–998.

Scavenius M., vHulsel L., Meijer J., Wendte H. & Gurgel R. (2007) In practice, the theory is different: a processual analysis of breastfeeding in north east Brazil. *Social Science and Medicine* **64**, 676–688.

Scholl T.O., Hediger M.L. & Ances I.G. (1990) Maternal growth during pregnancy and decreased infant birth weight. *American Journal of Clinical Nutrition* **51**, 790–793.

Scholl T.O., Hediger M.L., Cronk C.E. & Schall J.I. (1993) Maternal growth during pregnancy and lactation. *Hormone Research* **39** (Suppl. 3), 59–67.

Sellen D.W. (2001) Weaning, complementary feeding, and maternal decision making in a rural East African pastoral population. *Journal of Human Lactation* **17**, 233–244.

Seth A., Marwaha R.K., Singla B., Aneja S., Mehrotra P., Sastry A. *et al*. (2009) Vitamin D nutritional status of exclusively breast fed infants and their mothers. *Journal of Pediatric Endocrinology* **22**, 241–246.

Sian L., Krebs N.F., Westcott J.E., Fengliang L., Tong L., Miller L.V. *et al*. (2002) Zinc homeostasis during lactation in a population with a low zinc intake. *American Journal of Clinical Nutrition* **75**, 99–103.

Sing S., Darroch J.E. & Frost J.J. (2001) Socioeconomic disadvantage and adolescent women's sexual and reproductive behavior: the case of five developed countries. *Family Planning Perspectives* **33**, 251–258 & 289.

Sommer A., Davidson F.R. & Annecy A. (2002) Assessment and control of vitamin A deficiency: the Annecy Accords. *Journal of Nutrition* **132** (Suppl), 2845S–2850S.

Specker B.L., Black A., Allen L. & Morrow F. (1990) Vitamin B12 low milk concentrations are related to low serum, concentrations in vegetarian women and to methylmalonic aciduria in their infants. *American Journal of Clinical Nutrition* **52**, 1073–1076.

Spiro A. (2006) Gujarati women and infant feeding decisions. In *Maternal and Infant Nutrition and Nurture: Controversies and Challenges* (eds V. Hall Moran & F. Dykes), pp. 232–249. Quay Books: London.

Stapleton H. & Keenan J. (2009) Bodies in the making: reflections on women's consumption practices in pregnancy. In *Infant and Young Child Nutrition: Challenges to Implementing a Global Strategy* (eds F. Dykes & V. Hall Moran), pp. 119–145. Wiley-Blackwell: Oxford.

Symon A.G. & Wrieden W.L. (2003) A qualitative study of pregnant teenagers' perceptions of the acceptability of a nutritional education intervention. *Midwifery* **19**, 140–147.

Villamor E. & Cnattingius S. (2006) Interpregnancy weight change and risk of adverse pregnancy outcomes: a population-based study. *The Lancet* **368**, 1164–1170.

Vio F., Salazar G. & Infante C. (1991) Smoking during pregnancy and lactation and its effects on breast-milk volume. *American Journal of Clinical Nutrition* **54**, 1011–1016.

Wagnon J., Cagnard B., Bridoux-Henno L., Tourtelier Y., Grall J.Y. & Dabadie A. (2005) Breastfeeding and vegan diet. *Journal de Gynecologie, Obstetrique et Biologie de la Reproduction* **34**, 610–612.

Wambach K. & Riordan J. (2010) The cultural context of breastfeeding. In *Breastfeeding and Human Lactation* (eds J. Riordan & K. Wambach), pp. 799–816. Jones and Bartlett Publishers: London.

Weiss R., Fogelman Y. & Bennett M. (2004) Severe vitaminB12 deficiency in an infant associated with a maternal deficiency and strict vegetarian diet. *Journal of Pediatric Hematology and Oncology* **26**, 270–271.

WHO (1998) *Complementary Feeding of Young Children in Developing Countries: Review of the Current Scientific Knowledge*. WHO: Geneva.

WHO (2000) *Collaborative Study Team on the Role of Breastfeeding on the Prevention and of Infant Mortality*. WHO: Geneva.

WHO (2003) *Global Strategy for Infant and Young Child Feeding*. Geneva: WHO.

WHO (2007) *Assessment of Iodine Deficiency Disorders and Monitoring their Elimination. A Guide for Program Managers*, 3rd edn. WHO: Geneva.

WHO/Food and Agriculture Organization of the United Nations (FAO) (2004) *Vitamin and Mineral Requirements in Human Nutrition*, 2nd edn. WHO/FAO: Geneva.

Widstrom A.M., Werner S., Matthiesen A.S., Svensson K. & Uvnas-Moberg K. (1991) Somatostatin levels in plasma in non-smoking and smoking breastfeeding women. *Acta Paediatrica Scandinavica* **80**, 13–21.

Worsley A. & Skrzypiec G. (1998) Teenage vegetarianism: prevalence, social and cognitive contexts. *Appetite* **30**, 151–170.

Zimmermann M. & Delange F. (2004) Iodine supplementation of pregnant women in Europe: a review and recommendations. *European Journal of Clinical Nutrition* **58**, 979–984.

# 9 MANUSCRIPT 1

Hermoso, M., V. Vuvic, C. Vollhardt, C., A. Arsic, B. Roman-Viñas, I. Iglesia-Altaba, M. Gurinovic. & B. Koletzko. Iron and neurodevelopment and immunity in infants, children and adolescents: a systematic review. (Submitted)

# Iron and neurodevelopment and immunity in infants, children and adolescents: a systematic review

Maria Hermoso, MSc[1], Vesna Vucic, PhD[2], Christiane Vollhardt, PhD[1], Aleksandra Arsic, MSc[2], Blanca Roman-Viñas, MD, PhD[3], Iris Iglesia-Altaba, MSc[4], Mirjana Gurinovic, MD, PhD[2], Berthold Koletzko, MD, PhD[1]

[1] Division of Metabolic and Nutritional Medicine. Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich Medical Centre, Munich, Germany,

[2] Centre of Research Excellence in Nutrition and Metabolism. Institute for Medical Research. University of Belgrade, Belgrade, Serbia,

[3] Community Nutrition Research Centre of the Nutrition Research Foundation, Barcelona Science Park, University of Barcelona, Barcelona, Spain,

[4] Growth, Exercise, Nutrition and Development (GENUD) Research Group, Escuela Universitaria de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain,

**Correspondence:** Maria Hermoso, Division of Metabolic and Nutritional Medicine. Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich Medical Centre, Lindwurmstr. 4, 80337 Munich, Germany. Phone: 0049(0)8951602832. Fax: 0049(0)8951604938. E-mail: maria.hermoso@med.uni-muenchen.de

**Keywords:** iron, development, neurodevelopment, cognition, immunity, infants, children, adolescents, EURRECA

**Source of funding**

The work report herein has been carried out within the EURRECA Network of Excellence (http://www.eurreca.org), which is financially supported by the Commission of the European Communities, specific Research Technology and Development Programme Quality of Life and Management of Living Resources, within the Sixth Framework Programme, contract no. 036196. This report does not necessarily reflect the Commission's views or its future policy in this area. BK is the recipient of a Freedom to Discover Award of the Bristol Myers Squibb Foundation, New York, NY, USA.

**Conflict of interest:** The authors have declared no conflict of interest.

Abstract

A systematic review was conducted to summarize the evidence currently available from randomised controlled trials (RCT) concerning the effect of iron intake of infants, children and adolescents until 18 years of age on measures of neurodevelopment, including cognition and psychomotor development, and on immunity. The Cochrane Library, MEDLINE and EMBASE were searched up to and including February 2010. Studies were also identified by checking the bibliographies of the articles retrieved. All RCTs with an adequate control group in which iron supply was provided by natural food sources, fortified foods, formula, or supplements until the age of 18 years were considered for inclusion. No language restrictions were applied. 13 supplementation studies met the selection criteria for neurodevelopment and 7 for immunity. Most of the studies had a high or moderate risk of bias. A large variety of outcomes were reported. Overall, the studies showed a modest positive effect of iron supplementation on cognition and psychomotor outcomes, especially in anemic infants and children and after long-term supplementation periods (>2 months). A comparison of studies using immunity outcome was not possible due to heterogeneity. Currently available evidence is too incomplete to make evidence-based conclusions on the effect of iron on immunity.

**List of abbreviations**

ACT Alpha-1-Antil-Chymotrypsin
ADHD Attention Deficit Hyperactivity Disorder
ADHD-RS Attention Deficit Hyperactivity Disorder Rating Scale
AGP Alpha-1-Acid Glycoprotein
BRS Behavioural Rating Scale
BSID Bayley Scales of Infant Development
BTA Brief Test of Attention
CGI-S Clinical Global Impression- Severity
CPRS Conner's Parent Rating Scale
CRP C-Reactive Protein
CTRS Conner's Teacher Rating Scale
EURRECA European Recommendations Aligned
HVLT Hopkins Verbal Learning Test
ID Iron deficiency
IDA Iron deficiency anemia
IQ Intellectual intelligence quotient
MDI Mental Development Index
NAID Non anemic iron deficient
PDI Psychomotor Development Index
RCT Randomised controlled trials
SDMT Symbol Digit Modalities Test
URTI Upper Respiratory Tract Infections
VSAT Visual Search and Attention Test

## INTRODUCTION

During the first year of life, the body iron content increases markedly. In healthy term infants, iron stores at birth comprise most of the iron requirements for the first 4–6 months. From the 4th month, the requirement for dietary iron increases to an estimated 0.78 mg/day due to the stepwise depletion of endogenous stores and rapid growth with an expansion of blood volume and increased tissue and storage iron [1]. Rapid growth with high iron needs makes infants and young children a particular risk group for iron deficiency anemia (IDA), especially infants and young children aged 6-24 months [2]. Because of rapid growth and increased iron demands during puberty, adolescents are another risk group for development of IDA, especially adolescent girls due to menstrual losses [3, 4]. In addition to inadequate intake, factors which could influence iron status may include strenuous exercise, pregnancy, low socioeconomic status and ethnicity, as well as disease-induced malabsorption and chronic blood loss [5] or polymenorrhea in girls [6, 7]. Prevalence estimates of iron deficiency (ID) in adolescent girls range from 9% to 40%, depending on the population studied and the criteria used to define ID [8]. In children below 4 years of age the estimated prevalence of ID ranges from 20% in industrialized countries to 39% in non-industrialised countries [9]. Anaemia is considered to be a public health problem when the prevalence of low haemoglobin concentrations exceeds 5% in the population. The severity of the public health problem of anaemia is classified as mild (5.0–19.9%), moderate (20.0–39.9%) or severe (≥ 40%) according to the prevalence of anaemia in the population [10]. Worldwide, the estimated prevalence of IDA among preschool-aged children is 25%; 40% among schoolchildren, and 30–55% among adolescents [11].

ID and IDA can have a serious impact on infants' and children's health and later development: alteration of the immune status, adverse effects on morbidity, delayed behavioural and mental development, below average school achievements and growth retardation [12], as well as adverse effects on cognition that may or may not be reversible with iron treatment [8, 13]. However, although various studies have been performed using neurodevelopment outcome to assess iron requirement in infancy and childhood, results have been inconclusive. Different populations have been studied at different ages with different developmental tests, which makes difficult to compare the studies [14]. Particular interest has been given to preschool and school children. Most observational studies in children have found a significant association between IDA and poor cognitive and motor development [15, 16] showing that anaemia is associated with lower scores on testing of intelligence quotients. Children 9-11 years old with IDA obtained significantly lower scores in a standardized educational achievement test than did iron-replete children [17]. It is not clear, however, whether the iron deficiency causes the delay or merely whether the two findings are associated evidence of an underprivileged environment. It is also possible that an early neurodevelopment insult caused by IDA may not result in detectable psychomotor delay during infancy, but symptoms such as deficits in attention and school performance may appear later, in older children, young adolescents and adults [14]. There is increasing evidence that low iron status, as a systemic condition, adversely influences physiological

functioning not only due to reduced haemoglobin synthesis, but also because of decreased activity of iron containing enzymes in the brain [18]. Since in iron deficiency iron appears to be preferentially channelled to hemoglobin synthesis, the brain may become iron depleted when intake is insufficient even if the individual is not yet anemic [18]. A number of animal studies show that ID changes the myelination of neurones, neurometabolism, neurotransmitters and gene/protein profiles before and after iron repletion at weaning [19]. Human infants who experience iron deficiency during the first 6–12 months of life are likely to experience persistent effects of the deficiency that alter functioning in adulthood. A lack of sufficient iron intake may significantly delay the development of the central nervous system as a result of alterations in morphology, neurochemistry, and bioenergetics [20].

The role that iron plays in the neurodevelopment of anemic and non anemic iron deficient (NAID) infants, children and adolescents is not fully understood. Resistance to iron treatment was observed in most trials of children <2 years of age with IDA, but not in older children. In children >2 y of age and in adolescents with IDA, evidence suggests beneficial effects of iron treatment on cognitive or behavioral function; however, the insufficient number of studies, often associated with different confounders, prevents a thorough assessment.

Additionally, it is well known that ID causes impaired immune response and adverse effects on morbidity [12, 21], but the relation between iron intake and morbidity is still controversial.

The EURopean RECcommendations Aligned (EURRECA) Network of Excellence attempts to consolidate the basis for the definition of micronutrient requirements across Europe, taking into account relationships among intake, status and health outcomes [22]. Systematic reviews are being conducted on those micronutrients that are deemed to be of major importance for certain population groups, following a standardised methodology. This paper aims to review data from all available RCTs which met EURRECA's high quality standards, to ascertain whether there is a beneficial effect of iron on neurodevelopment, including psychomotor development and cognition, and on immune function in infants, children, and adolescents.

## METHODS

### Search methods for identification of studies

Structured electronic searches were conducted (over all years until February 2010) on MEDLINE, EMBASE (both on Ovid) and the Cochrane Library CENTRAL database. A general search strategy was devised which included terms for "[study designs in humans] AND [intake or status] AND [iron]". Both indexing and text terms were used and each search strategy was further adapted for the individual databases used. The search was not limited by language. To ensure comprehensive retrieval of relevant articles a multi-database search was conducted. The reference lists of retrieved articles and of published reviews were also checked for relevant studies.

### Criteria for the consideration of studies for this review

Studies had to fulfil the following two criteria to be included in the review: (1) investigate the effect of iron intake from supplements, fortified foods or natural dietary sources on any measures of neurodevelopment, including cognition and psychomotor development, or on immune response and (2) be randomised controlled trials with an adequate control (placebo or no intervention) in apparently healthy infants, children and adolescents from birth to 18 years of age at the time of the intervention. Subjects with ID or IDA but otherwise healthy were included. The effect of iron had to be clear: combined interventions with more than one micronutrient or with lifestyle intervention which did not study the effect of iron separately were not considered. Baseline data for the measured outcomes had to be reported.

**Selection of studies and data extraction**

First, studies were screened by two independent reviewers on the basis of title and abstract to exclude any reference clearly not meeting the review criteria. 10% was assessed in duplication. The duplicate checks were performed first and any discrepancies discussed before the remainder of the references were screened. Any potentially relevant references were then located as full texts to be assessed according to the inclusion criteria. Full texts were assessed by two independent reviewers and disagreements were settled through discussion. Only those papers meeting all the criteria were included in the review. Data were extracted into a standardised database. Data extracted included bibliographic details, methodological details, population characteristics, study groups details and outcome data. 10% of the total papers were extracted in duplicate and any disagreements were discussed to settle uniform data extraction.

**Assessment of risk of bias in included studies**

In order to assess the quality of the study and the risk of bias, the following indicators of internal validity specific to the RCT methodology were collected during data extraction: (1) method of sequence generation and allocation, (2) blinding, (3) potential funding bias, (4) number of participants at start, (5) dropouts and dropout reasons, (6) dose check, (7) dietary intake data reported, (8) outcome comparability and reproducibility, and (8) similarity of most and least exposed groups at baseline. Based on these indicators, two reviewers decided on the overall risk of bias. Disagreements were resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook [23].

## RESULTS

### The effect of iron neurodevelopment, including cognitive function and psychomotor development

Table 1 shows the key characteristics of the included studies assessing neurodevelopment, including cognitive function and psychomotor development, in infants, children and adolescents.

In total, thirteen studies were eligible and were included in the review. Only three of the studies were performed in infants. Two included infants until 12 months of age [24, 25], and

one of them included also children until 24 months of age [26]. The three of them were supplementation trials, with iron alone [24, 26] or with iron combined with zinc [25]. The studies were performed in Indonesia, Guatemala and Turkey. One of them was performed in non anemic infants [24], while the other two included anemic and non anemic infants [25, 26]. One study was a short-term intervention study and used a dosage of ferrous ascorbate of 5 mg/kg during 1 week [26], whereas the other two studies used doses of ferrous sulphate 10 mg/d and 1mg/kg&d during 6 and 3 months, respectively [24, 25].

Ten studies assessed cognitive function in children and/or adolescents 1-18 years old. Excepting one study with a fortified food product [27], all provided iron supplements: in form of ferrous sulphate alone (8 studies) [8, 13, 17, 24, 25, 28-32], ferrous sulphate combined with vitamin C [33], or ferrous fumarate combined with multivitamins [34]. Seven studies included children 1-13 years old [13, 17, 28, 29, 32-34], two included children and adolescents from 8 to 15 years of age [30, 31] and one study included adolescents 13-18 years old [8]. Two studies were performed on females only [8, 30] while the rest were performed on mixed population. Three studies were conducted in Europe: Greece [34], France [32] and England [33], one in the USA [8], and the other six studies in Asia: two in India [30, 31], two in Indonesia [13, 29] and two in Thailand [17, 28]. The dosages of supplemented iron varied widely between the studies. One of the trials provided 15 mg elemental iron daily, given as ferrous fumarate [34]. Doses between 10 and 60 mg of elemental iron daily in form of ferrous sulphate were used in 12 studies (see Table 1). Bruner et al (1996) used markedly higher doses, 2x2 tablets of 325 mg (1300 mg in total) ferrous sulphate daily in adolescent girls, that is equivalent to 260 mg iron daily [8]. Two studies conducted in Indonesia used different doses of iron according to the weight of the children, 2 and 3 mg/kg&day [13, 29]. Pollit et al. (1989) increased the dose from 10 mg/d in the first two weeks, to 20 mg/d in the following 14 weeks [17]. The duration of the interventions ranged from 8 weeks to 4 months. In two Indian studies girls were treated 2x60 days in a school year [30, 31]. In most of the studies, children received the iron supplement daily during the intervention, except in the study by Sungthong et al. where children received 60 mg elemental iron 5 times per week (one group) or the same dose once a week [28]. Two studies [30, 33] were performed in anemic children, one study in NAID girls [8], one in apparently healthy children [28] and five studies in both anemic and nonanemic children [29, 31, 34], two of them including a NAID group [13, 17]. In addition, Konofal et al. performed their study in hyperactive children with normal hemoglobin level, but with low ferritin status [32].

The three trials in infants used the Bayley Scales of Infant Development (BSID). Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores were derived from the results, and one trial additionally reported Behavioural Rating Scale (BRS). Assessment of neurodevelopment in older children varied between the studies. Idjradinata and Pollit (1993) performed a trial in children 12-18 months old using the Bayley Scales too [13], while Aukett and Parks (1986) used 24 items from the Denver developmental screening test validated by the Bayley scale in children 17-19 months old [33]. Two studies conducted

in India used the same tests for assessment of cognitive function in children 8-15 years old: Clerical Task test, Digit Span test, Mazes test and Visual Memory [30, 31]. Simple reaction time, continuous performance task and oddity learning tasks were used by Metallinos-Katsaras et al. (2004) [34], while Bruner et al. (1996) used Brief Test of Attention (BTA), Symbol Digit Modalities Test (SDMT), Visual Search and Attention Test (VSAT) and Hopkins Verbal Learning Test (HVLT) [8]. In three studies the authors measured the intelligence quotient (IQ) in children before and after intervention [17, 28, 29]. The study by Konofal et al. was performed in hyperactive children, using the following tests: Conners Parent Rating Scale (CPRS), Attention Deficit Hyperactivity Disorder (RS), Conners Teacher Rating Scale (CTRS) and Clinical Global Impression- Severity (CGI-S) [32]. The large diversity of tests employed in the different studies hampered any quantitative comparison of results or meta-analysis.

A trial in Guatemala that provided short term supplementation for 1 week to infants and children until 24 months of age found no benefit of iron supplement when testing with the BSID [26]. A study in Turkey with a longer supplementation period (3 months) showed no significant change in developmental test scores in non anemic infants [24]. In contrast, a study in Indonesia with a 6 months supplementation period and a considerably higher sample size of 680 subjects, compared to the study in Guatemala with 68 infants and the one in Turkey with 24, indicated a positive developmental outcome related to motor development as assessed by BSID in all infants [25]. The latter study also used the BRS to assess infants. No significant differences in BRS scales between the iron supplementation and placebo group were found.

A study in 12-18 months old children showed that iron treatment significantly improved BSID results, even reaching the performance level of iron-sufficient children, unlike the group treated with placebo ($p<0.001$) [13]. In another study, the supplementation with iron (or placebo) did not affect the results in NAID children or in non-anemic children. In a group of anemic toddlers of similar age, 17-19 months, 31% achieved 6 or more new skills after treatment with iron, vs. 12% in the placebo treated group ($p<0.05$), assessed by Denver developmental screening test [33]. In another trial, anemic children 3-4 years old made significantly fewer errors of commission (14% higher specificity, $p<0.05$) and exhibited 8% higher accuracy ($p<0.05$) after iron treatment than those given placebo, suggesting improved discrimination and selective attention, although no effects were found on the Oddity Learning task. These positive effects of iron were not seen among the children with good iron status [34].

In a study by Pollitt and coworkers (1989), the IQ and scholastic achievement significantly increased in anemic and to a lesser degree in non-anemic iron deficient children 9-11 years old, after 10 mg/d iron supplementation in the first 2 weeks and 20 mg/d in the following 14 weeks [17]. Interestingly, Sungthong et al. (2004) reported a higher increase in IQ in 6-13 years old children treated with 60 mg iron once weekly for 16 weeks (6±12 points) or placebo (6±12 points) than in children treated with the same dose of 60 mg iron 5 days a week (3±12), while no difference in learning achievement was found among groups [28]. No

statistically significant improvement in IQ was found in an Indonesian study on children who received 2 mg/kg&d iron for 3 months compared to the control group, but improved learning-achievement scores were detected in anemic children treated with iron [29].

Sixty days of supplementation with 30 or 40 mg iron per day in 8-15 years old anemic boys induced a significant improvement of all cognitive functions comparing to placebo group ($p<0.05$), except for Mazes test in the 30 mg/d group. No difference between the iron and placebo group was found in nonanemic boys [31]. Girls of the same age were treated for 2x60 days with 60 mg/d iron. Significantly better scores in Clerical Task test, Digit Span test and Mazes test were reported after 8 months in the iron vs. placebo treated group ($p<0.05$) of anemic girls, but not after 4 months [30]. Similar results were found by Seshadri and Gopaldas but, additionally, they reported improved Mazes test in non-anemic girls too, after supplementation with iron [31]. Markedly higher doses of 260 mg/d iron, given to NAID adolescent girls for 8 weeks, lead to significantly better results in a test of verbal learning and memory compared to girls in the placebo group ($p<0.02$) [8].

Twelve weeks of iron supplementation improved Attention Deficit Hyperactivity Disorder (ADHD) symptoms in 5-8 years old children with low serum ferritin levels. There was a progressive significant decrease in the ADHD Rating Scale (ADHD-RS) on the iron group ($p < 0.008$), but not in the placebo group. Improvement of CPRS (p 0.055) and CTRS (p 0.076) with iron supplementation therapy failed to reach significance. The mean CGI-S significantly decreased at 12 weeks ($p < 0.01$) in the iron treated group, but it did not change in the placebo group [32].

Table 1: Summary of main characteristics and main results of included trials assessing neurodevelopment, cognitive function and psychomotor development

| **Author,year of publication, country** | **Population characteristics (number of subjects, age)** | **Intervention (iron dose, number of subjects in each group)** | **Control** | **Duration of intervention (weeks of months)** | **Outcomes** | **Main results** |
|---|---|---|---|---|---|---|
| Aukett and Parks, 1986 [33] England | N=110 Anemic children 17-19 mo of age | Ferrous sulphate 24 mg/d + Vitamin C 10 mg/d n=48/54 | Vitamin C + placebo n=49/56 | 2 mo | Neurodevelopment psychomotor development 24 items from the Denver developmental screening test | More of the children who received the iron achieved the expected rate of development as compared to those not receiving iron. |
| Bruner et al. 1996 [8] USA-Baltimore | N=81 Non-anemic iron deficient adolescents 13-18 y of age | Ferrous sulphate 260 mg/d N=37/39 | Placebo n=36/39 | 2 mo | BTA-auditory SDMT-visual VSAT HVLT | Girls who received iron performed better on a test of verbal learning and memory than girls in the control group |
| Idjradinata and Pollitt, 1993 [13] Indonesia | N=126 Anemic and non anemic children 12-18 mo of age | Ferrous sulphate, 3 mg/d 1. IDA n=24/25 2. nonanemic ID n=29/29 3. iron sufficient n=22/24 | Placebo 1. IDA n=23/25, 2. nonanemic ID n=14/15, 3. iron sufficient n=22/23 | 4 mo | BSID (MDI, PDI) | The poor performance of 12-18 month-old iron deficient anaemic infants in the Bayley scales prior to the treatment. 4 mo treatment with FeSO4 improved performance to the level of performance of iron-sufficient intants |
| Kashyap et al. 1987 [30] India | N=207 Anemic school girls 8-15 y of age | Ferrous sulphate 60 mg/d n=65/83 | Placebo n=65/83 | 4 mo | Clerical Task, Digit Span Maze test, Visual Memory | Prophylactic supplementation of 60 mg elemental iron for 60 days at a stretch, twice in a school year, improved concentration, discrimination, perception and visual motor coordination |

| Author,year of publication, country | Population characteristics (number of subjects, age) | Intervention (iron dose, number of subjects in each group) | Control | Duration of intervention (weeks of months) | Outcomes | Main results |
|---|---|---|---|---|---|---|
| Konofal et al. 2008 [32] France | N=23<br>Children with ADHD, non anemic, iron deficient<br>5-8 y of age | Ferrous sulphate 80 mg/d<br>N=16/18 | Placebo<br>n=5/5 | 3 mo | CPRS<br>RS<br>CTRS<br>CGI-S | Iron supplementation improved ADHD symptoms in children with low serum ferritin levels |
| Lind et al. 2004 [25] Indonesia | N=680<br>Anemic and non anemic infants<br>6 mo of age | Ferrous sulphate 10 mg/d (n=167/170)<br>Ferrous sulphate 10 mg/d + Zinc sulphate 10 mg/d (n=160/170)<br>Zinc sulphate (n=161/170) | Placebo (n=162/170) | 6 mo | BSID (MDI, PDI and BRS) assessed at 12 mo of age | Single supplementation with iron significantly improved psychomotor development<br>Combined supplementation had no significant effect on development. |
| Lozoff et al. 1982 [26] Guatemala | N=68<br>Infants with and without mild IDA from 6 to 24 mo of age | Ferrous ascorbate 5 mg/kg&d (n=12/15 anemic, n=17/19 non anemic) | Placebo (n=12/13 anemic, 18/21 non anemic) | 1 w | BSID (MDI, PDI) assessed 1 week after start of treatment | Developmental deficits in anemic group prior to treatment.<br>Lack of rapid improvement with short term oral iron therapy. |
| Metallinos-Katsaras et al. 2004 [34] Greece | N=123<br>Anemic and non anemic<br>3-4 y of age | Ferrous fumarate + multivitamins 15 mg Fe/d n=27/27 | Multivitamins n=17/17 | 2 mo | Simple reaction time, continuous performance task, oddity learning task (ODL1), ODL2, ODL3 | Iron supplementation of iron-deficient anemic preschoolers improved cognitive function, specifically discrimination and selective attention |
| Pollitt et al. 1989 [17] Thailand | N=1389<br>Anemic, and non anemic children with ID<br>9-11 y of age | Ferrous sulphate 50mg Fe/d for 2 w and 100mg Fe/d for 14w<br>No numbers per group | Placebo<br>No numbers per group | 4 mo | IQ, Thai language, Mathematika | Positive association between iron status and IQ and language school achievement test. No support for the internal validity of the hypothesis that this association is causal |
| Seshadri et al. 1989 [31] India | N=178<br>Anemic and non anemic<br>8-15 y of age | Ferrous sulphate boys 30 mg/d, n=16/16<br>boys 40 mg/d, n=16/16<br>girls 60 mg/d n=65/65 | Placebo boys n=16/16 placebo girls n=65/65 | 2 mo | Clerical Task, Digit Span<br>Maze test, Visual Memory | In both supplemented group of anemic boys 69% improved cognitive function, except for mazes in 30 mg Fe group. No difference in placebo and iron |

| Author,year of publication, country | Population characteristics (number of subjects, age) | Intervention (iron dose, number of subjects in each group) | Control | Duration of intervention (weeks of months) | Outcomes | Main results |
|---|---|---|---|---|---|---|
| | | | | | | group.<br>In anemic girls Clerical test, mazes and overall test were better in iron treated than in placebo. In nonanemic girls mazes was better in iron treated than in placebo group |
| Soemantri et al. 1989 [29] Indonesia | N=130<br>Anemic and non anemic children from primary school<br>10 y of age | Ferrous sulphate 2mg kg&d<br>Anemic n=34/34<br>Non anemic n=37/37 | Placebo<br>n=59/59<br>Anemic n=24/24<br>Non anemic n=35/35 | 3 mo | IQ, language scores, math scores, biology scores, social science scores | Iron supplementation for 3 mo improved learning-achievement scores but not IQ in anemic children |
| Sungthong et al. 2004 [28] Thailand | N=397<br>Apparently healthy children<br>6-13 y of age | Ferrous sulphate 60 mg/d<br>1. group: 5 days/week n=139/140<br>group once/week n=130/134 | Placebo<br>n=122/123 | 4 mo | IQ, Thai language, Mathematika | Weekly iron supplementation increased IQ better than daily supplementation |
| Yalcin et al. 2000 [24] Turkey | N=24<br>Non anemic infants<br>6 mo of age | Ferrous sulphate 1mg/kg&d (n=7/11) | Placebo (n=9/13) | 3 mo | BSID (MDI, PDI) assessed at 9 mo of age | Iron supplementation did not change developmental test scores. |

**Effects of iron on immunity**

Table 2 shows the key characteristics of the included studies assessing immunity in children and adolescents.

Seven studies met the selection criteria and were included in this review. Iron was given as ferrous sulphate (10-60 mg of elemental iron daily), ferrous citrate (20 mg Fe/d), [35] or in form of ferrous fumarate with or without multivitamins (66 mg Fe/d) [12]. In the study by Longfils et al 10 mg of iron was given with fortified fish sauce daily as ferrous sulphate and citrate (1st group) or NaFe-EDTA (2nd group) [27]. This study included children, adolescents and young adults 9-21 years of age, while children in the other 6 studies were aged 1.5-13.5 years.

Four studies were carried out in Asia: two in Indonesia [36, 37] and two in Sri Lanka [38] and Cambodia [27]. The other three studies were conducted in Benin-Africa [12], Guatemala, South America [35] and Mexico, North America [21]. The duration of interventions ranged from 6 weeks to 12 months (Table 2).

The effect of iron on immune function was assessed in different ways. Dossa et al (2001) reported number of children with fever (>38oC), diarrhoea (3 or more liquid/semiliquid stools per day) or upper respiratory tract infections (URTI) during 6 days before and 6 days after the supplementation period [12]. Rosado et al. (1997) measured the same parameters twice weekly during the whole supplementation period, e.g. 12 months [21]. In addition to these parameters assessed weekly during the intervention, Longfils et al. (2008) measured C reactive protein (CRP) [27]. Rosales et al. (2004) measured CRP, alpha-1-acid glycoprotein (AGP) and alpha-1-antil-chymotrypsin (ACT) as markers of infections before and after iron supplementation [35]. In these four studies iron supplementation had no detectable effect on the measured markers of immune function. De Silva el al. (2003) assessed the number of infectious episodes and the number of sick days [38], while Chwang et al. (1988) measured morbidity scores of acute and chronic diseases, scored from 0 (healthy) to 3 (>3 days absence from school due to disease) [37]. Palupi et al (1997) used parasite infections as indicator of immune function, assessed as number of eggs per gram of stool at the beginning and the end of the study [36]. In these three studies iron supplementation significantly improved the chosen measures of immune function in children.

Table 2: Main characteristics and results of trials assessing effects of iron supply on immunity

| Author,year of publication, country | Population characteristics (number of subjects, age) | Intervention (iron dose, number of subjects in each group) | Control | Duration of intervention (weeks of months) | Outcomes | Main results |
|---|---|---|---|---|---|---|
| Chwang et al. 1988 Indonesia [37] | N=119<br>Anemic and non- anemic<br>8.2-13.5 y of age | Ferrous sulphate 2mg/kg&d<br>n=59/59 | Placebo<br>n=60/60 | 3 mo | Morbidity score of acute or chronic diseases | Iron supplementation improved level of morbidity in anemic but not in non anemic children |
| Dossa et al. 2001 [12] Benin | N=154<br>Stunted, anemic children<br>1.5-2.5 y of age | 1. group: Ferrous fumarate 66 mg/d n=37/35<br>2. group: Ferrous fumarate+ multivitamines 66 mg/d n=40/39 | 1. Group: Placebo n=39/39<br>2. Group: Multivitamines n=38/37 | 6 weeks | Diarrhea, fever, URTI | The morbidity status was comparable in all groups, before and after supplementation. Supplementation with iron with or without multivitamin-multiminerals failed to improve immune function in children. |
| Longfils et al. 2008 [27] Cambodia | N=140<br>Anemic subjects<br>9-21 y of age | Ferrous sulphate 10mg/d<br>1.group-FeSO4+citrate containing 10 mg elemental iron n=47/48<br>2.group-NaFe-EDTA containing 10 mg elemental iron n=47/48 | Placebo<br>n=45/46 | 5.25 mo | Diarrhea, URTI, CRP | Iron-fortified Khmer fish sauce added to Khmer food did not affect the immune function in children and adolescents. |
| Palupi et al. 1997 [36] Indonesia | N=299<br><br>2-5 y of age | Ferrous sulphate 30mg/d<br>1.group-ferous sulphate+aldendazole (400mg) n=95/95<br>2.group-ferous sulphate n=96/96 | Placebo<br>n=98/98 | 2.25 mo | Parasite infections | Iron supplementations administered once weekly reduced parasite infection in an Indonesian community |
| Rosado et al. 1997 [21] Mexico | N=108<br>18-36 mo of age | Ferrous sulphate 20mg/d<br>n=53/53 | Placebo<br>n=55/55 | 12 mo | Diarrhea, URTI | Iron supplementation did not significantly affect morbidity |
| Rosales et al. 2004 [35] Guatemala | N=77<br>Non anemic<br>8-11 y of age | Ferrous citrate 20mg/d<br>n=15/15 | Placebo<br>n=14/14 | 2 mo | CRP, AGP, ACT | Iron supplementation did not improve inflammation |
| de Silva et al. 2003 [38] Sri Lanka | N=434<br>Children with or without infection<br>5-10 y of age | Ferrous sulphate 60mg/d<br>1.Children with infection n=127/127<br>2.Children without infection n=134/134 | 1.Placebo-children with infection n=52/52<br>2. Placebo-children without infection n=50/50 | 2 mo | URTI and gastrointestinal infections | In both the infection group and the control group, the mean number of URTI episodes and the total number of days sick with an URTI during the period of intervention were significantly lower in the children who received iron supplements than in those who received placebo. |

**Quality of studies included**

Table 3 summarizes the methodological quality of the included studies assessing neurodevelopment and immunity, assessed as described in the section “Methods”. The results clearly show a high risk of bias in most of the studies. Only 2 studies [13, 25] had a low risk of bias. Two studies had a moderate risk of bias [8, 28]. The main reasons for having a high risk of bias were an inadequate sequence generation and/or allocation and the study funders. Among the trials dealing with immunity, only one study out of 7 was assessed as having a moderate risk of bias. Six studies had a high risk of bias, mainly due to inadequate sequence generation and allocation.

Table 3: Assessment of methodological quality of included RCTs on effects of iron supply

| Study (Author, year) | Adequate sequence generation | Allocation concealment adequate | Blinding adequate | Dropouts adequate and outcome data complete | Funder adequate | Lack of other potential threats to validity | Overall risk of bias |
|---|---|---|---|---|---|---|---|
| ***Neurovelopment including cognitive function and psychomotor development*** | | | | | | | |
| Aukett and Parks 1986 [33] | Unclear | Yes | Yes | Yes | Unclear | Yes | **High** |
| Bruner et al. 1996 [8] | Yes | Yes | Yes | Yes | No | Yes | **Moderate** |
| Idjradinata and Pollitt 1993 [13] | Yes | Yes | Yes | Yes | Yes | Yes | **Low** |
| Kashyap et al. 1987 [30] | Unclear | Unclear | Unclear | No | Yes | Yes | **High** |
| Konofal et al. 2008 [32] | Unclear | Unclear | Yes | Yes | Unclear | Unclear | **High** |
| Lind et al. 2004 [25] | Yes | Yes | Yes | Yes | Yes | Unclear | **Low** |
| Lozoff et al. 1982 [26] | Unclear | Unclear | Yes | Yes | Yes | Unclear | **High** |
| Metallinos-Katsaras et al. 2004 [34] | Unclear | Yes | Yes | Yes | No | Yes | **High** |
| Pollitt et al. 1989 [17] | Unclear | Unclear | Yes | Unclear | Yes | Yes | **High** |
| Seshadri et al. 1989 [31] | Unclear | Unclear | Unclear | Yes | Unclear | Yes | **High** |
| Soemantri et al. 1989 [29] | Unclear | Unclear | Yes | Yes | Yes | Yes | **High** |
| Sungthong et al. 2004 [28] | Yes | Unclear | Yes | Yes | Yes | Yes | **Moderate** |
| Yalcin et al. 2000 [24] | Unclear | Unclear | No | Yes | Unclear | Unclear | **High** |
| ***Immunity*** | | | | | | | |
| Chwang et al. 1988 [37] | Unclear | Unclear | Yes | Yes | Yes | Yes | **High** |
| Dossa et al. 2001 [12] | Yes | No | No | Yes | Yes | Yes | **High** |
| Longfils et al. 2008 [27] | Unclear | Unclear | Yes | Yes | No | Yes | **High** |
| Palupi et al. 1997[36] | Unclear | Unclear | Yes | Yes | No | Yes | **High** |
| Rosado et al. 1997 [21] | Unclear | Yes | Yes | Yes | Yes | Yes | **Moderate** |
| Rosales et al. 2004 [35] | Unclear | Yes | No | Yes | Unclear | Yes | **High** |
| de Silva et al. 2003 [38] | Unclear | Unclear | Yes | Unclear | Yes | Yes | **High** |

## DISCUSSION

### Evidence

This review of 20 RCTs has assessed the effects of iron supplementation on measures of neurodevelopment, including measures of cognition and psychomotor development, and on immunity, in infants, children and adolescents from birth to 18 years of age. The overwhelming feature of this review is the utter paucity of relevant studies that could be identified and included in the review, especially for infants until 12 months of age.

Only one study identified provided iron supplements for a short period of time (one week) [26]. Based on this single study, there is no evidence of a positive effect of short term supplementation in the mental and psychomotor development of anemic infants and children with and without IDA of 6 to 24 months of age.

For children aged 1 to 5 years of age, there is some evidence from 3 RCTs which show a benefit of iron supplementation during 2 to 4 months on the mental and psychomotor development of both anemic and non anemic children [13, 34] and of anemic children [33].

Seven RCTs in anemic and non-anemic children over 5 years of age provided evidence for a positive effect of iron supplementation on different measures of cognition [8, 17, 28-32].

Given the data base available, it is not possible to derive firm evidence-based conclusions on the effect of iron intake on neurodevelopment. The data on iron status and morbidity are even more controversial. One important reason for this lack of evidence is that a large number of the trials originally had to be excluded because they did not have an adequate control group according to our criteria. This was especially relevant for interventions with fortified formula and fortified foods, which limited the review to supplementation trials except for one trial with a fortified food [27]. Furthermore, the presence of other confounding variables, such as other nutritional deficiencies, low socioeconomic status, studies carried out in areas with endemic malaria etc. could markedly have influenced the obtained results.

The large heterogeneity of outcomes reported related to immunity impedes any comparison of studies.

Another remarkable feature of the collected studies is the heterogeneity of study populations. This further complicated matters by making it difficult or impossible to compare and combine the available results in a meaningful way. The heterogeneity of outcome measures precludes pooling study results quantitatively or even qualitatively in the case of immunity. The assessment of validity shows that the methodological quality of most studies is limited.

### Comparison with other reviews

In 2005, Sachdev and collaborators published a systematic review with a meta-analysis examining the effects of iron supplementation in relation to development in children [15]. The review included 17 trials of oral iron supplementation, fortified milks and cereal, and parenteral iron (all RCTs) involving 3646 participants. The authors concluded that iron supplementation had modest but significant beneficial effects on mental development for

children who were anemic or iron deficient at baseline and for all children >7 years old, particularly for IQ scores. In younger children (aged <27 months), no effect of iron supplementation on mental development was detected. Motor development was not found to be improved through iron supplementation.

An extensive review by Iannotti et al. on the benefits and risks of iron supplementation of infants and children under 5 years of age in developing countries, published in 2006, concluded that providing additional iron via daily or weekly supplementation to iron-deficient children under 5 years may have some positive effects on developmental indicators, especially among children who are anemic or iron deficient at baseline [39].

A Cochrane review by Logan et al (2001) aimed to assess the effects of iron supplementation provided to iron deficient infants and children under 3 years of age, beginning supplementation before 1 year of age, on measures of psychomotor development or cognitive function [40]. 7 RCTs providing oral or intramuscular iron were included in this review, 5 of which assessed psychomotor development between 5 and 11 days of commencement of therapy. It was concluded that there was no convincing evidence that administration of iron improves scores on tests of psychomotor development within 6-11 days of treatment. The effect of longer interventions unfortunately remained unclear from the results of the other 2 studies where the assessment was done more than 30 days after commencement of therapy.

A recent review with meta-analysis by Szajewska et al. (2010) analysed the effects of non-anemic infants and young children on the mental performance and psychomotor development of children (<3 years) [41]. The included RCTs did not individually show a beneficial effect of iron supplementation during early life. The authors performed 2 meta-analyses of 3 RCTs involving 561 infants and children. It was concluded that iron supplementation in infants may positively influence children's psychomotor development, whereas it does not seem to alter their mental development or behaviour.

Falkingham et al. (2010) recently published a systematic review assessing the effects of iron supplementation on cognition in older children (>6 years) and adults [42]. They included 14 RCTs and concluded that iron supplementation improved attention and concentration in all studies irrespective of baseline iron status. In anemic groups of children supplementation improved IQ but had no effect on non-anemic participants. No effect of iron supplementation was found on memory, psychomotor skills or scholastic achievement. However, the authors emphasised the limited number of included studies, which were generally conducted in small samples of subjects, had a short duration and were methodologically weak.

No published reviews on the effect of iron on immunity could be identified.

**Strengthens and weakness of this review**

In children and adolescents, 4 out of 10 studies on cognitive function and 4 out of 7 studies on immune function, included in this review had a supplementation period shorter than 12 weeks of supplementation (Tables 1 and 2), which is thought to be sufficient to alter the iron status. Some studies were included of too short a duration; these could possibly negate the

effects of iron supplementation, but in this case, these studies showed similar effects on cognition compared to studies with longer supplementation periods. Additionally, a 3-month long study [29] showed no effects of supplementation on IQ in anemic (and non anemic) children, which the authors explained by several factors such as mucosal block or other unidentified causes that were not detected during the study (e.g. genetic factors that control absorption). Although a much longer duration of intervention is probably needed for outcomes such as scholastic achievement, where iron status at learning may be different from iron status at assessment of performance [42], we found improved learning results after iron supplementation.

Comparing morbidity outcomes in studies shorter and longer than 12 weeks of supplementation, similarly inconsistent results are found compared to cognitive outcomes. In the longest study, for instance, which lasted 12 months, Rosado et al. (1997) did not find significantly lower frequency of infections in the iron supplemented group, while de Silva et al. (2003) reported significantly less acute respiratory infections after 8 weeks of iron supplementation than in the placebo group.

An important weakness of this review is the high risk of bias of most of the included papers. The sequence generation and/or allocation were not well addressed in 9 and 8 papers respectively, as well as funders in 4 studies on cognition (Table 3). Only 2 studies were assessed as having a low risk of bias. A high risk of bias was assessed in 6 out of 7 studies, due to inadequate sequence generation (6), allocation (5) and funding (3 studies).

The tests for assessment of neurodevelopment are similar in infants and young children (Bayley test) while in older children the comparison is much more complicated. Immune function was also assessed in different ways. Different iron status at baseline (anemic, non anemic and non anemic iron deficient subjects), and different socioeconomic status of the participants make it difficult to assess the effects of iron on cognitive and immune functions.

**Recommendations for practice and research**

Until more convincing evidence exist that iron supplementation can significantly improve neurodevelopment, including measures of cognitive function and psychomotor development in infants, children and adolescents, policy should focus on prevention of iron deficiency anemia.

Randomised controlled trials in infants, children and adolescents should be conducted in a way that facilitates comparison with other studies and metaanalysis. Reporting those studies in a standardised way would also contribute to a more evidence based nutrition.

**Acknowledgments**

The original conception of the systematic review was undertaken by the EURRECA Network and coordinated by partners based at Wageningen University (WU), the Netherlands and the University of East Anglia (UEA), United Kingdom. Susan Fairweather-Tait (UEA), Lisette de Groot (WU), Pieter van' t Veer (WU), Kate Ashton (UEA), Amélie Casgrain (UEA), Adriënne Cavelaars (WU), Rachel Collings (UEA), Rosalie Dhonukshe-Rutten (WU), Esmée Doets

(WU), Linda Harvey (UEA) and Lee Hooper (UEA) designed and developed the review protocol and search strategy. The authors are grateful to all EURRECA members and partners involved in database searching, screening and data entry for iron: Rachel Collings, Amelie Casgrain, and Joy Ngo.

**REFERENCES**

[1] Institute of Medicine. Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc Washington DC: National Academy Press; 2000.

[2] Leung AK, Chan KW. Iron deficiency anemia. Adv Pediatr. 2001;48:385-408.

[3] Thane CW, Bates CJ, Prentice A. Risk factors for low iron intake and poor iron status in a national sample of British young people aged 4-18 years. Public Health Nutrition. 2003;6:485-96.

[4] Iglesia I, Doets EL, Bel-Serrat S, Román B, Hermoso M, Peña Quintana L, et al. Physiological and public health basis for assessing micronutrient requirements in children and adolescents. The EURRECA network. Maternal and Child Nutrition. 2010;6:84-99.

[5] Clark SF. Iron deficiency anemia. Nutr Clin Pract. 2008;23:128-41.

[6] Ferrara M, Coppola L, Coppola A, Capozzi L. Iron deficiency in childhood and adolescence: retrospective review. Hematology. 2006;11:183-6.

[7] Gibson RS, Heath AL, Ferguson EL. Risk of suboptimal iron and zinc nutriture among adolescent girls in Australia and New Zealand: causes, consequences, and solutions. Asia Pac J Clin Nutr. 2002;11 Suppl 3:S543-52.

[8] Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet. 1996;348:992-6.

[9] World Health Organization. WHO 55th Assembly (WHA55.25, Agenda item 13.10): Infant and Young Child Nutrition. 2002.

[10] World Health Organization. Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. Geneva2001.

[11] World Health Organization, Food and Agricultural Organization of the United Nations. Guidelines on food fortification with micronutrients. In: Lindsay Allen, Bruno de Benoist, Omar Dary, Richard Hurrell, editors.2006.

[12] Dossa RA, Ategbo EA, de Koning FL, van Raaij JM, Hautvast JG. Impact of iron supplementation and deworming on growth performance in preschool Beninese children. Eur J Clin Nutr. 2001:223-8.

[13] Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet. 1993;341:1-4.

[14] Scientific Committee on Food of the European Commission. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae. Brussels: Scientific Committee on Food. European Commission; 2003.

[15] Sachdev H, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr. 2005;8:117-32.

[16] Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr. 2001;131:649S-66S; discussion 66S-68S.

[17] Pollitt E, Hathirat P, Kotchabhakdi NJ, Missell L, Valyasevi A. Iron deficiency and educational achievement in Thailand. Am J Clin Nutr. 1989;50:687-96; discussion 96-7.

[18] Benton D. Micronutrient status, cognition and behavioral problems in childhood. Eur J Nutr. 2008;47:38-50.

[19] Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64:S34-S43.

[20] Beard JL. Why Iron Deficiency Is Important in Infant Development. J Nutr. 2008;138:2534-6.

[21] Rosado JL, López P, Muñoz E, Martinez H, Allen LH. Zinc supplementation reduced morbidity, but neither zinc nor iron supplementation affected growth or body composition of Mexican preschoolers. The American journal of clinical nutrition1997. p. 13-9.

[22] Ashwell M, Lambert JP, Alles MS, Branca F, Bucchini L, Brzozowska A, et al. How we will produce the evidence-based EURRECA toolkit to support nutrition and food policy. Eur J Nutr. 2008;47 Suppl 1:2-16.

[23] Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.0.2 [updated September 2009] ed2009.

[24] Yalcin SS, Yurdakok K, Acikgoz D, Ozmert E. Short-term developmental outcome of iron prophylaxis in infants. Pediatr Int. 2000;42:625-30.

[25] Lind T, Lonnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nutr. 2004;80:729-36.

[26] Lozoff B, Brittenham GM, Viteri FE. The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. J Pediatr. 1982;100:351-7.

[27] Longfils P, Monchy D, Weinheimer H, Chavasit V, Nakanishi Y, Schumann K. A comparative intervention trial on fish sauce fortified with NaFe-EDTA and FeSO4+citrate in iron deficiency anemic school children in Kampot, Cambodia. Asia Pac J Clin Nutr. 2008;17:250-7.

[28] Sungthong R, Mo-Suwan L, Chongsuvivatwong V, Geater AF. Once-weekly and 5-days a week iron supplementation differentially affect cognitive function but not school performance in Thai children. J Nutr. 2004;134:2349-54.

[29] Soemantri AG, Gopaldas T, Seshadri S, Pollitt E. Preliminary findings on iron supplementation and learning achievement of rural Indonesian children. Am J Clin Nutr. 1989;50:698-702.

[30] Kashyap P, Gopaldas T. Impact of hematinic supplementation on cognitive function in underprivileged school girls (8-15 yrs of age). Nutrition Res. 1987;7:1117-26.

[31] Seshadri S, Gopaldas T. Impact of iron supplementation on cognitive functions in preschool and school-aged children: the Indian experience. Am J Clin Nutr. 1989;50:675-84; discussion 85-6.

[32] Konofal E, Lecendreux M, Deron J, Marchand M, Cortese S, Zaïm M, et al. Effects of iron supplementation on attention deficit hyperactivity disorder in children. Pediatr Neurol. 2008;38:20-6.

[33] Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. Arch Dis Child. 1986;61:849-57.

[34] Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E. Effect of iron supplementation on cognition in Greek preschoolers. Eur J Clin Nutr. 2004;58:1532-42.

[35] Rosales FJ, Kang Y, Pfeiffer B, Rau A, Romero-Abal ME, Erhardt JG, et al. Twice the recommended daily allowance of iron is associated with an increase in plasma alpha-1 antichymotrypsin concentrations in Guatemalan school-aged children. Nutrition Res. 2004;24:875-87.

[36] Palupi L, Schultink W, Achadi E, Gross R. Effective community intervention to improve hemoglobin status in preschoolers receiving once-weekly iron supplementation. Am J Clin Nutr. 1997;65:1057-61.

[37] Chwang L, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr. 1988;47:496-501.

[38] de Silva A, Atukorala S, Weerasinghe I, Ahluwalia N. Iron supplementation improves iron status and reduces morbidity in children with or without upper respiratory tract infections: a randomized controlled study in Colombo, Sri Lanka,. Eur J Clin Nutr. 2003;77:234-41.

[39] Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr. 2006;84:1261-76.

[40] Logan S, Martins S, Gilbert R. Iron therapy for improving psychomotor development and cognitive function in children under the age of three with iron deficiency anaemia. Cochrane Database Syst Rev. 2001:CD001444.

[41] Szajewska H, Ruszczynski M, Chmielewska A. Effects of iron supplementation in nonanemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials. Am J Clin Nutr. 2010;91:1684-90.

[42] Falkingham M, Abdelhamid A, Curtis P, Fairweather-Tait S, Dye L, Hooper L. The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta-analysis. Nutr J. 2010;9:4-19.

# 10 APPENDIX A: SEARCH STRATEGIES

## 10.1 FINAL IRON SEARCH STRATEGY – EMBASE

Date of search: 6/02/2009 and 6/02/2010

| # | Searches | Results | Search Type |
|---|---|---|---|
| 1 | infant nutrition.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name] | 2103 | Advanced |
| 2 | exp infant nutrition/ | 25456 | Advanced |
| 3 | Infant Nutrition/ | 1940 | Advanced |
| 4 | artificial milk/ or breast milk/ or bottle feeding/ or breast feeding/ | 23313 | Advanced |
| 5 | lactation/ | 12685 | Advanced |
| 6 | random*.ti,ab. | 389782 | Advanced |
| 7 | factorial*.ti,ab. | 8131 | Advanced |
| 8 | (crossover* or cross over* or cross-over*).ti,ab. | 39188 | Advanced |
| 9 | placebo*.ti,ab. | 109036 | Advanced |
| 10 | (doubl* adj blind*).ti,ab. | 84173 | Advanced |
| 11 | (singl* adj blind*).ti,ab. | 7399 | Advanced |
| 12 | Crossover Procedure/ | 20937 | Advanced |
| 13 | Double Blind Procedure/ | 71265 | Advanced |
| 14 | Randomized Controlled Trial/ | 165330 | Advanced |
| 15 | Single Blind Procedure/ | 7943 | Advanced |
| 16 | animal/ or nonhuman/ or animal experiment/ | 3415144 | Advanced |
| 17 | human/ | 6387488 | Advanced |
| 18 | ((iron or ferrous or ferric or ferr*) adj3 (intake* or diet* or supplement* or deplet* or status or expos* or concentration*)).ti,ab. | 12627 | Advanced |
| 19 | *Ferritin Blood Level/ | 511 | Advanced |
| 20 | *Transferrin Receptor/ | 1991 | Advanced |
| 21 | *Hemoglobin/ | 12370 | Advanced |

| # | Searches | Results | Search Type |
|---|---|---|---|
| 22 | ((serum adj3 ferritin) or (plasma adj3 ferritin) or (serum adj3 apoferritin) or (plasma adj3 apoferritin) or (serum adj3 "transferrin receptor*") or (plasma adj3 "transferrin receptor*") or (serum adj3 TFR*) or (plasma adj3 TFR*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name] | 5311 | Advanced |
| 23 | (hemoglobin* or haemoglobin*).ti,ab. | 62809 | Advanced |
| 24 | *Iron Intake/ or *Iron Depletion/ or *Iron Deficiency/ | 3095 | Advanced |
| 25 | (iron or ferrous or ferric or ferr*).ti,ab. | 96859 | Advanced |
| 26 | (intake* or diet* or fortif* or supplement* or deplet* or status or concentration* or expos*).ti,ab. | 1855418 | Advanced |
| 27 | supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp food intake/ or exp mineral intake/ | 179562 | Advanced |
| 28 | exp nutritional status/ or nutritional deficiency/ | 21122 | Advanced |
| 29 | 11 or 8 or 12 or 14 or 7 or 13 or 6 or 9 or 15 or 10 | 502578 | Advanced |
| 30 | 16 and 17 | 527284 | Advanced |
| 31 | 16 not 30 | 2887860 | Advanced |
| 32 | 29 not 31 | 440615 | Advanced |
| 33 | 23 or 21 or 22 or 20 or 19 | 69947 | Advanced |
| 34 | 27 or 26 or 28 | 1932328 | Advanced |
| 35 | 33 or 34 | 1974813 | Advanced |
| 36 | 35 and 25 | 41757 | Advanced |
| 37 | 24 or 18 or 36 | 42652 | Advanced |
| 38 | 32 and 37 | 2191 | Advanced |
| 39 | 4 or 34 or 3 or 5 | 1941284 | Advanced |
| 40 | 33 or 39 | 1983747 | Advanced |
| 41 | 25 and 40 | 41853 | Advanced |
| 42 | 18 or 24 or 41 | 42746 | Advanced |
| 43 | 42 and 32 | 2193 | Advanced |

## 10.2 FINAL IRON SEARCH STRATEGY – MEDLINE

Date of search 6/02/2009 and 6/02/2010

| # | Searches | Results | Search Type |
|---|---|---|---|
| 1 | randomized controlled trial.pt. | 261515 | Advanced |
| 2 | controlled clinical trial.pt. | 77893 | Advanced |
| 3 | randomized.ab. | 172980 | Advanced |
| 4 | clinical trials as topic.sh. | 140006 | Advanced |
| 5 | randomly.ab. | 125849 | Advanced |
| 6 | randomised.ab. | 33552 | Advanced |
| 7 | (animals not (human and animals)).sh. | 4293014 | Advanced |
| 8 | ((iron or ferric or ferrous or ferr*) adj3 (intake* or diet* or supplement* or deplet* or status or expos* or concentration*)).ti,ab. | 14306 | Advanced |
| 9 | (hemoglobin* or haemoglobin*).ti,ab. | 84956 | Advanced |
| 10 | ((serum adj3 ferritin) or (plasma adj3 ferritin) or (serum adj3 apoferritin) or (plasma adj3 apoferritin) or (serum adj3 "transferrin receptor*") or (plasma adj3 "transferrin receptor*") or (serum adj3 TFR*) or (plasma adj3 TFR*)).ti,ab. | 6042 | Advanced |
| 11 | *iron, dietary/ | 806 | Advanced |
| 12 | (iron or ferrous or ferric or ferr*).ti,ab. | 116185 | Advanced |
| 13 | (intake* or diet* or fortif* or supplement* or deplet* or status or concentration* or expos*).ti,ab. | 2148582 | Advanced |
| 14 | nutritional support/ or dietary supplements/ or nutritional requirements/ or exp nutritional status/ or exp deficiency diseases/ or supplementation/ | 108850 | Advanced |
| 15 | diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or diet/ or food, fortified/ or nutrition assessment/ or nutritive value/ | 94854 | Advanced |
| 16 | 6 or 4 or 1 or 3 or 2 or 5 | 578036 | Advanced |
| 17 | 16 not 7 | 516057 | Advanced |
| 18 | 10 or 9 | 89036 | Advanced |
| 19 | 13 or 15 or 14 | 2231529 | Advanced |
| 20 | 11 or 12 | 116199 | Advanced |
| 21 | 18 or 19 | 2289421 | Advanced |
| 22 | 21 and 20 | 46409 | Advanced |

| # | Searches | Results | Search Type |
|---|---|---|---|
| 23 | 22 or 8 | 46409 | Advanced |
| 24 | 23 and 17 | 2311 | Advanced |
| 25 | Breast Feeding/ or exp Infant Food/ | 26392 | Advanced |
| 26 | Bottle Feeding/ | 2725 | Advanced |
| 27 | 25 or 26 | 27172 | Advanced |
| 28 | Infant Formula/ | 1023 | Advanced |
| 29 | 27 or 28 | 27172 | Advanced |
| 30 | 19 or 29 | 2248563 | Advanced |
| 31 | 18 or 30 | 2306432 | Advanced |
| 32 | 8 or 31 | 2306432 | Advanced |
| 33 | 32 and 20 | 46494 | Advanced |
| 34 | 33 and 17 | 2321 | Advanced |

## 10.3 FINAL IRON SEARCH STRATEGY – COCHRANE

Date of search: 9/02/2009 and 6/02/2010

| ID | Search | Hits |
|---|---|---|
| #1 | iron | 3316 |
| #2 | MeSH descriptor Iron, Dietary explode all trees | 162 |
| #3 | MeSH descriptor Iron explode tree 4 | 1320 |
| #4 | (#1 OR #2 OR #3) | 3316 |
| #5 | MeSH descriptor Hemoglobins, this term only | 1976 |
| #6 | hemoglobin* or haemoglobin* | 9043 |
| #7 | ferritin or apoferritin | 1007 |
| #8 | MeSH descriptor Ferritins explode all trees | 580 |
| #9 | "transferrin receptor" | 104 |
| #10 | TfR | 31 |
| #11 | MeSH descriptor Receptors, Transferrin, this term only | 62 |

| ID | Search | Hits |
|---|---|---|
| #12 | (#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11) | 9474 |
| #13 | intake* or diet* or fortif* or supplement* or deplet* or status or concentration* or expos* | 168530 |
| #14 | (#12 OR #13) | 172972 |
| #15 | (#14 AND #4) | 2603 |
| #16 | "kidney disease" or hemodialys* or dialys* or "renal failure" or "heart failure" or CKD or chemotherap* or cancer* or malignanc* or thalassemia or "sickle cell" | 79705 |
| #17 | (#15 AND NOT ( "kidney disease" OR hemodialys* OR dialys* OR "renal failure" OR "heart failure" OR CKD OR chemotherap* OR cancer* OR malignanc* OR thalassemia OR "sickle cell" )) | 2054 |

# 11 ACKNOWLEDGEMENTS

This work has been carried out within the EURRECA Network of Excellence (www.eurreca.org) which is financially supported by the Commission of the European Communities, specific Research Technology and Development (RTD) Programme Quality of Life and Management of Living Resources, within the Sixth Framework Programme, contract no. 036196.

I wish to express my gratitude to my supervisor, Prof. Dr. med. Berthold Koletzko, for giving me the framework to develop my doctoral thesis, and for his support throughout the completion of this project.

My thanks to all EURRECA colleagues throughout Europe, for the stimulating collaboration, and to my colleagues at the Dr. von Hauner Children's Hospital in Munich.

Thank you to my dear family, for their permanent support, generosity and understanding.