TECHNICAL REPORT

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Dietary Reference Values for nutrients

Summary report

European Food Safety Authority (EFSA)

Update: 4 September 2019[1]

Abstract

Dietary reference values (DRVs) is an umbrella term for the complete set of nutrient reference values which include population reference intakes (PRIs), the average requirements (ARs), adequate intakes (AIs) and reference intake (RIs) ranges for macronutrients. These values indicate the amount of a nutrient which must be consumed on a regular basis to maintain health in an otherwise healthy individual (or population). In 2005, the European Commission asked EFSA to review the advice of the Scientific Committee for Food (SCF) dated 1993 on DRVs for the European population, to ensure that Community action in the area of nutrition was underpinned by the latest scientific evidence. The task was entrusted to the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). The Panel set the foundations for this task in an inaugural opinion published in 2010, which addressed the general principles for deriving and applying DRVs. A total of 34 scientific opinions were then published over 9 years, covering water, fats, carbohydrates and dietary fibre, protein, energy, as well as 14 vitamins and 15 minerals. This summary report brings together the summaries of the individual opinions, together with synthetic tables and annexes. It provides an overview of the outcome of EFSA's scientific deliberations for easy reference by end-users. This report is not meant to replace the original opinions. For the detailed reasoning behind individual values, the reader is invited to consult the full opinions.

Key words: dietary reference values; nutrients; macronutrients; micronutrients

Requestor: EFSA

Correspondence: nutri@efsa.europa.eu

[1] The present document is an update of the original version published in 2017. It includes the last two assessments on DRVs for sodium and chloride published in September 2019.

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Preface

Dietary Reference Values (DRVs) is an umbrella term for the complete set of nutrient reference values which include the Population Reference Intakes (PRIs), the Average Requirements (ARs), Adequate Intakes (AIs) and Reference Intake (RIs) ranges for macronutrients. These values indicate the amount of a nutrient which must be consumed on a regular basis to maintain health in an otherwise healthy individual (or population). They are set on the assumption that the requirements for energy and all other nutrients are already satisfied. DRVs also include the tolerable upper intake levels (ULs), which represent the maximum average daily intake level of nutrients considered to be unlikely to pose a risk of adverse health effects. DRVs are key concepts in the nutrition field. They provide the scientific bases on which nutrition recommendations are built. They are the references used in diet assessment and diet planning, at the population and individual level. They also serve as the basis for setting reference values in food labelling, and in establishing food based dietary guidelines. For these reasons, they will be helpful to health professionals, scientists, risk managers, policy makers, industry and many more.

In 2005, the European Commission asked EFSA to review the advice of the Scientific Committee for Food (SCF)[2] dated 1993 on DRVs for the European population, to ensure that Community action in the area of nutrition was underpinned by the latest scientific evidence. The task was entrusted to the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). The Panel set the foundations for this task in an inaugural opinion published in 2010[2], which addressed the general principles for deriving and applying DRVs (EFSA NDA Panel, 2010a). A total of 34 scientific opinions were then published over 9 years, covering water, fats, carbohydrates and dietary fibre, protein, energy, as well as 14 vitamins and 15 minerals.

EFSA DRVs opinions share a common structure, which reflects the process by which DRVs are set. Initial sections provide background information on the nutrient under consideration, addressing its chemistry, functions, physiology and metabolism, its interaction with other nutrients, as well as a review of available biomarkers of intake and status. Data on sources of the nutrient and its intake in European countries are then summarised, gathered from national reports or derived from EFSA Comprehensive European Food Consumption database[3] and Food Composition database.[4] Each opinion also compiles an overview of DRVs and recommendations set by other bodies. The Panel then reviews existing literature on the physiological needs of the nutrient, as well as its association with health outcomes, including the risk of chronic diseases, in order to identify criteria on which to base the DRVs. Finally, the Panel integrates the available evidence and derives DRVs, where possible.[5]

Because of the length of the individual opinions, it is impractical to publish them as a single report. Therefore, the present summary report puts together the summaries of the individual opinions, together with synthetic tables and annexes. It provides an overview about the outcome of EFSA's scientific deliberations, for easy reference by end-users. This report is not meant to replace the original opinions. For the detailed reasoning behind individual values, the reader is invited to consult the full opinions.[6]

Many gave their time and energy to the DRVs opinions. EFSA and its NDA Panel are heartily grateful to all of them. Over the past 12 years, WG members on DRVs and NDA Panel members were involved, with a special tribute to Albert Flynn and Ambroise Martin, former chairs of the NDA Panel. This project would not have been completed without the dedication, commitment and high level of expertise of the EFSA Nutrition Unit, in particular Juliane Kleiner, Leng Heng, Anja Brönstrup, Céline Dumas, Lucia Fabiani, Jelena Gudelj Rakic, Agnès de Sesmaisons Lecarré and Silvia Valtueña Martínez, as well as the trainees and interim staff who have been involved. The help of other EFSA Units, in particular the

[2] SCF (Scientific Committee on Food), 1993. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st Series. Food - Science and Technique, European Commission, Luxembourg, 248 pp

[3] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

[4] https://www.efsa.europa.eu/en/data/food-composition

[5] At the time the task was initiated, opinions on tolerable upper intake levels for vitamins and minerals had already been published (http://www.efsa.europa.eu/en/science/nda/nda_opinions.html). Therefore, ULs for micronutrients are not addressed in the scientific opinions compiled in the present report.

[6] A link to the full opinion is provided at the beginning of the summary for each individual nutrient.

Evidence Management and Assessment and Methodological Support Units, need to be acknowledged as well. EFSA NDA Panel also wants to extend its gratitude to stakeholders for their contribution during the public consultations on DRVs opinions, and to European Commission's Directorate General Santé. Every individual involved in reviewing, interpreting and translating scientific evidence into DRVs made a substantial contribution to public health and to the science and practice of nutrition.

Dominique Turck
Chair of the NDA Panel

Valeriu Curtui
Head of EFSA Nutrition Unit

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1. Background and Terms of Reference as provided by the requestor

Background as provided by the European Commission

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example, such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and, if necessary, to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an Opinion on the nutrient and energy intakes for the European Community[7]. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF Opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

Terms of Reference as provided by the European Commission

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[8] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, trans fatty acids;
- Protein;
- Dietary fibre

[7] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

[8] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).[9]

[9] This part of the request does not refer to the setting of DRVs and has been addressed in the following opinion: EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on establishing Food-Based Dietary Guidelines. EFSA Journal 2010; 8(3):1460. [42 pp.]. doi:10.2903/j.efsa.2010.1460.
Available online: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1460/full

2. General principles

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1458/full

This Opinion focuses on the general principles for development and application of Dietary Reference Values (DRVs) - quantitative reference values for nutrient intakes for healthy individuals and populations which may be used for assessment and planning of diets (EFSA NDA Panel, 2010a). Similarly to the earlier Scientific Committee on Food (SCF) report in 1993 the Panel proposes to derive the following Dietary Reference Values:

- *Population Reference Intakes (PRI):* the level of (nutrient) intake that is adequate for virtually all people in a population group.
- *Average Requirement (AR):* the level of (nutrient) intake that is adequate for half of the people in a population group, given a normal distribution of requirement.
- *Lower Threshold Intake (LTI):* the level of intake below which, on the basis of current knowledge, almost all individuals will be unable to maintain "metabolic integrity", according to the criterion chosen for each nutrient. In addition, the Panel also proposes to derive the following Dietary Reference Values :
- *Adequate Intake (AI):* the value estimated when a Population Reference Intake cannot be established because an average requirement cannot be determined. An Adequate Intake is the average observed daily level of intake by a population group (or groups) of apparently healthy people that is assumed to be adequate.
- *Reference Intake ranges for macronutrients (RI):* the intake range for macronutrients, expressed as % of the energy intake. These apply to ranges of intakes that are adequate for maintaining health and associated with a low risk of selected chronic diseases. The Panel will not address the Tolerable Upper Intake Level (UL) as this has been assessed previously. The Tolerable Upper Intake Level is the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans.[10]

Some of the Reference Values - the Average Requirement, Population Reference Intake and the Lower Threshold Intake - relate to nutrient requirements that are defined by specific criteria of nutrient adequacy. In defining nutrient requirements the selection of criteria to establish nutrient adequacy is an important step. For most nutrients a hierarchy of criteria for nutrient adequacy can be established, ranging from prevention of clinical deficiency to optimisation of body stores, or status.

Which criterion, or combination of criteria, will be the most appropriate is decided on a case-by-case basis.

Within any life stage group, nutrient requirements vary between individuals and the Average Requirement, Population Reference Intake and Lower Threshold Intake represent different points on the distribution of individual requirements. Nutrient requirements also differ with age, sex and physiological condition, due to differences in the velocity of growth for the younger age groups, and age-related changes in nutrient absorption and body functions and/or functional capacity, such as renal function. Especially in older subjects, variability in functional capacity and in energy expenditure appears higher than in younger adults, particularly for elderly above 75 years.

Because of this, Dietary Reference Values are developed for different life stage and sex groups. The Panel proposes to define the age ranges used for each nutrient on a case-by-case basis depending on the available data.[11] For the age group < 6 months requirements are considered to be equal to the

[10] A report on tolerable upper intake levels for vitamins and minerals was published in February 2006 (http://www.efsa.europa.eu/en/science/nda/nda_opinions.html).

[11] The following age range can be defined arbitrarily: infants ≥ 6 to < 12 months; young children ≥ 1 to < 4 years; children ≥ 4 years to < 7 years; children ≥ 7 years to < 11 years; children ≥ 11 years to < 15 years; children ≥ 15 years to < 18 years; adults ≥ 18 to < 75 years; older adults ≥ 75 years. However, the Panel may use different grouping, depending on the available data and/or on the specific age-related variations in requirement of the nutrient under consideration.

supply from breast-milk, except on a case-by-case basis where this does not apply. Separate reference values are established for pregnant and lactating women, taking into account the additional nutrient requirement for the formation of new tissues, or to compensate for the nutrients lost to the body in the form of human milk, respectively, and considering the physiological adaptations that occur during these conditions.

Interpolation or extrapolation between population groups are used in instances where no data are available for defined age and sex groups (Appendix B). Scaling methods using isometric (linear with body weight) or allometric (body weight to the power of a chosen exponent) or interpolation based on other non-predefined parameters are being used. Which method is the most appropriate is decided on a case-by-case basis.

Reference heights and weights are useful when more specificity about body size and nutrient requirements are needed than that provided by life stage categories.[12]

Dietary reference values can be used for different purposes, such as in diet assessment and diet planning, both at the population and individual level, but also as a basis for reference values in food labelling, and in establishing food based dietary guidelines.

In dietary assessment of groups the Average Requirement can be used to estimate the prevalence of inadequate intakes of micronutrients (the Average Requirement cut-point method), if the distribution of nutrient intakes is normal, and intakes are independent from requirements. The Population Reference Intake should not be used for this purpose as this would result in overestimation of the proportion of the group at risk of inadequacy. Probabilistic methods, taking into account both the intake and requirement variation might be used as an alternative, and in case distributions are skewed.

For macronutrients with a defined reference intake range for individuals, the distribution of usual intake of individuals may be assessed to ascertain what proportion of the group lies outside the reference lower and upper limits of the range. In case of energy, the mean usual intake of energy of a defined group, relative to the average requirement, may be used in assessing the adequacy.

For assessment of adequacy of nutrient intakes in individuals Dietary Reference Values are of limited use. Usual intakes below the AR are likely inadequate, and below the Lower Threshold Intake very probably inadequate, while chronic intakes above the Tolerable Upper Intake Level may be associated with an increased risk of adverse effects. For a valid assessment of the adequacy of an individual's usual intake, combined information with anthropometric, biochemical (status) and clinical data is needed.

In dietary planning for groups the usual intake distribution should be between the AR and UL to avoid inadequate, respectively excessive intakes. For nutrients such as vitamins, minerals, and protein, the PRI can be a practical starting point. However, target median intakes higher than the Population Reference Intake might be considered, especially in case of a skewed intake distribution. For macronutrients the distribution of usual intake of individuals should be such as to minimise the proportion of the group that lies outside the reference lower and upper limits of the range. For energy, the reference intake (estimated average energy requirement) of the group based on sex, age, height, weight, and physical activity level of the group may be used as a planning goal.

The goal of planning diets for individuals is to have a low probability of inadequacy while minimising potential risk of excess for each nutrient. For nutrients such as vitamins, minerals, and protein, this is done by ensuring that the usual intake meets the Population Reference Intake or Adequate Intake while not exceeding the Tolerable Upper Intake Level. Population Reference Intakes would be an overestimation for most individuals. For macronutrients which have a reference intake range, the usual intake of individuals should be between the lower and upper bounds of the reference range. For energy, the reference intake (average energy requirement) based on an individual's sex, age, height, weight, and physical activity level may be used as an initial planning goal; however, body weight must be monitored and intake adjusted as appropriate.

[12] The reference heights and weights used are outlined in the respective opinions. For convenience, the reference weights used for scaling are presented in Appendix B, Table 17.

3. Energy, macronutrients and water

3.1. Energy

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3005/full

The DRVs for food energy provide a best estimate of the food energy needs of population groups within Europe (EFSA NDA Panel, 2013a). They are given as average requirements (ARs) of specified age and sex groups and are of limited use for individuals. The reference values need to be adapted to specific objectives, such as dietary assessment, dietary planning, labelling dietary reference values, or development of food-based dietary guidelines. In addition, there is a need to define and characterise the target population.

In this Opinion, total energy expenditure (TEE) in the steady state of a healthy body mass was chosen as the criterion on which to base the AR for energy. In practice, the adequacy of usual energy intakes is best monitored by measuring body mass. In terms of regulation of body mass, the overall energy balance over a prolonged period of time needs to be considered. TEE expended over 24 hours is the sum of basal energy expenditure, the energy expenditure of physical activity and the thermic effect of food. In this Opinion, resting energy expenditure (REE) was used as a proxy for the slightly lower basal energy expenditure, as most studies measured REE. TEE is best measured with the doubly labelled water (DLW) method, which provides energy expenditure data over biologically meaningful periods of time and under normal living conditions.

One approach to determine the AR for energy is to use of regression equations which describe how TEE measured with the DLW method varies as a function of anthropometric variables (such as body mass and height) for defined population groups and for an activity constant that accounts for the level of physical activity. However, this approach has been criticised because of the inability of such TEE prediction models to account for the variations in energy expenditure of physical activity in a transparent way. In addition, limited TEE data generated with the DLW method are available, and they may not be representative for the European population; moreover, some age groups are underrepresented. Another approach for estimating TEE is by the factorial method in which the energy spent in various activities is added to measured or predicted REE. This is achieved by using the physical activity level (PAL), which is defined as the ratio of TEE to REE per 24 hours and reflects the part of TEE that is due to physical activity. Accordingly, TEE is predicted as PAL x REE. During growth, pregnancy and lactation, additional energy is needed for the synthesis and deposition of new tissues, and for milk production.

In this Opinion, TEE of children and adults was estimated factorially to account for the diversity in body size, body composition and habitual physical activity among children and adult populations with different geographic, cultural and economic backgrounds.

To estimate REE, predictive equations were used, derived from regression analysis of measured REE, body masses and heights from groups of subjects. Body mass is the most important determinant of REE and all predictive equations use this parameter. In addition to body mass, height, sex, age and ethnicity can affect REE significantly and numerous equations have been developed to take into account one or several of these parameters. Based on the accuracy of various equations in specified population groups, five widely used equations (Harris and Benedict, 1919; Schofield et al., 1985; Mifflin et al., 1990; Muller et al., 2004; Henry, 2005) were considered as equally valid for estimating the REE of healthy adults in Europe. For healthy children and adolescents, the equations of Schofield et al. (1985) and Henry (2005) derived from large datasets and covering wide age groups were considered to be the most suitable.

PAL can be estimated either from time-allocated lists of daily activities expressed as physical activity ratio values or from the ratio of TEE (measured by the DLW method) to REE (either measured or estimated). However, the same limitations apply to the derivation of PAL values from DLW data as to the estimates of TEE with this method. Within the general population, PALs associated with sustainable lifestyles have been observed to range between 1.35 and 2.5, and to decrease only marginally with age. When assigning PAL values to descriptions of activities/lifestyles (such as light, moderate or heavy activity), the range of PAL values in each lifestyle category is large. Thus, the allocation of lifestyles to

defined PAL values can only be considered a rough indication of PAL, but may be useful for decisions about which PAL values to apply in various circumstances and applications.

In the absence of arguments for the selection of one predictive equation best fitted to adults in the European Union (EU), REE was calculated with five widely applied predictive equations using individual data of measured body heights of adults obtained in 13 representative national surveys in EU Member States, with corresponding body masses calculated for a body mass index (BMI) of 22 kg/m^2, i.e. the midpoint of the range of healthy BMI of adults as defined by the WHO. This yielded a range of ARs calculated for PAL values from 1.4 to 2.4 in steps of 0.2, and demonstrated the magnitude of uncertainty inherent in these values. However, for practical reasons, only one AR is proposed for a defined age and sex group with a healthy BMI of 22, and for PAL values selected to approximate corresponding lifestyles (Table 1). The predictive equations of Henry (2005) were used to estimate REE because, at present, the underlying database is the most comprehensive as regards number of subjects, their nationalities and age groups. To derive TEE as REE x PAL, PAL values of 1.4, 1.6, 1.8 and 2.0 were chosen to approximately reflect low active (sedentary), moderately active, active and very active lifestyles. Because of a lack of anthropometric data from EU countries for age groups from 80 years onwards, average requirements were not calculated for adults ≥ 80 years.

For infants from birth to six months of age, energy requirements were considered to be equal to the energy supply from human milk, and no DRV is proposed. For infants aged 7–11 months, the ARs were estimated from equations for TEE, adding the energy needs for growth (Table 1). TEE was based on measurements using the DLW method in healthy, full-term infants, exclusively breast-fed for the first four months of life and with adequate body mass. Body masses from the WHO Growth Standards were used to derive ARs for infants growing along the trajectory of this standard (WHO Multicentre Growth Reference Study Group, 2006). Estimates of the energy requirement for growth were based on protein and fat gains reported in the literature.

The ARs of children from one year upwards are based on predicted REE and adjusted PAL for growth (Table 1). REE was calculated using the predictive equations of Henry (2005) and Schofield et al. (1985) and median body masses and heights taken from the WHO Growth Standards (for children up to two years) (WHO Multicentre Growth Reference Study Group, 2006) or from harmonised growth curves of EU children (for children from 3 to 17 years) (van Buuren et al., 2012). For the same reasons as outlined for adults, and because the results obtained with these two equations were very similar, only the predictive equations of Henry (2005) were applied for the estimation of REE values. PAL values of 1.4, 1.6, 1.8 and 2.0 were used for three age groups (1–3 years, > 3– < 10 years, and 10–18 years). Energy expenditure for growth was accounted for by a 1% increase in PAL values for each age group.

For pregnant women, a mean gestational increase in body mass of 12 kg was considered to be associated with optimal maternal and fetal health outcomes. The additional amount of energy required during pregnancy to support this increase in body mass was estimated using the cumulative increment in TEE estimated with the DLW technique plus the energy deposited as protein and fat. Based on these data, the average additional energy requirement for pregnancy is 320 MJ (76,530 kcal) which equates to approximately 0.29 MJ/day (70 kcal/day), 1.1 MJ/day (260 kcal/day) and 2.1 MJ/day (500 kcal/day) during the first, second and third trimesters, respectively (Table 1).

For women exclusively breastfeeding during the first six months after birth, the additional energy requirement during lactation was estimated factorially as 2.1 MJ/day (500 kcal/day) over pre-pregnancy requirements, taking into account a requirement of 2.8 MJ/day (670 kcal/day) for milk production and an energy mobilisation from maternal tissues of 0.72 MJ/day (170 kcal/day) (Table 1). No additional energy requirement is proposed for women lactating beyond the sixth month because volumes of milk produced during this period are highly variable and depend on the infant's energy intake from complementary foods.

3.2. Carbohydrates and dietary fibre

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1462/full

Nutritionally, two broad categories of carbohydrates can be differentiated: "glycaemic carbohydrates", i.e. carbohydrates digested and absorbed in the human small intestine, and "dietary fibre", non-digestible carbohydrates passing to the large intestine (EFSA NDA Panel, 2010c).

The main glycaemic carbohydrates are monosaccharides, disaccharides, malto-oligosaccharides, and starch. In this Opinion the term "sugars" is used to cover monosaccharides and disaccharides. The term "added sugars" refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, highfructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term "sugars". However, they are partly metabolised and included in "carbohydrates" according to the European legislation.

In this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin, including nonstarch polysaccharides (NSP) – cellulose, hemicelluloses, pectins, hydrocolloids (i.e., gums, mucilages, -glucans), resistant oligosaccharides – fructo-oligosaccharides (FOS), galactooligosaccharides (GOS), other resistant oligosaccharides, resistant starch – consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches, and lignin associated with the dietary fibre polysaccharides.

Main dietary sources of sugars are fruits, berries, fruit juices, some vegetables, milk and milk products, and foods containing added sucrose and starch hydrolysates (e.g., glucose syrup, highfructose syrup) such as carbonated beverages and sweets. Main dietary sources of starch are bread and other cereal products, potatoes, tubers and pulses.

Data from dietary surveys show that average carbohydrate intakes in European countries in children and adolescents varied between 43 to 58 E%, and from 38 to 56 E% in adults. Average intakes of sugars varied between 16 to 36 E% in children and adults.

Whole grain cereals, pulses, fruit, vegetables and potatoes are the main sources of dietary fibre. Average dietary fibre intakes varied from 10 to 20 g per day in young children (< 10 to 12 years), from 15 to 30 g per day in adolescents, and from 16 to 29 g per day in adults. Average intakes of dietary fibre per MJ ranged from 1.7 to 2.5 g per MJ in (young) children and from 1.8 to 2.9 g per MJ in adults.

Total and glycemic carbohydrates

As energy balance is the ultimate goal, dietary reference values for carbohydrate intake cannot be made without considering other energy delivering macronutrients and will be given as percentage of total energy intake (E%). The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. Generally, an intake of 50 to 100 g per day will prevent ketosis. An intake of 130 g per day for both children (> 1 year) and adults has been estimated to be sufficient to cover the needs of glucose for the brain. However, these levels of intake are not sufficient to meet energy needs in the context of acceptable intake levels of fat and protein.

Intervention studies provide evidence that high fat (> 35 E%), low carbohydrate (< 50 E%) diets are associated to adverse short- and long-term effects on body weight, although data are not sufficient to define a Lower Threshold of Intake (LTI) for carbohydrates. Similarly, high carbohydrate diets tend to induce adverse effects on the blood lipid profile, but there is an insufficient scientific basis for setting a Tolerable Upper Intake Level (UL) for total carbohydrates. The Panel therefore comes to the conclusion that only a Reference Intake range can be given for total carbohydrate intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns).

Based on the above considerations the Panel proposes 45 to 60 E% as the Reference Intake range for carbohydrates (Table 2). Diets with glycaemic carbohydrate contents of 45 to 60 E%, in combination with reduced intakes of fat and saturated fatty acids (SFA), are compatible with the improvement of metabolic risk factors for chronic disease, as well as with mean carbohydrate intakes observed in some European countries. This intake range applies to both adults and children older than one year of age.

Sugars

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when oral hygiene and fluoride prophylaxis are insufficient. However, available data do not allow the setting of an upper limit for intake of (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by frequency of consumption, oral hygiene, exposure to fluoride, and various other factors.

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for intake of (added) sugars based on their effects on body weight.

Observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to intake of added sugars *per se.* The available data are not sufficient to set an upper limit for (added) sugar intake.

Although there is some evidence that high intakes (> 20 E%) of sugars may increase serum triglyceride (TG) and cholesterol concentrations, and that > 20 to 25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake.

Evidence on the relationship between patterns of consumption of sugar-containing foods and dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines.

The Panel notes that a number of authorities have established upper limits for population average intake or individual intake of added sugars of < 10 E% but others have not. Typically, such recommendations reflect a judgement of what level of sugar intake is practically achievable within the context of a nutritionally adequate diet based on known patterns of intake of foods and nutrients in specific populations. It is also noted that the average intake of (added) sugars in some EU Member States exceeds 10 E%, especially in children.

Dietary Fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g per day to be adequate for normal laxation in adults (Table 2). There is limited evidence to set adequate intakes for children. The Panel considers that the Adequate Intake (AI) for dietary fibre for children should be based on that for adults with appropriate adjustment for energy intake. A fibre intake of 2 g per MJ is considered adequate for normal laxation in children from the age of one year (Table 2).

The Panel notes that in adults there is evidence of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g. reduced risk of coronary heart disease and type 2 diabetes and improved weight maintenance. Such evidence should be considered when developing food-based dietary guidelines.

Glycaemic index and glycaemic load

Although there is some experimental evidence that a reduction of the dietary glycaemic index and glycaemic load may have favourable effects on some metabolic risk factors such as serum lipids, the evidence for a role in weight maintenance and prevention of diet-related diseases is inconclusive.

3.3. Fats

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1461/full

Dietary fats or lipids include triacylglycerols, phosphatidylcholine and cholesterol (EFSA NDA Panel, 2010d). Along with proteins, carbohydrates, and alcohol, fats are a major energy source for the body. Fatty acids are also involved in many other vital processes in the body (e.g. structural components of cell membranes, precursors for bioactive molecules, regulators of enzyme activities, regulation of gene expression).

Fatty acids can be classified according to their number of double bonds. Saturated fatty acids (SFA) have no double bonds, while monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. These double bonds can have either the *cis* or *trans* configuration. Most unsaturated fatty acids in the diet have the *cis* configuration, but *trans* fatty acids (TFA) are also present as either *trans*-MUFA or *trans*-PUFA. *Trans*-PUFA have at least one *trans* double bond and may therefore also have double bonds in the *cis* configuration.

In most countries, separate dietary recommendations exist for total fat intake, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and *trans* fatty acids. For this purpose, polyunsaturated fatty acids are frequently subdivided into n-6 polyunsaturated fatty acids, n-3 polyunsaturated fatty acids, and n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA). This latter class of fatty acids has 20 or more carbon atoms. Except for the n-3 LCPUFA, recommendations are expressed as percentage of total dietary energy (E%) or as grams per day.

Due to its physical properties, cholesterol is also a fat. It does not provide energy, but plays a central role in many metabolic processes. Recommendations are expressed in milligrams per day (mg/day) or in milligrams per megajoule (mg/MJ).

Total fat

Fat is an important dense source of energy and facilitates the absorption of fat-soluble dietary components such as vitamins. Fats and oils are also important sources of essential fatty acids (EFA). High-fat diets may decrease insulin-sensitivity and are positively associated with changes in fasting and postprandial factor VII, which may increase cardiovascular risk. However, a precise dose–response relationship cannot be defined. There is evidence that a moderate fat intake (< 35 E%) is accompanied by a reduced energy intake and therefore moderate weight reduction and/or prevention of weight gain. However, there are not sufficient data to define a Lower Threshold Intake (LTI) or Tolerable Upper Intake Level (UL) for total fat. The Panel concludes that only a Reference Intake range can be established for total fat intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns). At the lowest observed intake of total fat (20 E%) in European countries no overt signs of deficiencies have been observed nor adverse effects on blood lipids. Total fat intakes > 35 E% may be compatible with both good health and normal body weight depending on dietary patterns and the level of physical activity. The Panel proposes to set for adults a lower bound of the Reference Intake range of 20 E% and an upper bound of 35 E% (Table 2).

Fat intake in infants, which is high during the breastfeeding period, can gradually be reduced in the second half of the first year of life from the start of the complementary feeding period up to three years of age: 40 E% in the 6 to 12 month period and 35 to 40 E% in the 2nd and 3rd year of life (Table 2). Fat intakes below 25 E% have been associated with low vitamin levels in some young children.

Saturated fatty acids

SFA are synthesised by the body and are not required in the diet. Therefore, no Population Reference Intake (PRI), Average Requirement (AR), Lower Threshold Intake (LTI), or Adequate Intake (AI) is set.

There is a positive, dose-dependent relationship between the intake of a mixture of saturated fatty acids and blood low density lipoprotein (LDL) cholesterol concentrations, when compared to carbohydrates. There is also evidence from dietary intervention studies that decreasing the intakes of products rich in saturated fatty acids by replacement with products rich in n-6 polyunsaturated fatty acids (without changing total fat intake) decreased the number of cardiovascular events. As the relationship between

saturated fatty acids intake and the increase in LDL cholesterol concentrations is continuous, no threshold of saturated fatty acids intake can be defined below which there is no adverse effect. Thus, also no Tolerable Upper Intake Level can be set.

The Panel concludes that saturated fatty acids intake should be as low as is possible within the context of a nutritionally adequate diet[13] (Table 2). Limiting the intake of saturated fatty acids should be considered when establishing nutrient goals and recommendations.

Cis-monounsaturated fatty acids (*cis*-MUFA)

Cis-monounsaturated fatty acids are synthesised by the body, have no known specific role in preventing or promoting diet-related diseases, and are therefore not indispensable constituents of the diet. The Panel proposes not to set any Dietary Reference Value for *cis*-monounsaturated fatty acids.

Cis-polyunsaturated fatty acids (*cis*-PUFA)

In view of the different metabolic effects of the various dietary *cis*-polyunsaturated fatty acids, the Panel proposes not to formulate a Dietary Reference Value for the intake of total *cis*-polyunsaturated fatty acids. Also, the Panel proposes not to set specific values for the n-3/n-6 ratio as there are insufficient data on clinical and biochemical endpoints in humans to recommend a ratio independent of absolute levels of intake.

n-6 polyunsaturated fatty acids (n-6 PUFA)

Linoleic acid (LA) cannot be synthesised by the body, is required to maintain "metabolic integrity", and is therefore an EFA. However, there are not sufficient scientific data to derive an Average Requirement, a Lower Threshold Intake or a Population Reference Intake.

There is a negative (beneficial), dose-dependent relationship between the intake of linoleic acid and blood LDL cholesterol concentrations, while this relationship is positive for HDL cholesterol concentrations. In addition, linoleic acid (LA) lowers fasting blood triacylglycerol concentrations when compared to carbohydrates. There is also evidence that replacement of saturated fatty acids by n-6 polyunsaturated fatty acids (without changing total fat intake) decreases the number of cardiovascular events in the population. As the relationship between linoleic acid intake and the blood lipid profile is continuous, no threshold value of linoleic acid intake can be identified below which the risk for cardiovascular events increases.

The Panel proposes to set an Adequate Intake for linoleic acid of 4 E%, based on the lowest estimated mean intakes of the various population groups from a number of European countries, where overt LA deficiency symptoms are not present (Table 2).

Arachidonic acid (ARA) is synthesised by the body from linoleic acid and is therefore not an essential fatty acid despite its important role in maintaining "metabolic integrity". The Panel proposes not to set any Dietary Reference Value for arachidonic acid.

Finally, there is at present no consistent evidence that the intake of any of the n-6 polyunsaturated fatty acids has detrimental effects on health (e.g. in promoting diet-related diseases). The Panel proposes not to set a Tolerable Upper Intake Level UL for total or any of the n-6 polyunsaturated fatty acids.

n-3 polyunsaturated fatty acids (n-3 PUFA)

Alpha-linolenic acid (ALA) cannot be synthesised by the body, is required to maintain "metabolic integrity", and is therefore considered to be an EFA. However, there are not sufficient scientific data to derive an Average Requirement, a Lower Threshold Intake or a Population Reference Intake. The Panel proposes to set an Adequate Intake for alpha-linolenic acid of 0.5 E%, based on the lowest estimated mean intakes of the various population groups from a number of European countries, where overt alpha-linolenic acid deficiency symptoms are not present (Table 2). There is no convincing evidence that the

[13] "Nutritionally adequate diets" are the subject of food-based dietary guidelines and mean a dietary pattern which provides all essential nutrients in adequate amounts as well as energy delivering macronutrients in proportions that are known to maintain health

intake of alpha-linolenic acid has detrimental effects on health (e.g. in promoting diet-related diseases). The Panel, therefore, proposes not to set a Tolerable Upper Intake Level for alpha-linolenic acid.

The human body can synthesise eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from alpha-linolenic acid. Intervention studies have demonstrated beneficial effects of preformed n-3 LCPUFA on recognised cardiovascular risk factors, such as a reduction of plasma triacylglycerol concentrations, platelet aggregation, and blood pressure. These effects were observed at intakes 1 g per day, well above levels that were associated with lower cardiovascular disease (CVD) risk in epidemiological studies. With respect to cardiovascular diseases, prospective epidemiological and dietary intervention studies indicate that oily fish consumption or dietary n-3 LCPUFA supplements (equivalent to a range of 250 to 500 mg of eicosapentaenoic acid plus docosahexaenoic acid daily) decrease the risk of mortality from coronary heart disease (CHD) and sudden cardiac death. An intake of 250 mg per day of eicosapentaenoic acid plus docosahexaenoic acid appears to be sufficient for primary prevention in healthy subjects. Therefore, and taking into account that available data are insufficient to derive an Average Requirement, the Panel proposes to set an Adequate Intake of 250 mg for eicosapentaenoic acid plus docosahexaenoic acid for adults based on cardiovascular considerations (Table 2).

To this intake 100 to 200 mg of preformed docosahexaenoic acid should be added during pregnancy and lactation to compensate for oxidative losses of maternal dietary docosahexaenoic acid and accumulation of docosahexaenoic acid in body fat of the fetus/infant (Table 2).

In older infants, docosahexaenoic acid intakes at levels of 50 to 100 mg per day have been found effective for visual function in the complementary feeding period and are considered to be adequate for that period. The Panel proposes an Adequate Intake of 100 mg docosahexaenoic acid for older infants (> 6 months of age) and young children below the age of 24 months (Table 2).

The currently available evidence does not permit to define an age specific quantitative estimate of an adequate dietary intake for eicosapentaenoic acid and docosahexaenoic acid for children aged 2 to 18 years. However, dietary advice for children should be consistent with advice for the adult population (i.e., 1 to 2 fatty fish meals per week or ~250 mg of eicosapentaenoic acid plus docosahexaenoic acid per day).

Trans fatty acids (TFA)

Trans fatty acids are not synthesised by the human body and are not required in the diet. Therefore, no Population Reference Intake, Average Requirement, or Adequate Intake is set.

Consumption of diets containing *trans*-monounsaturated fatty acids, like diets containing mixtures of saturated fatty acids, increases blood total and LDL cholesterol concentrations in a dose-dependent manner, compared with consumption of diets containing *cis*-monounsaturated fatty acids or *cis*-polyunsaturated fatty acids. Consumption of diets containing *trans*-monounsaturated fatty acids also results in reduced blood HDL cholesterol concentrations and increases the total cholesterol to HDL cholesterol ratio. The available evidence indicates that *trans* fatty acids from ruminant sources have adverse effects on blood lipids and lipoproteins similar to those from industrial sources when consumed in equal amounts. Prospective cohort studies show a consistent relationship between higher intakes of *trans* fatty acids and increased risk of coronary heart disease. The available evidence is insufficient to establish whether there is a difference between ruminant and industrial *trans* fatty acids consumed in equivalent amounts on the risk of coronary heart disease.

Dietary *trans* fatty acids are provided by several fats and oils that are also important sources of essential fatty acids and other nutrients. Thus, there is a limit to which the intake of *trans* fatty acids can be lowered without compromising adequacy of intake of essential nutrients. Therefore, the Panel concludes that *trans* fatty acids intake should be as low as is possible within the context of a nutritionally adequate diet (Table 2). Limiting the intake of *trans* fatty acids should be considered when establishing nutrient goals and recommendations.

Conjugated linoleic acids (CLA)

There is no convincing evidence that any of the conjugated linoleic acids isomers in the diet play a role in prevention or promotion of diet-related diseases. The Panel therefore proposes not to set any Dietary Reference Value for conjugated linoleic acids.

Cholesterol

Cholesterol is synthesised by the body and is not required in the diet. Therefore, no Population Reference Intake, Average Requirement, or Adequate Intake is set. Although there is a positive dose-dependent relationship between the intake of dietary cholesterol with blood LDL cholesterol concentrations, the main dietary determinant of blood LDL cholesterol concentrations is saturated fat intake. Furthermore, most dietary cholesterol is obtained from foods which are also significant sources of dietary saturated fatty acids, e.g. dairy and meat products.

Therefore the Panel decided not to propose a reference on cholesterol intake beside its conclusion on the intake of saturated fatty acids.

3.4. Protein

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2557/full

Dietary proteins are the source of nitrogen and indispensable amino acids which the body requires for tissue growth and maintenance (EFSA NDA Panel, 2012). The main pathway of amino acid metabolism is protein synthesis. In this opinion, "protein" is total nitrogen x 6.25 and protein requirements are based on nitrogen content. Protein digestion takes place in the stomach and in the small intestine. In healthy humans, the absorption and transport of amino acids is usually not limited by the availability of digestive enzymes or transport mechanisms, but some protein escapes digestion in the small intestine and is degraded in the colon through bacterial proteolysis and amino acid catabolism. By the time digesta are excreted as faeces, they consist largely of microbial protein. Therefore, when assessing protein digestibility, it is important to distinguish between faecal and ileal digestibility, as well as apparent and true nitrogen and amino acid digestibility.

The concept of protein requirement includes both total nitrogen and indispensable amino acid requirements. The quantity and utilisation of indispensable amino acids is considered to be an indicator of the dietary protein quality, which is usually assessed using the Protein Digestibility-Corrected Amino Acid Score (PD-CAAS). It is important to determine to what extent the nitrogen from dietary protein is retained in the body. Different values for the efficiency of protein utilisation have been observed for maintenance and for tissue deposition/growth; at maintenance, the efficiency of nitrogen utilisation for retention is about 47% in healthy adults in nitrogen balance on mixed diets.

Foods of animal origin with a high protein content are meat, fish, eggs, milk and dairy products. Bread and other grain-based products, leguminous vegetables, and nuts are plant foods high in protein. Most of the animal sources are considered high quality protein having an optimal indispensable amino acid composition for human needs and a high digestibility, whereas the indispensable amino acid content of plant proteins and/or their digestibility is usually lower. In European countries the main contributors to dietary protein intake are meat and meat products, grains and grain-based products, and milk and dairy products.

Data from dietary surveys show that the average protein intakes in European countries vary between 67 to 114 g/d in adult men and 59 to 102 g/d in women, or about 12 to 20% of total energy intake (E%) for both sexes. Few data are available for the mean protein intakes on a body weight basis, which vary from 0.8 to 1.25 g/kg body weight per day for adults.

In order to derive Dietary Reference Values (DRVs) for protein the Panel decided to use the nitrogen balance approach to determine protein requirements. Nitrogen balance is the difference between nitrogen intake and the amount lost in urine, faeces, via the skin and other routes. In healthy adults who are in energy balance the protein requirement (maintenance requirement) is defined as that amount of dietary protein sufficient to achieve zero nitrogen balance. The requirement for dietary

protein is considered to be the amount needed to replace obligatory nitrogen losses, after adjustment for the efficiency of dietary protein utilisation and the quality of the dietary protein. The factorial method is used to calculate protein requirements for physiological conditions such as growth, pregnancy or lactation in which nitrogen is not only needed for maintenance but also for the deposition of protein in newly formed tissue or secretions (milk).

According to a meta-analysis of available nitrogen balance data as a function of nitrogen intake in healthy adults, the best estimate of average requirement for healthy adults was 105 mg N/kg body weight per day (0.66 g high quality protein/kg per day). The 97.5th percentile was estimated as 133 mg N/kg body weight per day (0.83 g high quality protein/kg per day) from the distribution of the logarithm of the requirement, with a coefficient of variation (CV) of about 12%. The Panel considers that the value of 0.66 g/kg body weight per day can be accepted as the Average Requirement (AR) and the value of 0.83 g/kg body weight per day as the Population Reference Intake (PRI) derived for proteins with a PD-CAAS value of 1.0 (Table 3). This value can be applied to usual mixed diets in Europe which are unlikely to be limiting in their content of indispensable amino acids. For older adults, the protein requirement is considered to be equal to that for adults. The lower energy requirement of sedentary elderly people means that the protein to energy ratio of their requirement may be higher than for younger age groups.

For infants, children and adolescents, the Panel accepted the approach of WHO/FAO/UNU (2007) in which estimates of the protein requirements from six months to adulthood were derived factorially as the sum of requirements for maintenance and growth corrected for efficiency of protein utilisation. An average maintenance value of 0.66 g protein/kg body weight per day was applied. Average daily needs for dietary protein for growth were estimated from average daily rates of protein deposition, calculated from studies on whole-body potassium deposition, and from an efficiency of utilisation of dietary protein for growth of 58%. The PRI was estimated based on the average requirement plus 1.96 SD using a combined SD for growth and maintenance (Table 3).

For pregnant women, the Panel accepted the factorial method for deriving protein requirements during pregnancy which was based on the newly deposited protein in the fetus and maternal tissue, and on the maintenance requirement associated with the increased body weight. Because of the paucity of data in pregnant women and because it is unlikely that the efficiency of protein utilisation decreases during pregnancy, the efficiency of protein utilisation was taken to be 47% as in non-pregnant women. Thus, for pregnant women a PRI for protein of 1, 9 and 28 g/d in the first, second and third trimesters, respectively, is proposed in addition to the PRI for non-pregnant women (Table 3).

For lactation, the Panel accepted the factorial method which requires assessing milk volumes produced and the content of both protein nitrogen and non-protein nitrogen, as well as calculating the amount of dietary protein needed for milk protein production. As the efficiency of protein utilisation for milk protein production is unknown, the same efficiency as in the non-lactating adult (47%) was assumed. The PRI was estimated by adding 1.96 SD to give an additional 19 g protein/d during the first six months of lactation (exclusive breastfeeding), and 13 g protein/d after six months (partial breastfeeding) (Table 3).

The Panel also considered several health outcomes that may be associated with protein intake. The available data on the effects of an additional dietary protein intake beyond the PRI on muscle mass and function, on body weight control and obesity (risk) in children and adults, and on insulin sensitivity and glucose homeostasis do not provide evidence that can be considered as a criterion for determining DRVs for protein. Likewise, the available evidence does not permit the conclusion that an additional protein intake might affect bone mineral density and could be used as a criterion for the setting of DRVs for protein.

Data from food consumption surveys show that actual mean protein intakes of adults in Europe are at, or more often above, the PRI of 0.83 g/kg body weight per day. In Europe, adult protein intakes at the upper end (90–97.5th percentile) of the intake distributions have been reported to be between 17 and 27 E%. The available data are not sufficient to establish a Tolerable Upper Intake Level (UL) for protein. In adults an intake of twice the PRI is considered safe.

DRVs have not been derived for indispensable amino acids since amino acids are not provided as individual nutrients but in the form of protein. In addition, the Panel notes that more data are needed to obtain sufficiently precise values for indispensable amino acid requirement.

3.5. Water

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1459/full

Water is consumed from different sources, which include drinking water (tap and bottled water), beverages, moisture content of foods, and water produced by oxidative processes in the body (EFSA NDA Panel, 2010b) . Water intake from beverages and foods is defined as total water intake, while the sum of total water intake and oxidation water constitutes total available water.

Water is essential for practically all functions of the body and is particularly important for thermoregulation.

A water intake which balances losses and thereby assures adequate hydration of body tissues is essential for health and life.

The water content of the body and the distribution of body water over the intracellular and extracellular compartments of the body changes with age, but is under tight homeostatic control for an individual in a given stage of life.

Loss of body weight, denoting loss of body water, of about 1% is normally compensated within 24 hours. Without compensation and further increases of losses of body water, reductions in physical and cognitive performance, in thermoregulation and cardiovascular function occur. A loss of 10% or more of body water can be fatal.

Water intoxication with life-threatening hypo-osmolarity is rare but can occur in rapid rehydration, with near-drowning in fresh water and in overconsumption of water, which exceeds the kidney's maximal excretion rate of 0.7–1.0 L/hour.

Water requirement varies between individuals and according to environmental conditions. Therefore, only adequate intakes have been defined for specific age groups from a combination of observed intakes in population groups with desirable osmolarity values of urine and desirable water volumes per energy unit consumed.

The Panel has decided that the reference values for total water intake should include water from drinking water, beverages of all kind, and from food moisture.

The Panel concludes that on the basis of available data, adequate intakes can be defined for infants in the first half of the first year of life based on water intake from human milk in exclusively breast-fed infants (100–190 mL/kg per day).

For older infants adequate intakes can be derived from observed intakes of human milk and typical patterns of complementary food and beverages. The Panel concludes that a total water intake of 800 to 1,000 mL/day is adequate for the age period 6 to 12 months. For the second year of life an adequate total water intake of 1,100 to 1,200 mL/day is defined by interpolation, as intake data are not available.

The Panel concludes that adequate intakes of water for children can be derived from observed intakes, corrected for a desirable water-energy relationship and corrected for inter-individual variation, particularly from those studies in which the water contribution by food has been or can be assessed (see section 3): 1,300 mL/day for boys and girls 2 to 3 years of age; 1,600 mL/day for boys and girls 4 to 8 years of age; 2,100 mL/day for boys 9 to 13 years of age; 1,900 mL for girls 9 to 13 years of age (Table 2). Adolescents of 14 years and older are considered as adults with respect to adequate water intake and the adult values apply (Table 2).

The Panel concludes that available data for adults permit the definition of adequate intakes and that these adequate intakes should be based both on observed intakes and on considerations of achievable or desirable urine osmolarity. Adequate total water intakes for females would have to be 2.0 L/day (95th percentile 3.1 L) and for males 2.5 L/day (95th percentile 4.0 L) (Table 2). The Panel defines the same adequate intakes for the elderly as for adults. Despite a lower energy requirement, the water requirement in the elderly per unit of dietary energy becomes higher because of a decrease in renal concentrating capacity.

The Panel did not find data on habitual water intake in pregnant women and proposes the same water intake as in non-pregnant women plus an increase in proportion to the increase in energy intake (300 mL/day) (Table 2).

The Panel recommends adequate water intakes for lactating women of about 700 mL/day above the adequate intakes of non-lactating women of the same age (Table 2).

These adequate intakes apply only to conditions of moderate environmental temperature and moderate physical activity levels (PAL = 1.6). Water losses incurred under extreme conditions of external temperature and physical exercise, which can be up to about 8,000 mL/day have to be replaced with appropriate amounts. In such instances concomitant losses of electrolytes have to be replaced adequately to avoid hypo-osmolar disturbances.

Too high intakes of water which cannot be compensated by the excretion of very dilute urine (maximum urine volumes of about one litre/hour in adults) can lead to hyponatraemic, hypo-osmolar water intoxication with cerebral oedema. No maximum daily amount of water that can be tolerated by a population group can be defined, without taking into account individual and environmental factors.

Table 1: ARs for energy

Age[b]	AR for Energy (MJ[a]/d)									
			at PAL=1.4[c]		at PAL=1.6[c]		at PAL=1.8[c]		at PAL=2.0[c]	
	M	F	M	F	M	F	M	F	M	F
6 mo	2.5	2.3								
7 mo	2.7	2.4								
8 mo	2.8	2.5								
9 mo	2.9	2.6								
10 mo	3.0	2.7								
11 mo	3.1	2.8								
1 y			3.3	3.0						
2 y			4.3	4.0						
3 y			4.9	4.6						
4 y			5.3	4.9	6.0	5.6	6.8	6.3		
5 y			5.6	5.2	6.4	5.9	7.2	6.7		
6 y			5.9	5.5	6.7	6.3	7.6	7.1		
7 y			6.3	5.8	7.2	6.7	8.1	7.5		
8 y			6.7	6.2	7.6	7.1	8.6	7.9		
9 y			7.0	6.6	8.1	7.5	9.1	8.4		
10 y					8.1	7.6	9.1	8.6	10.1	9.5
11 y					8.5	8.0	9.6	9.0	10.7	10.0
12 y					9.1	8.4	10.2	9.4	11.4	10.5
13 y					9.8	8.8	11.0	9.9	12.2	11.0
14 y					10.5	9.1	11.8	10.2	13.1	11.4
15 y					11.3	9.3	12.7	10.5	14.1	11.7
16 y					11.9	9.5	13.4	10.6	14.9	11.8
17 y					12.3	9.5	13.8	10.7	15.4	11.9
18–29 y			9.8	7.9	11.2	9.0	12.6	10.1	14.0	11.2
30–39 y			9.5	7.6	10.8	8.7	12.2	9.8	13.5	10.8
40–49 y			9.3	7.5	10.7	8.6	12.0	9.7	13.4	10.7
50–59 y			9.2	7.5	10.5	8.5	11.9	9.6	13.2	10.7
60–69 y			8.4	6.8	9.6	7.8	10.9	8.8	12.1	9.7
70–79 y			8.3	6.8	9.5	7.7	10.7	8.7	11.9	9.6
Pregnancy										
1st trimester	+ 0.29[d]									
2nd trimester	+ 1.1[d]									
3rd trimester	+ 2.1[d]									
Lactation										
0-6 mo *post partum*	+ 2.1[d]									

d, day; F, female; M, male; mo, months; PAL, physical activity level; y, years

(a): 1 MJ = 238.83 kcal

(b): for adults, ARs for energy are calculated by multiplying estimates of resting energy expenditure (REE), predicted from anthropometric measures (Henry, 2005), with PAL values. ARs for energy were not calculated for adults ≥ 80 years because of a lack of anthropometric data from EU countries for this age group.

(c): PAL values of 1.4, 1.6, 1.8 and 2.0 reflect low active (sedentary), moderately active, active and very active lifestyles (EFSA NDA Panel, 2013).

(d): in addition to the AR for energy of non-pregnant, non-lactating women

Table 2: RIs for total fat and carbohydrates and AIs for fatty acids, dietary fibre and water

Age group (years)	Total carbohydrates (E%)[a]	Dietary fibre (g/d)[b]	Total fat (E%)[a]	SFA	LA (E%)[b]	ALA (E%)[b]	EPA+DHA (mg/d)[b]	DHA (mg/d)[b]	TFA	Age group (years)	Water (L/d)[b],[c]	
											M	F
7–11[d]			40[b]	ALAP	4	0.5		100	ALAP	6–12 mo	0.8–1.0	
1	45–60	10	35–40	ALAP	4	0.5		100	ALAP	1	1.1–1.2	
2–3	45–60	10	35–40	ALAP	4	0.5	250		ALAP	2–3	1.3	
4–6	45–60	14	20–35	ALAP	4	0.5	250		ALAP	4–8	1.6	
7–10	45–60	16	20–35	ALAP	4	0.5	250		ALAP	9–13	2.1	1.9
11–14	45–60	19	20–35	ALAP	4	0.5	250		ALAP	14–17	2.5	2.0
15–17	45–60	21	20–35	ALAP	4	0.5	250		ALAP			
≥ 18	45–60	25	20–35	ALAP	4	0.5	250		ALAP	≥ 18	2.5	2.0
Pregnancy												
			20–35	ALAP	4	0.5	250	+100–200[e]	ALAP			2.3
Lactation												
			20–35	ALAP	4	0.5	250	+100–200[e]	ALAP			2.7

ALA; α-linolenic acid; ALAP, as low as possible; d, day; DHA, docosahexaenoic acid; E% percentage of energy intake; EPA, eicosapentaenoic acid; F, female; L, liter; LA, linoleic acid; M, male; mo, months, SFA, saturated fatty acids; TFA, trans-fatty acids

(a): RI, reference intake range
(b): AI, adequate intake
(c): includes water from beverages of all kind, including drinking and mineral water, and from food moisture
(d): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(e): in addition to combined intakes of EPA and DHA of 250 mg/day

Table 3: ARs and PRIs for protein

Age	AR for Protein (g/kg bw[a] per day)		PRI for Protein (g/kg bw[a] per day)	
	M	F	M	F
0.5 y	1.12		1.31	
1 y	0.95		1.14	
1.5 y	0.85		1.03	
2 y	0.79		0.97	
3 y	0.73		0.90	
4 y	0.69		0.86	
5 y	0.69		0.85	
6 y	0.72		0.89	
7 y	0.74		0.91	
8 y	0.75		0.92	
9 y	0.75		0.92	
10 y	0.75		0.91	
11 y	0.75	0.73	0.91	0.90
12 y	0.74	0.72	0.90	0.89
13 y	0.73	0.71	0.90	0.88
14 y	0.72	0.70	0.89	0.87
15 y	0.72	0.69	0.88	0.85
16 y	0.71	0.68	0.87	0.84
17 y	0.70	0.67	0.86	0.83
18–59 y	0.66		0.83	
≥ 60 y	0.66		0.83	
Pregnancy				
1st trimester		+0.52 g/d[b]		+1 g/d[c]
2nd trimester		+7.2 g/d[b]		+9 g/d[c]
3rd trimester		+23 g/d[b]		+28 g/d[c]
Lactation				
0–6 mo *post partum*		+ 15 g/d[b]		+19 g/d[c]
>6 mo *post partum*		+ 10 g/d[b]		+13 g/d[c]

bw, body weight; d, day; F, female; M, male; y, years

(a): to be multiplied by reference body weights to calculate values in g/day
(b): in addition to the AR for protein of non-pregnant, non-lactating women
(c): in addition to the PRI for protein of non-pregnant, non-lactating women

4. Minerals

4.1. Calcium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4101/full

Calcium is an integral component of the skeleton; approximately 99% of total body calcium is found in bones and teeth as calcium hydroxyapatite, where it has a structural role (EFSA NDA Panel, 2015g). The remaining 1% of calcium found in the body acts as an essential intracellular messenger in cells and tissues.

Intestinal calcium absorption occurs through both an active, saturable, transcellular process and a non-saturable, passive process. Active transport is controlled by $1,25(OH)_2D$ and passive transport is paracellular. Calcium absorption varies considerably throughout the lifespan, being higher during periods of rapid growth and lower in old age. Calcium absorption is affected by vitamin D status; it has been shown to be low in patients with vitamin D deficiency, but there is uncertainty about the serum concentration of 25(OH)D that is required for optimal calcium absorption. Unabsorbed dietary calcium is lost in the faeces. The main routes of obligatory (endogenous) calcium loss are urine, faeces, and skin and sweat (dermal losses).

If the dietary supply of calcium is insufficient to meet physiological requirements, calcium is resorbed from the skeleton to maintain blood concentrations within the range required for normal cellular and tissue functions. This causes a reduction in bone mass, which leads to osteopenia and osteoporosis, and an associated increased risk of fracture.

Hypercalcaemia, defined by serum calcium concentrations > 2.75 mmol/L (11 mg/dL), is unlikely to occur with high intake of calcium from the diet alone but can be caused by high-dose calcium supplements, especially when accompanied by vitamin D supplements, as these can increase calcium absorption.

The main dietary sources of calcium in European countries differ, although dairy products are generally the most important food group. Rich food sources of calcium include dairy products, dark green vegetables, legumes, nuts, fish with soft bones (e.g. canned sardines) and calcium-fortified foods. Hard water also makes a significant contribution to calcium intake.

Evidence from human studies on the relationship between calcium intake and various health outcomes was reviewed and found to be inconsistent. It was not possible to use measures of bone health for deriving calcium requirements. A variety of endpoints are used to assess the effect of calcium intake on bone health, depending on the population group of interest, including skeletal growth, bone mineral density and fracture rates. However, as genotype, weight-bearing exercise and vitamin D status are important determinants of bone health, they may act as confounders in calcium dose–response studies. The Panel concluded that measures of bone health (skeletal growth, bone mineral density and fractures) could not be used to derive DRVs for calcium. Similarly, evidence related to cardiovascular outcomes and cancer was not helpful for deriving DRVs for calcium.

Calcium balance data collected from a number of carefully controlled metabolic studies undertaken in North American adults aged 25 years and over were analysed to determine the value at which calcium intake equals calcium losses via urine and faeces. The mean value at which calcium intake equals excretion is 715 mg/day. An allowance for dermal losses of calcium, which were not included in the balance data, of 40 mg/day was added to derive an AR of 750 mg/day (Table 4, Table 6). The upper bound of the 95% prediction interval at the estimated population mean at null balance (which represents the 97.5th percentile of the distribution of the individual predictions for each level of calcium intake) was 904 mg/day, and when dermal losses are added this gives a PRI of 950 mg/day (Table 5, Table 7).

In infants aged 7–11 months, an AI was derived by estimating the average amount of calcium absorbed by exclusively breast-fed infants (120 mg/day) and extrapolating upwards using isometric scaling. Assuming an absorption of 60%, the AI is 280 mg/day.

In children aged 1–17 years, a factorial approach was employed where the quantity of dietary calcium that is sufficient for calcium accretion in bone and for replacement of obligatory body losses in 50% of the population was the criterion upon which the AR is based. ARs for children aged 1–3, 4–10 and 11–17 years are 390, 680 and 960 mg/day, respectively (Table 4, Table 6). Assuming a coefficient of variation (CV) of 10%, the PRIs for children aged 1–3, 4–10 and 11–17 years are 450, 800 and 1,150 mg/day, respectively (Table 5, Table 7).

The AR for young adults (18–24 years), who still accumulate calcium in bones, is 860 mg/day (Table 4, Table 6). This is the intermediate value between children aged 11–17 years and adults. Assuming a CV of 10%, the PRI is 1,000 mg/day (Table 5, Table 7).

Taking into consideration adaptive changes in calcium metabolism that occur during pregnancy and lactation, the PRI for non-pregnant women also applies to pregnant and lactating women of the same age groups (Table 6, Table 7).

4.2. Chloride

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5779/full

Chloride (Cl^-) is the predominant anion in intracellular fluid and one of the most important extracellular anions (EFSA NDA Panel et al., 2019b). It contributes to many body functions including the maintenance of osmotic and acid–base balance, muscular and nervous activity, and the movement of water and solutes between fluid compartments.

Dietary chloride deficiency is rare. Sodium chloride added during industrial food processing, discretionary use or food preservation is the major source of dietary chloride in Western diets. Other sources of chloride include inherently food-borne sources, and chloride-containing food additives, in which chloride may be associated with cations other than sodium.

In healthy people, chloride is efficiently absorbed in the gut. Following absorption, chloride anions are freely transported in the blood, where their concentration is maintained within a narrow range. Renal excretion of chloride is coupled to that of sodium and potassium. The overall regulation of chloride balance is linked to that of sodium through hormonal control by the renin–angiotensin–aldosterone system and cortisol. The close interrelationship between sodium and chloride physiology and intakes are reflected by high correlations between sodium and chloride urinary excretion. Studies which quantified 24-h urinary excretion of sodium and chloride in subjects from Western populations indicate that, on a molar basis, both electrolytes are excreted in similar amounts.

As for sodium, the amount of chloride excreted in the urine of an individual varies widely within the day and between days. In a long-term controlled feeding trial, a daily variation in chloride excretion with a seven-day rhythm was observed, which indicates that the day-to-day variation in chloride excretion is partly independent of chloride intake.

Because of its tight homeostatic regulation, serum chloride concentration is not a sensitive marker of chloride intake or status. Values outside the reference range are typically related to disorders affecting water and electrolyte balances. Overall, there are no appropriate biomarkers for chloride status that can be used for setting DRVs for chloride.

A few studies have measured chloride intake and losses and related chloride 'balance' in various experimental settings. These studies have important limitations. No balance studies can be used to set DRVs for chloride. There is evidence that chloride can contribute to the effect of sodium chloride on blood pressure.

Data from studies on hypertensive rats, and some clinical observations, suggest that the full expression of sodium chloride-dependent elevation in blood pressure relies on the concomitant presence of both sodium and chloride. An independent effect of chloride on cardiovascular risk has also been explored in

observational studies using serum/plasma chloride concentration. However, serum/plasma chloride concentration cannot be used as a marker of chloride intake. No studies are available which investigate the association between chloride intake or urinary excretion and cardiovascular disease-related health outcomes.

There are no data that can be used to determine Average Requirements and population reference intakes for chloride. Hence, the Panel considered that reference values for chloride can be set at the value equimolar to the reference values for sodium for all population groups, and are as follows: 1.7 g/ day for children aged 1–3 years, 2.0 g/day for children aged 4–6 years, 2.6 g/day for children aged 7–10 years, 3.1 g/day for children aged 11–17 years and 3.1 g/day for adults including pregnant and lactating women (Table 8). Consistent with the reference values for sodium, these levels of chloride intake are considered to be safe and adequate for the general EU population, under the consideration that the main dietary source of chloride intake is sodium chloride. For infants aged 7–11 months, an adequate intake of 0.3 g/day is set (Table 8).

4.3. Chromium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3845/full

In 1993, the Scientific Committee for Food was unable to define a specific physiological requirement of chromium and did not propose DRVs for chromium, but other authorities have subsequently proposed DRVs for chromium (EFSA NDA Panel, 2014d).

Trivalent chromium (Cr(III)) has been reported as an essential trace element in that it has been postulated to be necessary for the efficacy of insulin in regulating the metabolism of carbohydrates, lipids and proteins. However, at present, the mechanism(s) for these roles and the essential function of chromium in metabolism have not been substantiated. The postulation of chromium's essentiality for humans was almost entirely based on case reports of patients on long-term total parenteral nutrition (TPN) who developed metabolic and neurological defects, which were reported to respond to supplementation with Cr(III). The Panel noted that the chromium concentrations in the TPN solutions that induced the presumed deficiency symptoms were not reported in all the patients studied. In the three studies in which the concentration of chromium in the TPN solution was reported, the daily chromium supply was between 5 and 10 µg; at an absorption efficiency of 5% this amount of infused chromium is equivalent to an oral intake of 100–200 µg/day. The Panel notes that this intake is well above the estimated mean daily intakes in the 17 European countries for which data were available to perform an assessment of chronic dietary chromium intake. On the basis of these case reports, the Panel concludes that it is unclear whether deficiency of chromium has occurred in these patients and whether chromium deficiency occurs in healthy populations.

The Panel considered the criteria for the essentiality of a trace element and noted that attempts to create chromium deficiency in animal models have not produced consistent results, that there is no evidence of essentiality of Cr(III) as a trace element in animal nutrition and that Cr(III) requirements could not be established for animal feed. The Panel considered that there is a possibility that Cr(III) is an essential trace element for humans, but that there is, as yet, no convincing evidence of this. The evidence from reported improvements associated with chromium supplementation in patients on TPN is arguably the most convincing, but overall these data do not provide sufficient information on the reversibility of the possible deficiencies and on the nature of any dose–response curve in order to identify a dietary requirement for humans. The existence and functional characterisation of a chromium–oligopeptide complex (chromodulin) is still unclear.

The Panel concludes that no Average Requirement and no Population Reference Intake for chromium for the performance of physiological functions can be defined.

Nevertheless, as for fluoride, DRVs might be derived if a consistent dose–response relationship could be established between dietary chromium intake and a beneficial health outcome. A comprehensive search of the literature published between January 1990 and October 2011 was performed to identify relevant health

outcomes upon which DRVs for chromium may potentially be based. Several studies that assessed the effect of chromium supplementation on glucose and/or lipid metabolism were retrieved in the literature search. In most studies, chromium intake from the diet was not assessed, and information on total chromium intake is therefore not available. In one cross-over study for which total chromium intake was available, there was no significant difference in the parameters of glucose metabolism between the placebo and chromium-supplemented periods in normoglycaemic subjects. The Panel considered that there is no evidence of beneficial effects associated with chromium intake in healthy subjects. The Panel concludes that the setting of an Adequate Intake for chromium is also not appropriate.

4.4. Copper

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4253/full

Copper is an essential micronutrient required for electron transfer processes (EFSA NDA Panel, 2015h). It is a central component of many enzymes, including those involved in neurotransmitter synthesis, in energy metabolism and in collagen and elastin cross-linking.

The main food group contributing to the copper intake of all population groups except infants is grains and grain-based products. Another important contributor to copper intake is the food group meat and meat products.

Based on balance studies and other studies, the Panel considered that copper absorption from the diet is around 50% for all age and life-stage groups.

The primary site of copper absorption is the upper small intestine. Uptake is through a carrier protein, Ctr1, and once in the cell, the copper is directed towards its target via one of a series of chaperone proteins that ensure the metal is present in a non-toxic form. In the gut, the major pathway of transport into the portal circulation is via a Cu-ATPase, ATP7A. In the portal circulation, copper is bound to histidine, albumin or possibly transcuprein and transported to the liver, where it is incorporated into ceruloplasmin, which is then secreted into the systemic circulation. It is taken up into the liver through Ctr1 and, if it is not incorporated into ceruloplasmin, it is stored as metallothionein. Excess copper is excreted in bile after transport across the apical membrane of the hepatocytes via another ATPase, ATP7B. This copper is not reabsorbed. In humans, between 80 and 95% of the copper in plasma is ceruloplasmin, with the remainder being a low-molecular weight form. It is not certain which of these two pools, ceruloplasmin or low-molecular weight copper complexes, makes the major contribution to uptake by organs other than the liver, although it is more likely to be low-molecular weight copper complexes than ceruloplasmin, with the latter playing a major role in the release of iron from the liver.

If the dietary supply of copper is less than adequate, the body upregulates transfer systems to make more copper available. If these are not able to rectify the problem, the result is copper deficiency. Clinical symptoms are not common in humans, and generally are seen as a consequence of mutations in the genes involved in copper metabolism. Symptoms of copper deficiency include anaemia that is refractory to iron supplementation, neurological defects and cutis laxa ("floppy" skin). There are also changes in hair colour and texture, and an increased risk of aneurysm as a consequence of impaired collagen and elastin synthesis.

The Panel noted that there are no biomarkers of copper status that are sufficiently robust, sensitive and specific to be used for deriving requirements for copper. The Panel also considered whether health outcomes can be used to derive DRVs for copper. However, it was concluded that the limited evidence available on copper intake and cardiovascular disease-related outcomes and cancer cannot be used for setting DRVs for copper.

There have been several balance studies examining the relationship between copper intake and losses in men, but few in women and children. Studies differed with regard to experimental conditions, and many studies had limitations and their results varied. Nevertheless, the Panel considered that they may be used,

for men at least, in conjunction with data on observed intakes in the European Union (EU) to inform the setting of DRVs for copper.

The Panel decided to derive Adequate Intakes (AIs) based on observed intakes in several EU countries. Mean copper intakes in eight EU countries range from 1.27 to 1.67 mg/day in men aged 18 years and older and from 1.15 to 1.44 mg/day in non-pregnant women aged 18 years and older. The Panel noted that midpoints of ranges for intake estimates in three age groups of adults and in both sexes are in good agreement with medians, for the corresponding sex and age groups, of the average intakes estimated per survey. The Panel noted that there is, at present, insufficient evidence to set different DRVs according to age in adults, but decided to set different AI values for women and men, as intakes are lower for women. For men, based on observed intakes and taking into account that zero copper balance was reported at a copper intake of approximately 1.6 mg/day in men, the Panel proposed an AI of 1.6 mg/day (Table 5). For women, based on observed intakes, the Panel proposed an AI of 1.3 mg/day (Table 7).

For infants aged 7–11 months, based on results from four surveys in infants, the Panel proposed an AI of 0.4 mg/day. The Panel noted that upwards extrapolation by allometric scaling of estimated copper intake in exclusively breast-fed infants aged 0–6 months results in an estimated intake at 7–11 months of 0.36 mg/day, which supports the AI of 0.4 mg/day (Table 5, Table 7).

For boys and girls aged 1 to < 3 years, considering the absence of a strong basis for a distinct value according to sex and the distribution of observed mean intakes of 0.60–0.86 mg/day in boys and 0.57–0.94 mg/day in girls, the Panel selected the midpoint of average intakes and set an AI of 0.7 mg/day (Table 5, Table 7).

In children aged 3 to < 10 years, mean observed intakes range from 0.92 to 1.44 mg/day in boys and from 0.82 to 1.30 mg/day in girls. The Panel considered the distribution of the observed mean intakes and set an AI of 1.0 mg/day for boys and girls aged 3 to < 10 years (Table 5, Table 7). In children aged 10 to < 18 years, mean observed intakes range from 1.16 to 1.59 mg/day in boys and from 0.98 to 1.41 mg/day in girls. Considering the rather large differences in intakes of boys and girls, the Panel decided to set separate AI values. Taking into account the distribution of observed average intakes, the Panel proposed an AI of 1.3 mg/day for boys (Table 5) and of 1.1 mg/day for girls aged 10 to < 18 years (Table 7).

In pregnancy, taking into account the requirement for the developing fetus and its placenta, the additional requirement for copper was calculated to be 0.06 mg/day. Considering that about 50% of ingested copper is absorbed, and in anticipation of copper requirements for lactation, the Panel proposed that the AI of non-pregnant women be increased by 0.2 mg/day during pregnancy (Table 7).

For lactation, taking into account that copper absorption is about 50%, an increment of 0.56 mg/day would be required to compensate for copper losses in breast milk. The Panel assumed that this can be mitigated in part by the increased AI in pregnancy. Thus, the Panel proposed that the AI of non-pregnant women be increased by 0.2 mg/day during lactation (Table 7).

4.5. Fluoride

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3332/full

Fluoride has no known essential function in human growth and development and no signs of fluoride deficiency have been identified (EFSA NDA Panel, 2013e). Though fluoride is not essential for tooth development, exposure to fluoride leads to incorporation into the hydroxyapatite of the developing tooth enamel and dentin. The resulting fluorohydroxyapatite is more resistant to acids than hydroxyapatite. Thus, teeth which contain fluoroapatite are less likely to develop caries. Apart from incorporation of fluoride into the dentin and enamel of teeth before eruption, dietary fluoride exerts an anticaries effect on erupted teeth through contact with enamel during consumption, excretion into saliva and uptake into biofilms on teeth. In addition, fluoride interferes with the metabolism of oral microbial cells, by directly inhibiting, for example,

glycolytic enzymes and cell membrane-associated H+ ATPases in microbial cells after entry of hydrofluoric acid into their cytoplasm.

In bone, the partial substitution of fluoride for hydroxyl groups of apatite alters the mineral structure of the bone. Depending on the dose, fluoride can delay mineralisation. There is evidence from animal studies for a biphasic effect of fluoride on bone strength, with increases in both bone strength and bone fluoride content at moderately high fluoride intake, and a decrease with higher fluoride intake.

Major dietary fluoride sources are water and water-based beverages or foods reconstituted with fluoridated water, tea, marine fish, and fluoridated salt. Fluoride absorption occurs by passive diffusion in both the stomach (20–25%) and the small intestine. On average 80–90% of ingested fluoride is absorbed. In adults, up to 50% of absorbed fluoride is associated with calcified tissues, mainly bone, a small amount reaches soft tissues, and the remainder is excreted, predominantly via the kidney and to a small extent via sweat and faeces.

The role of fluoride in the prevention of caries has been known for many years. In epidemiological studies performed before the 1970s, when fluoride in drinking water was practically the only relevant source of fluoride intake, it was shown that the prevalence of caries was negatively correlated with the fluoride concentration of water. The fluoride concentration at which the caries preventive effect approached its maximum was 1 mg/L, and at that level only 10% of the population was affected by mild dental fluorosis. The average daily fluoride intake of a child in a community with this "optimal" drinking water fluoride concentration of 1 mg/L was determined as being approximately 0.05 mg fluoride/kg body weight per day from both water and diet.

Since then, many studies have reviewed the efficacy of fluoride in different forms (water, milk, salt, tablets/drops, chewing gum) in preventing dental caries. However, very few of these studies provide information on total dietary fluoride intake, and the outcome measure for caries may have been affected by additional uses of non-dietary fluoride. Therefore, they do not permit a conclusion to be drawn on a dose–response relationship between dietary fluoride intake and caries risk.

The available data on the relationship between fluoride intake or intake deduced from the fluoride content of toenails and bone health did not provide evidence for a beneficial effect of fluoride on bone health.

As fluoride is not an essential nutrient, no Average Requirement for the performance of essential physiological functions can be defined. Because of the beneficial effect of dietary fluoride on the prevention of caries, the Panel considered that the setting of an Adequate Intake (AI) is appropriate and that data on the dose-response relationship between caries incidence and consumption of drinking water with different fluoride concentrations are sufficient to set an AI of 0.05 mg/kg body weight per day (Table 5, Table 7). The AI covers fluoride intake from all sources, including non-dietary sources such as toothpaste and other dental hygiene products.

No data are available to define a dose–response relationship between fluoride intake and caries for adults. The Panel considered that the AI for children of 0.05 mg/kg body weight per day can also be applied to adults, including pregnant and lactating women (Table 5, Table 7). For pregnant and lactating women the AI is based on the body weight before pregnancy and lactation (Table 7).

Reliable and representative data on the total fluoride intake of the European population are not available. The available data on fluoride intake are variable but generally at or below 0.05 mg/kg body weight per day.

4.6. Iodine

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3660/full

Iodine is an essential nutrient for mammals, required as a mandatory structural and functional element of thyroid hormones (EFSA NDA Panel, 2014f). Through these hormones, iodine has an important role in energy-yielding metabolism and on the expression of genes that impact many physiological functions, including embryogenesis and growth, and the development of neurological and cognitive functions.

The clinical effects of iodine deficiency, referred to as iodine deficiency disorders, are the result of insufficient intakes leading to insufficient thyroid function. Iodine deficiency disorders are seen at all stages of development and are particularly of concern in pregnancy and infancy. Chronic iodine deficiency may lead to compensatory thyroid hypertrophy with an enlargement of the thyroid gland denoted as goitre.

Intestinal absorption efficiency of ingested iodine is considered to be high (> 90%). The thyroid is the major storage site for iodine in the body. Goitrogenous substances in foods, drinking water or cigarette smoke may inhibit the thyroidal uptake of iodide or its incorporation into the tyrosine precursors of thyroid hormones. The synthesis of normal quantities of thyroid hormones requires an adequate dietary intake of iodine. The kidney is the main route of excretion of iodine. In a steady state, the urinary iodine (UI) excretion represents more than 90% of the dietary intake and is therefore a good indicator of recent iodine intakes. UI concentration has also been used to define the iodine status of a population. Concentrations of thyroglobulin, thyroid hormone T4 (thyroxine or 3,5,3,5-tetraiodothyronine) and thyroid-stimulating hormone in serum are also considered useful biomarkers of iodine status depending on age and population group and their iodine status. Thyroid volume and goitre prevalence are useful long-term clinical indicators of iodine status.

Iodine occurs in food and water mainly as iodide. The iodine concentration of water and foods is highly variable. The richest iodine sources are marine products, eggs, milk, and food products derived from them, and iodised salt. Dietary assessment methods do not accurately quantify habitual iodine intakes.

Studies of iodine balance have provided highly variable results, with null balances observed at very different levels of intakes. In addition, balance studies performed in countries with a higher habitual iodine intake compared with most European countries are difficult to extrapolate to the European context. The same is true for results on iodine accumulation by the thyroid. Insufficient evidence is available to determine iodine requirements by a factorial approach taking into account iodine needs for hormone production and iodine storage in the thyroid as well as basal iodine losses in urine, faeces and sweat.

Health outcomes such as cognitive function in children, cancer and sub-clinical thyroid dysfunction in older adults were also considered but were found not to be suitable for deriving DRVs for iodine.

For the setting of DRVs for iodine it was considered that the prevalence of thyroid volume enlargement in a population can be used to define a threshold of UI excretion and UI concentration above which the prevalence of abnormal increases of thyroid volume is minimised. This threshold is based on observational studies of goitre prevalence in Central America in the 1960s and in European school-aged children. In the latter study, a prevalence of goitre below 5% was almost systematically observed in all study areas when the UI concentration was above 100 µg/L. Even though this threshold has been established in school-aged children, the Panel also accepted it for adults. A UI concentration of 100 µg/L corresponds to an approximate iodine intake of 150 µg/day in adults. It was concluded that an Adequate Intake (AI) for iodine for adult men and women can be set at 150 µg/day (Table 5, Table 7). Accepting the threshold for UI concentration of 100 µg/L also for infants aged 7–11 months and for children and taking into account age-specific urinary volumes and body weights, AIs of 70 µg/day to 130 µg/day were derived (Table 5, Table 7).

For pregnant women, T4 production is estimated to be increased by a mean of 37 µg/day, corresponding to an additional iodine demand of 25 µg/day for hormone synthesis in the thyroid. The additional requirements due to the development of the fetus, placenta and amniotic fluid were considered very low

when related to the whole pregnancy (equivalent to a net transfer of 1 µg iodine/day). Adding to this requirement the iodine needed for fetal synthesis of thyroid hormones, the total additional iodine requirement is rounded to 50 µg/day. Provided that thyroid status and iodine stores before pregnancy are adequate, an AI of 200 µg/day is proposed for pregnant women (Table 7). The Panel considered that the information available on the relationship between iodine intake or status of pregnant women and clinical outcomes, such as maternal thyroid function, infants being born small for gestational age or infants' neuro-behavioural impairment, cannot be used for setting DRVs for pregnant women.

The Panel noted that iodine concentrations in breast milk of European women vary widely and that large iodine stores exist in conditions of adequate iodine status before pregnancy and lactation. The Panel therefore considered that a full compensation for the iodine secreted in breast milk may not be justified for the derivation of DRVs for iodine for lactating women. Therefore, for lactating women the same AI is proposed as for pregnant women, i.e. 200 µg/day (Table 7).

4.7. Iron

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4254/full

Iron is required for oxygen transport, electron transfer, oxidase activities and energy metabolism (EFSA NDA Panel, 2015a). The main components of the body that contain iron are erythrocyte haemoglobin and muscle myoglobin, liver ferritin, and haem and non-haem enzymes.

Dietary iron consists of haem (from animal tissues) and non-haem (including ferritin) iron. Foods that contain relatively high concentrations of iron include meat, fish, cereals, beans, nuts, egg yolks, dark green vegetables, potatoes and fortified foods.

Iron is inefficiently and variably absorbed, depending on dietary and host-related factors. Iron absorption occurs primarily in the duodenum. A proportion of non-haem iron in foods is solubilised in the gastrointestinal lumen, reduced by duodenal cytochrome b reductase to Fe^{2+} and transported into the enterocyte by the transmembrane divalent metal transporter 1. There, iron is either stored as ferritin, some of which is subsequently lost when the cells are sloughed, is taken up by mitochondria for the synthesis of haem, or is transported across the basolateral membrane by ferroportin where it is carried in the circulation as diferric-transferrin after oxidation to Fe^{3+} by hephaestin. The mechanisms of absorption of haem iron and ferritin iron are uncertain, but once taken up iron is released from haem iron by haem oxygenase and then follows the same pathways as non-haem iron.

Homeostasis is mediated via the regulation of iron absorption, as there are no active pathways for excreting iron. In healthy individuals, the mucosal uptake and transfer of iron is inversely related to systemic serum ferritin concentrations, and control is exerted via the expression of the hepatic hormone hepcidin.

If the supply of iron is insufficient to meet physiological requirements, iron stores will be mobilised and iron deficiency will develop once the stores are exhausted. Iron deficiency anaemia (a microcytic anaemia with haemoglobin concentrations below normal) is the most common nutritional deficiency disorder, being found in all countries of the world. Subjects at greatest risk are those with high iron requirements owing to growth (infants, children, pregnant women) or high losses (women with high menstrual losses), or those with impaired absorption, e.g. in the presence of infection/inflammation.

The risk of systemic iron overload from dietary sources is negligible with normal intestinal function. Chronic iron overload may occur as a result of specific clinical conditions and genetic mutations, but there is no evidence that heterozygotes for haemochromatosis are at an increased risk of iron overload.

The Panel considers that health outcomes cannot be used to derive DRVs for iron because of the uncertainties in intake measurements, the poor correlation between intake and iron status, and the presence of confounders that prevent the determination of dose–response relationships and the assessment of risks associated with deficiency or excess.

A factorial approach was used to derive dietary iron requirements. Data on iron turnover and total obligatory iron losses from the body (including skin, sweat, urine and faeces) obtained from radioisotope dilution measurements were used to determine iron requirements in men and premenopausal women. Although these data were collected from a North American population group, the Panel agreed to use them as a basis for the estimation and probability modelling of the mean and approximate variability of distribution percentiles for the iron losses of adult men and premenopausal women in the European Union (EU) population. Summary statistics were estimated for the main variables related to iron losses for men and premenopausal women and for associations among the variables which were considered to be explanatory for iron losses. From these, a regression model equation for iron losses (as mg/day) was fitted to the data using a set of potentially relevant variables. This stage included an assessment of outliers and goodness of fit. The regression model was then used to derive a distribution for iron losses, combining the model equation with parametric distributions fitted to the sampling observations of each of the explanatory variables.

Dietary (haem and non-haem) iron absorption was estimated from a probability model, based on measures of iron intake and status in a representative group of men and women from the UK National Diet and Nutrition Survey. This provides estimates of total iron absorption from a mixed Western style diet at any level of iron status. The Panel selected a target value of 30 µg/L for serum ferritin concentration. At this level, the predicted iron absorption is 16% in men and 18% in premenopausal women. The Panel decided to use 16% for adults (except premenopausal women) and children aged 12–17 years when converting physiological requirements into dietary intakes, based on the assumption that the relationship between serum ferritin concentration and efficiency of absorption holds for all age groups, as there are no indications that age will affect the relationship.

In men, the 50th percentile of the model-based distribution of obligatory iron losses is 0.95 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of 1.48, 1.61 and 1.72 mg/day. Using 16% iron absorption to convert the physiological requirement into the dietary requirement results in a calculated dietary requirement at the 50th percentile of 5.9 mg/day and of 10.8 mg/day at the 97.5th percentile. After rounding, an AR of 6 mg/day (Table 4, Table 6) and a PRI of 11 mg/day were set (Table 5, Table 7). In the absence of information on the iron requirement for postmenopausal women and despite their lower body weight, the Panel decided to set the same DRVs for postmenopausal women as those set for adult men.

In premenopausal women, the 50th percentile of the model-based distribution of obligatory iron losses is 1.34 mg/day. The 90th, 95th and 97.5th percentiles are, respectively, equal to iron losses of 2.44, 2.80 and 3.13 mg/day. Using 18% absorption to convert the physiological iron requirement into the dietary requirement results in a calculated dietary requirement at the 50th percentile of 7.4 mg/day. Intakes meeting the dietary iron requirement of approximately 90, 95 and 97.5% of the premenopausal women are calculated as 13.6, 15.6 and 17.4 mg/day, respectively. After rounding, the Panel derived an AR of 7 mg/day (Table 6) and a PRI of 16 mg/day for premenopausal women (Table 7). The Panel considers that the PRI meets the dietary requirement of 95% of women in their reproductive years and is derived from a group of premenopausal women, some of whom used oral contraceptives, as is the case in the EU. The Panel decided that women with very high iron losses should not be included in the premenopausal group, as this would result in unrealistically high DRVs for the majority of this population group.

In infants aged 7–11 months, the requirement for absorbed iron is 0.79 mg/day to replace obligatory losses (0.19 mg/day) and increase haemoglobin mass, tissue iron and storage iron (0.6 mg/day). Assuming 10% absorption, this gives an AR of 8 mg/day (Table 4, Table 6) and, based on a coefficient of variation (CV) of 20%, which allows for high individual variation relating to growth rate, iron losses, absorption and dietary patterns, the PRI is 11 mg/day (Table 5, Table 7). In children aged 1–6 years, the AR is 5 mg/day (Table 4, Table 6), calculated from the sum of the requirements for growth (0.25 mg/day for ages 1–3 years and 0.27 mg/day for ages 4–6 years) and obligatory losses of 0.022 (1–3 years) and 0.012 (4–6 years) mg/kg body weight per day, and absorption of 10%. Based on a CV of 20%, the PRI is 7 mg/day (Table 5, Table 7). In children aged 7–11 years, requirements for growth increase to 0.39 mg/day, but losses per kilogram of body weight do not change. Assuming 10% absorption, the AR (after rounding) is 8 mg/day (Table 4, Table 6) and, based on a CV of 20%, the PRI is 11 mg/day (Table 5, Table 7).

In boys and girls aged 12–17 years, the requirements for absorbed iron are 1.27 and 1.13 mg/day, respectively, calculated from losses of 0.012 mg/kg body weight per day and menstrual blood losses of 0.25 mg/day in girls, and growth needs of 0.61 mg/day for boys and 0.26 mg/day for girls. Assuming 16% absorption, the AR (after rounding) is 8 mg/day (Table 4) for boys and 7 mg/day for girls (Table 6). The PRI for boys is 11 mg/day based on a CV of 20% (Table 5). In girls, because of the uncertainties related to the rate and timing of physiological development and the onset of menarche, and because of the skewed distribution of menstrual losses, the Panel decided to set the PRI as the mean of the calculated dietary requirement of 97.5% of girls aged 12–17 years (9.9 mg/day) and the PRI for premenopausal women (16 mg/day). After rounding, the PRI is 13 mg/day for girls (Table 7).

In pregnancy, iron intake should cover basal losses during the first trimester, taking into account the cessation of menstruation. The requirements then increase exponentially, and this is associated with a dramatic increase in the efficiency of iron absorption. The total quantity of iron required for a singleton pregnancy is 835 mg. If the serum ferritin concentration is 30 µg/L at conception, around 120 mg of stored iron can be mobilised to support the pregnancy, which means that the total dietary requirement of iron is 715 mg. If the relevant percentage absorption figures determined from a study in pregnant women are applied to the entire pregnancy (7.2% during weeks 0–23, 36.3% during weeks 24–35 and 66.1% during weeks 36–40 for non-haem iron, plus 25% absorption for haem iron throughout the whole pregnancy), the total quantity of iron absorbed from a diet providing 13 mg iron/day is 866 mg. The Panel notes that using the absorption figures from single-meal studies in fasting mothers may be an overestimate, but, nevertheless, the quantity of iron absorbed is well in excess of the estimated 715 mg calculated by a factorial approach, and the progressive fall in serum ferritin concentration will be accompanied by an increased efficiency of absorption, irrespective of other homeostatic mechanisms. The Panel therefore considers that no additional iron is required in pregnancy (Table 6, Table 7).

During lactation, the quantity of iron secreted in breast milk is approximately 0.24 mg/day. When this is added to basal losses of 1.08 mg/day (obtained from data in postmenopausal women), the requirement for absorbed iron during the first months of lactation is calculated to be 1.3 mg/day, assuming that menstruation has not yet resumed. This requirement is slightly less than in nonpregnant, non-lactating women, but, for depleted iron stores to be replenished and to cover losses of iron when menstruation is re-established, the Panel considers that the AR and PRI for lactating women are the same as for non-pregnant women of childbearing age (Table 6, Table 7).

4.8. Magnesium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4186/full

Magnesium is an alkaline earth metal. It occurs as the free cation Mg^{2+} in aqueous solutions or as the mineral part of a large variety of compounds, including chlorides, carbonates and hydroxides (EFSA NDA Panel, 2015c). Magnesium is a cofactor of more than 300 enzymatic reactions, acting either on the enzyme itself as a structural or catalytic component or on the substrate, especially for reactions involving ATP, which make magnesium essential in the intermediary metabolism for the synthesis of carbohydrates, lipids, nucleic acids and proteins, as well as for specific actions in various organs in the neuromuscular or cardiovascular system.

Magnesium deficiency can cause hypocalcaemia and hypokalaemia, leading to neurological or cardiac symptoms when it is associated with marked hypomagnesaemia. Owing to the widespread involvement of magnesium in numerous physiological functions and the metabolic interactions between magnesium and other minerals, it is difficult to relate magnesium deficiency to specific symptoms.

Magnesium absorption takes place in the distal intestine, mainly as the ionised form. Percentage absorption is generally considered to be 40–50%, but figures from 10 to 70% have also been reported. Magnesium absorption can be inhibited by phytic acid and phosphate and enhanced by the fermentation of soluble

dietary fibre, although the physiological relevance of these interactions at adequate intakes remains to be established.

The majority of the body magnesium content is stored in bone (about 60%) and muscle (about 25%). A small amount is present in the serum, mainly as the free cation. Most cells are able to actively and rapidly buffer magnesium loss or accumulation through the involvement of specific magnesium transporters. The kidney plays a major role in magnesium homeostasis and maintenance of serum concentration. Urinary magnesium excretion is increased by high natriuresis, osmotic load and metabolic acidosis, and reduced by metabolic alkalosis, parathyroid hormone and, possibly, calcitonin. A large proportion of the magnesium content of faeces stems from unabsorbed magnesium. Endogenous magnesium is lost through bile, pancreatic and intestinal juices, and intestinal cells, and part of this can be reabsorbed. Magnesium losses through sweat are modest and very variable, depending on the techniques used for sweat collection, and losses through menstruation are negligible.

There is some evidence that urinary magnesium concentration reflects magnesium intake. Urinary, faecal, serum and erythrocyte magnesium concentrations have been used for the assessment of magnesium status, with serum magnesium concentration being the most frequently used marker. However, the Panel considers that the usefulness of serum magnesium concentration as a marker of intake or status is questionable and that there are at present no appropriate biomarkers for magnesium status that can be used for deriving DRVs for magnesium.

The Panel notes that a recent pooled analysis of balance studies in adults suggests that zero magnesium balance may occur at a magnesium intake of 165 mg/day. The Panel also notes that results of some large-scale and long-term prospective observational studies point to an inverse relationship between magnesium intake and the risk of diabetes mellitus type 2.

Foods rich in magnesium are nuts, whole grains and grain products, fish and seafood, several vegetables, legumes, berries, banana and some coffee and cocoa beverage preparations. The magnesium content of tap/bottled water can make a significant contribution to intake. On the basis of data from 13 dietary surveys in nine European Union (EU) countries, dietary intake of magnesium was estimated by EFSA using food consumption data from the EFSA Comprehensive European Food Consumption Database and composition data from the EFSA Food Composition Database.

For both sexes combined, average magnesium intake ranged from 72 to 120 mg/day (25–45 mg/MJ, 9.2–12.7 mg/kg body weight per day) in infants (< 1 year of age); from 153 to 188 mg/day (35–45 mg/MJ, 12.7–15.8 mg/kg body weight per day) in children aged 1 to < 3 years; from 184 to 281 mg/day (28–43 mg/MJ, 7.6–13.0 mg/kg body weight per day) in children aged 3 to < 10 years; from 213 to 384 mg/day (28–44 mg/MJ, 4.2–7.7 mg/kg body weight per day) in children aged 10 to < 18 years; and from 232 to 439 mg/day (35–51 mg/MJ, 3.4–5.3 mg/kg body weight per day) in adults (≥ 18 years). The main food groups contributing to magnesium intake were grains and grain-based products, milk and milk products, and coffee, cocoa, tea and infusions.

Considering all the evidence available, i.e. from balance studies and prospective observational studies, the Panel decided to set an Adequate Intake (AI) based on observed intakes in several EU countries. For adults of all ages, the Panel proposed to set AIs according to sex. Considering the distribution of observed average intakes (males 264–439 mg/day; females 232–357 mg/day), the Panel proposed an AI for all adult men over 18 years of 350 mg/day (Table 5) and for all adult women an AI of 300 mg/day (Table 7), after rounding.

The Panel also decided to set an AI for infants aged 7–11 months and children based on observed intakes in several EU countries. For infants aged 7–11 months, an AI in line with the proposal of the SCF (1993) of 80 mg/day was set (Table 5, Table 7). This value represents, after rounding, the midpoint (78 mg/day) of the range between 35 mg/day (magnesium intake estimated by extrapolation using isometric scaling from intakes in breast-fed infants aged 0–6 months) and 120 mg/day (highest value of the range of observed mean intakes in the EU countries for which data are available). For children aged 1 to < 10 years, considering the absence of a strong basis for a distinct value according to sex and the distribution of observed mean intakes, AIs were set at the midpoint of average intakes (170 mg/day for boys and girls

aged 1 to < 3 years, and 230 mg/day for boys and girls aged 3 to < 10 years) (Table 5, Table 7). For children aged 10 to < 18 years, considering the rather large differences in magnesium intakes between boys and girls, the Panel proposed to set AIs according to sex, and to select the midpoints of average intakes as AIs, i.e. 300 mg/day for boys (Table 5) and 250 mg/day for girls (Table 7).

Considering that pregnancy induces only a small increase in magnesium requirement, which is probably covered by adaptive physiological mechanisms, the Panel considers that the AI for non-pregnant women also applies to pregnant women (Table 7). For lactating women, considering that 25 mg/day is secreted with breast milk during the first six months of exclusive breastfeeding and that there is the possibility of adaptation of magnesium metabolism, at the level of both absorption and elimination, the Panel considers that the AI for non-pregnant non-lactating women also applies to lactating women (Table 7).

4.9. Manganese

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3419/full

In 1993, the Scientific Committee for Food set an Acceptable Range of Intakes for adults at 1–10 mg/day, considering observed intakes of manganese in European countries and data from balance studies (EFSA NDA Panel, 2013f). A few other authorities have set Adequate Intakes (AIs) for manganese, based on similar considerations.

Manganese is an essential dietary element for mammals. It is a component of metalloenzymes such as superoxide dismutase, arginase and pyruvate carboxylase, and is involved in amino acid, lipid and carbohydrate metabolism. A specific manganese deficiency syndrome has not been described in humans.

Absorption of manganese in the intestine is low (< 10%). Regulation at the level of absorption appears to be one of the adaptive responses to dietary manganese intake and such regulation allows manganese homeostasis to be maintained over a wide range of intakes. A reduction in the biological half-life of manganese has been observed with increased dietary manganese intakes indicating the role of whole-body turnover rate in manganese homeostasis. Elimination of manganese is primarily via the faeces.

The assessment of manganese intake or status using biological markers is difficult owing to the rapid excretion of manganese into bile, to homeostatic mechanisms and to the lack of sensitivity of biomarkers over the normal range of intakes. Therefore, there are no reliable and validated biomarkers of manganese intake or status.

Nuts, chocolate, cereal-based products, crustaceans and molluscs, pulses, and fruits and fruit products are rich sources of manganese. The main contributors to the manganese intake of adults are cereal-based products, vegetables, fruits and fruit products and beverages. In the EU, estimated mean manganese intakes of adults range from 2 to 6 mg/day, with a majority of values around 3 mg/day. Estimated mean manganese intakes range from 1.5 to 3.5 mg/day in children, and from 2 to 6 mg/day in adolescents.

Several balance studies have been undertaken to establish manganese requirements. These studies demonstrate that the body adapts quickly to changes in manganese intake. Although balance may be maintained at intakes below 2.5 mg/day, null or positive balances have consistently been observed with manganese intakes above 2.5 mg/day. Manganese balance may be influenced by the overall diet, variations in individual rates of absorption or excretion, differences in body contents and adaptation to varying dietary levels, which make comparisons between subjects and studies difficult.

No data on manganese intakes and health outcomes were identified for the setting of DRVs.

As the evidence to derive an Average Requirement and thus a Population Reference Intake is considered insufficient, an Adequate Intake (AI) is proposed. Observed mean intakes of adults in the EU are typically around 3 mg/day. In addition, null or positive balances have consistently been observed with intakes of manganese above 2.5 mg/day. An AI of 3 mg/day for adults is, therefore, proposed (Table 5, Table 7). The adult AI also applies to pregnant and lactating women (Table 7). For infants aged from 7 to 11 months,

the Panel decides to set a range for the AI of 0.02–0.5 mg/day (Table 5, Table 7). This reflects the wide range of manganese intakes that appear to be adequate, based on upwards extrapolation of manganese intakes in fully breast-fed infants, observed intake of manganese in infants aged 6 and 12 months and the value estimated from extrapolation of the adult AI using isometric scaling. For children and adolescents, an AI is proposed based on extrapolation from the adult AI using isometric scaling (i.e. extrapolation based on reference body weights of the respective age groups) and rounding to the nearest 0.5. The respective AIs vary from 0.5 mg/day in young children aged 1–3 years to 3.0 mg/day in adolescent boys and girls (Table 5, Table 7).

4.10. Molybdenum

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3333/full

Molybdenum is an essential component of certain enzymes that catalyse redox reactions and contain, in addition to molybdenum, other prosthetic groups such as flavin adenine dinucleotide or haem (EFSA NDA Panel, 2013d). In humans, sulphite oxidase, xanthine oxidoreductase, aldehyde oxidase and mitochondrial amidoxime reducing component require molybdenum linked with a pterin (molybdopterin) as the cofactor. These enzymes are involved in the metabolism of aromatic aldehydes and the catabolism of sulphurcontaining amino acids and heterocyclic compounds, including purines, pyrimidines, pteridins and pyridines.

In humans, a single case report of a syndrome suggestive of dietary molybdenum deficiency in a patient on total parenteral nutrition for several months has been reported, but clinical signs of molybdenum deficiency in otherwise healthy humans have not been observed. A distinct molybdenum deficiency syndrome has not been observed in animals when subjected to molybdenum restriction, despite considerable reduction in the activity of molybdoenzymes.

Water-soluble molybdates are efficiently and rapidly absorbed from the digestive tract at a wide range of intakes, and the body is able to adapt to this wide intake range by regulating excretion via the urine. Storage of molybdenum in mammals is low, and most tissue molybdenum is thought to be associated with molybdoenzymes.

There are no suitable biomarkers of molybdenum status. Biochemical changes observed in subjects with molybdopterin cofactor deficiency, a genetic disorder, or in the one subject reported with possible molybdenum deficiency, have not been observed in healthy individuals on varying levels of molybdenum intake. Low activity of molybdoenzymes in tissues, or changes in substrate/product relationships, are considered as insufficiently specific to be used as biomarkers of status.

Molybdenum is present in nearly all foods in trace amounts as soluble molybdates. Foods high in molybdenum are pulses, cereal grains and grain products, offal (liver, kidney) and nuts. Cereals and cereal-based products including bread are the major food contributors to the dietary molybdenum intake of adults. Mean molybdenum intakes, as assessed in duplicate diet or food portion studies, total diet studies and market basket studies, vary over a wide range, i.e. 58 µg/day to 157 µg/day, for adults in various European countries. Mean intakes are at or above 100 µg/day in five of the eight European countries for which data are available. Molybdenum intakes of children are only available from two European countries.

In 1993, the Scientific Committee for Food did not publish DRVs for molybdenum. More recently, other authorities have set DRVs for molybdenum and these are based on the maintenance of molybdenum homeostasis as measured in balance studies, taking into account molybdenum bioavailability from various food sources, or are based on observed molybdenum intakes with a mixed diet.

Various balance studies have been performed to establish molybdenum requirements. However, only one balance study in adults was considered to be of sufficient duration, and was performed with a constant diet and under controlled conditions. In this study carried out in four men, balance was reported to be near zero from day 49 until day 102 of the depletion period when intakes were as low as 22 µg/day. Biochemical

changes or symptoms suggestive of molybdenum deficiency were not observed and the possibility that humans may be able to achieve molybdenum balance at even lower intakes cannot be excluded. Results of two balance studies with some methodological limitations were reported in children, but these studies cannot be used to derive an average molybdenum requirement for children. Data on molybdenum intakes and health outcomes were unavailable for the setting of DRVs for molybdenum.

As the evidence to derive an Average Requirement (AR), and thus a Population Reference Intake, was considered insufficient, an Adequate Intake (AI) is proposed. An AI of 65 µg/day is proposed for adult men and women based on mean molybdenum intakes at the lower end of the wide range of observed intakes from mixed diets in Europe (Table 5, Table 7). Given the scarcity of data on molybdenum intakes in pregnant and lactating women, it is suggested that the adult AI also applies to pregnant and lactating women (Table 7). For infants from seven months and children, it was decided that an AR could not be established, and an AI is proposed based on extrapolation from the adult AI using isometric scaling and reference body weights of the respective age groups. The respective AIs vary between 10 µg/day in infants aged 7–11 months and 65 µg/day in adolescent boys and girls (Table 5, Table 7).

4.11. Phosphorus

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4185/full

Phosphorus is involved in many physiological processes, such as in the cell's energy cycle, in regulation of the body's acid–base balance, as a component of the cell structure, in cell regulation and signalling, and in the mineralisation of bones and teeth (EFSA NDA Panel, 2015b). About 85% of the body's phosphorus is in bones and teeth, 14% is in soft tissues, including muscle, liver, heart and kidney, and only 1% is present in extracellular fluids. Phosphorus homeostasis is intricately linked to that of calcium because of the actions of calcium-regulating hormones, such as parathyroid hormone (PTH) and 1,25-dihydroxy-vitamin D ($1,25(OH)_2D$), at the level of the bone, the gut and the kidneys.

Phosphorus absorption occurs through passive diffusion and sodium-dependent active transport and via paracellular and cellular pathways. In adults, limited data suggest that net phosphorus absorption ranges from 55 to 80% of intake. Phosphorus absorption is affected by the total amount of phosphorus in the diet and also by the type of phosphorus (organic versus inorganic), the food origin (animal- versus plant-derived) and the ratio of phosphorus to other dietary components. Absorption is regulated by $1,25(OH)_2D$ and PTH.

Hypophosphataemia, defined by a serum inorganic phosphorus concentration of < 0.80 mmol/L (2.48 mg/dL), only rarely occurs because of inadequate dietary phosphorus intake, and is generally due to metabolic disorders.

The major dietary contributors to phosphorus intake are foods high in protein content, i.e. milk and milk products followed by meat, poultry and fish, grain products and legumes. Based on data from 13 dietary surveys in nine European Union countries, mean phosphorus intakes range from 265 to 531 mg/day in infants, from 641 to 973 mg/day in children aged 1 to < 3 years, from 750 to 1,202 mg/day in children aged 3 to < 10 years, from 990 to 1,601 mg/day in children aged 10 to < 18 years and from 1,000 to 1,767 mg/day in adults (≥ 18 years).

Balance studies in adults were considered to be heterogeneous and to have many limitations. Overall, balance studies, including those in children and pregnant women, could not be used for setting DRVs for phosphorus. In addition, it was considered that estimations of phosphorus absorption from the diet, as well as losses of phosphorus via urine and faeces, vary over a wide range, so that the factorial approach cannot be used for deriving the requirement for phosphorus.

Evidence from human studies on the relationship between phosphorus intake and various health outcomes was also reviewed. It was considered that data on measures of bone health, cancer-related outcomes and

evidence related to all-cause mortality and cardiovascular outcomes could not be used to derive DRVs for phosphorus.

Data on the molar ratio of calcium to phosphorus in intact bone of healthy adults suggest a range of approximately 1.6:1 to 1.8:1. Using the calcium to phosphorus molar ratio in bone of 1.6:1 to 1.8:1 and adjusting for the proportion of calcium and phosphorus found outside bone, a molar ratio of calcium to phosphorus in the adult body of about 1.37:1 to 1.55:1 is estimated. In addition, data from measurements of whole-body calcium and phosphorus contents in Caucasian men and women indicate that the calcium to phosphorus molar ratio in the whole body ranges from 1.48:1 to 1.69:1 in women and from 1.57:1 to 1.89:1 in men. The Panel thus considered that the ratio of calcium to phosphorus in the whole body ranges from about 1.4:1 to 1.9:1 and proposed, in the absence of other consistent evidence, that DRVs for phosphorus be set based on the approximate molar ratio of calcium to phosphorus in the body. The fractional absorption of phosphorus is higher than that of calcium. However, as phosphorus absorption has been reported to vary over a wide range, it was considered that the actual amounts of calcium to phosphorus that are available for absorption from the diet and that may be retained in the body cannot be determined. In the absence of this information, the Panel proposed to set DRVs for phosphorus based solely on the range of the molar ratio of calcium to phosphorus in the whole body. The Panel considered that the data are insufficient to derive Average Requirements and Population Reference Intakes (PRIs) for phosphorus and proposed to set Adequate Intakes (AIs) for all population groups. Based on the AI (for infants aged 7–11 months) and the PRIs (for all other ages) for calcium and considering a molar calcium to phosphorus ratio of 1.4:1 to 1.9:1, adequate quantities of phosphorus were calculated in mg/day. The Panel chose the lower bound of this range (i.e. a ratio of 1.4:1 which results in the higher phosphorus intake value) for setting an AI for phosphorus, taking into account estimated phosphorus intakes in Western countries which are considerably higher than the calculated values.

The AI is 160 mg/day for infants aged 7–11 months and between 250 mg/day and 640 mg/day for children (Table 5, Table 7). For adults, the AI is 550 mg/day (Table 5, Table 7). Taking into consideration adaptive changes in phosphorus metabolism that may occur during pregnancy and lactation, it was considered that the AI for adults also applies to pregnant and lactating women (Table 7).

4.12. Potassium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4592/full

Potassium is an essential mineral in the human diet. It is the predominant osmotically active element inside cells. It plays a major role in the distribution of water inside and outside cells, assists in the regulation of the acid–base balance, and contributes to establishing a membrane potential which supports electrical activity in nerve fibres and muscle cells (EFSA NDA Panel, 2016e). Potassium has a role in cell metabolism, participating in energy transduction, hormone secretion, and the regulation of protein and glycogen synthesis.

Potassium is present in all natural foods, in particular starchy roots or tubers, vegetables, fruits, whole grains, dairy products and coffee. Based on the data from 13 dietary surveys in nine countries of the European Union, average potassium intakes ranged between 821 and 1,535 mg (21 and 39 mmol)/day in infants (< 1 year), between 1,516 and 2,005 mg (39 and 51 mmol)/day in children aged 1 to < 3 years, between 1,668 and 2,750 mg (43 and 70 mmol)/day in children aged 3 to < 10 years, between 2,093 and 3,712 mg (54 and 95 mmol)/day in children aged 10 to < 18 years, and between 2,463 and 3,991 mg (63 and 102 mmol)/day in adults (≥ 18 years).

Potassium deficiency, presenting as hypokalaemia, is defined as a serum potassium concentration lower than 3.5 mmol/L and is usually caused by increased potassium losses (e.g. via diarrhoea, vomiting or excessive renal losses) or intracellular shift of potassium (e.g. during alkalosis). Hypokalaemia resulting from insufficient dietary intake is rare and may be associated with severe hypocaloric diets, or with a

relative insufficiency caused by an increased requirement of potassium for the synthesis of tissue during recovery from malnutrition.

About 90% of dietary potassium is absorbed, mainly in the small intestine. Body potassium content is regulated by the balance between dietary intake and renal excretion. Urine is the major route of potassium excretion, while the remaining part is eliminated in the faeces and, to a lesser extent, in the sweat. Urinary potassium excretion, based on 24-h urine collection, is regarded as a reliable biomarker of dietary intake in adults on a population basis.

Most of body potassium is located in the muscle, with lower amounts present in the bone, liver, skin and red blood cells. Because of tight homeostatic mechanisms, blood potassium concentrations and total body potassium content are only minimally affected by variations in dietary potassium intake. The Panel therefore considers that there is no suitable biomarker of potassium status which can be used for setting DRVs for potassium in the general population.

Potassium intake has been reported to be associated with several health outcomes, particularly cardiovascular endpoints. Overall, the Panel considers that randomised controlled trials and an observational cohort study carried out in a European adult population provide evidence that a potassium intake of 3,500 mg (90 mmol)/day has beneficial effects on blood pressure in adults. Furthermore, there is consistent evidence from observational cohort studies that potassium intakes below 3,500 mg (90 mmol)/day are associated with a higher risk of stroke. Evidence on the association between potassium intake and coronary heart disease is unclear and inconsistent. Evidence in relation to diabetes mellitus type 2, kidney stones and bone health were also reviewed but the available data could not be used to derive DRVs for potassium.

The Panel decides to set DRVs for potassium based on the relationship between potassium intake and blood pressure and stroke. Currently, available data cannot be used to determine the average requirement of potassium but can be used as a basis for deriving an adequate intake (AI). A potassium intake of 3,500 mg (90 mmol)/day can be considered adequate for the adult population and an AI of 3,500 mg (90 mmol)/day for adult men and women is proposed (Table 5, Table 7).

No data are available on which to base an average potassium requirement for infants and children. The Panel derives AIs extrapolated from the AI for adults, taking into account differences in reference body weight (isometric scaling) and including a growth factor to take into account requirements for growth. The AI set for infants aged 7–11 months is 750 mg (19 mmol)/day (Table 5, Table 7). For children, AIs range from 800 mg (20 mmol)/day (1–3 years old) to 3,500 mg (90 mmol)/day (15–17 years old) (Table 5, Table 7).

The Panel considers that the requirement for the daily accretion rate of potassium in fetal and maternal tissues can be met by the adaptive changes which maintain potassium homeostasis during pregnancy. The AI for pregnant women is set at 3,500 mg (90 mmol)/day, the same as for non-pregnant women (Table 7).

Considering evidence which indicates that total body potassium content decreases in lactating women, a conservative approach is taken and the amount of potassium needed to compensate for the losses of potassium through breast milk is added to the AI for adult. Thus, an AI of 4,000 mg (102 mmol)/day is proposed for lactating women (Table 7).

4.13. Selenium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3846/full

In the diet, selenium is mainly present in organic compounds, as L-selenomethionine and L-selenocysteine, with lower amounts in inorganic compounds, as selenate and selenite (EFSA NDA Panel, 2014e). Because quantification and speciation of selenium in foods is complex and because there is considerable variation

in the selenium content of foods, food composition tables are often inaccurate, resulting in imprecise estimates of selenium intake.

A total of 25 selenoproteins with a variety of functions, including antioxidant effects, T-cell immunity, thyroid hormone metabolism, selenium homeostasis and transport, and skeletal and cardiac muscle metabolism, have been identified in humans. Selenoprotein P (SEPP1) plays a central role in selenium supply to tissues and participates in the regulation of selenium metabolism in the organism.

Selenium in its various forms appears to be well absorbed from the diet. Upon absorption, selenocysteine, selenate and selenite are available for the synthesis of selenoproteins. Selenomethionine is non-specifically integrated into the methionine pool and can substitute for methionine in proteins. Selenomethionine may also be converted to selenocysteine and enter the functional selenium body pool. The production of methylated selenium compounds in the liver, which are excreted predominantly in the urine, participates in the regulation of selenium metabolism in the organism.

Selenium deficiency affects the expression and function of selenoproteins and has been involved in the degeneration of organs and tissues leading to the manifestation of Keshan and Kashin-Beck diseases.

Plasma selenium includes selenium in selenoproteins (the functional pool of selenium), and other plasma proteins in which selenomethionine non-specifically substitutes for methionine. Thus, plasma selenium is not a direct marker of the functional selenium body pool. Measures of glutathione peroxidases (GPxs) activity can be used as a biomarker of selenium function. However, the activity of GPxs reaches a steady state with levels of selenium intake that are lower than those required for the levelling off of SEPP1. The latter is considered the most informative biomarker of selenium function on the basis of its role in selenium transport and metabolism and its response to different forms of selenium intake. Intervention studies using different levels of selenium intake showed that plasma SEPP1 concentration levels off in response to increasing doses of selenium. The levelling off of plasma SEPP1 was considered to be indicative of an adequate supply of selenium to all tissues and to reflect saturation of the functional selenium body pool, ensuring that selenium requirement is met. This criterion was used for establishing DRVs for selenium in adults.

Evidence from human studies on the relationship between selenium intake and plasma SEPP1 concentration was reviewed. The Panel noted uncertainties with respect to estimates of background selenium intake in most studies. Habitual selenium intakes of 50–60 µg/day were not sufficient for SEPP1 concentration to reach a plateau in Finnish individuals, while selenium intakes of 100 µg/day and above were consistently associated with plasma SEPP1 concentration at a plateau in population groups from Finland, the UK and the USA. In a study in healthy individuals from New Zealand, selenium intakes of around 60–70 µg/day were required for SEPP1 concentration to level off.

Although this was the only study that quantified background selenium intake from the analysed selenium content of consumed foods, the Panel noted the large variability in the results of this study. In another study among Chinese subjects, a selenium intake of 0.85 µg/kg body weight per day led to the levelling off of plasma SEPP1 concentration. The Panel noted, however, that there were uncertainties related to the intake estimates and to the extrapolation of results from Chinese individuals to the European population. The Panel also noted uncertainties in extrapolating values derived from studies that administered selenium as L-selenomethionine to dietary selenium including other forms of selenium.

Given the uncertainties in available data on the relationship between total selenium intake and SEPP1 concentration, they were considered insufficient to derive an Average Requirement for selenium in adults. Instead, an Adequate Intake (AI) of 70 µg/day for adult men and women was set (Table 5, Table 7). A review of observational studies and randomised controlled trials that investigated the relationship between selenium and health outcomes did not provide evidence for additional benefits associated with selenium intake beyond that required for the levelling off of SEPP1.

No specific indicators of selenium requirements were available for infants, children or adolescents.

For infants aged 7–11 months, an AI of 15 µg/day was derived by extrapolating upwards from the estimated selenium intake with breast milk of younger exclusively breast-fed infants and taking into account

differences in reference body weights (Table 5, Table 7). For children and adolescents, the AIs for selenium were extrapolated from the AI for adults by isometric scaling and application of a growth factor. The AIs range from 15 µg/day for children aged one to three years to 70 µg/day for adolescents aged 15– 17 years (Table 5, Table 7).

There is evidence suggesting adaptive changes in the metabolism of selenium during pregnancy, and it was considered that these changes cover the additional selenium needs during this period. The Panel proposes that the AI set for adult women also applies to pregnancy (Table 7). Based on an average amount of selenium secreted in breast milk of 12 µg/day and an absorption efficiency of 70% from usual diets, an additional selenium intake of 15 µg/day was considered to replace these losses. Thus, an AI of 85 µg/day is proposed for lactating women (Table 7).

4.14. Sodium

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5778/full

Sodium (Na^+) is the dominant cation in the extracellular fluid (ECF) of the body (EFSA NDA Panel et al., 2019a). The functions of sodium lie in its participation in the control of the volume and systemic distribution of total body water; enabling the cellular uptake of solutes; and the generation via interactions with potassium of transmembrane electrochemical potentials.

Dietary sodium deficiency is rare in healthy European populations. Sodium chloride and other sodium salts are ubiquitous in the diet, and there are adaptive physiological mechanisms that reduce the losses of sodium in urine, faeces and sweat at low levels of sodium intake. Sodium chloride added during industrial food processing and discretionary use or food preservation is the major source of dietary sodium in Western diets. Other sources of sodium include inherently native sources and sodium-containing food additives, in which sodium may be associated with anions other than chloride.

In healthy people, almost all dietary sodium is absorbed, even at very high level of intake. Following absorption, sodium ions are distributed by portal and systemic circulations, where their concentrations are maintained within a narrow range. Up to 95% of sodium body content is in the ECF, including a large proportion in bone, skin and muscle. The pool of sodium in bone, muscle and skin has been proposed to be a sodium depot or reserve, but could also have a homeostatic and adaptive role as an extra-renal clearance depository for handling excessive systemic accumulation of sodium. The excretion and retention (i.e. homeostasis) of sodium is effected by an integrated neurohormonal control from centres located in the hypothalamus. The kidney is the main organ mediating the excretion and retention of sodium. It efficiently excretes sodium in response to high dietary intakes and salvages sodium when dietary intake is low. By contrast, sodium losses in the faeces are relatively stable and typically limited to a few mmol/day. The amount of sodium lost in sweat can vary widely, depending on, for example environmental conditions or the levels of physical activity.

The Panel reviewed the reliability of the methods and biomarkers used to assess sodium intake. Urinary sodium excretion in 24-h collections is considered the most reliable biomarker of sodium daily intake. However, a single 24-h urine collection may not reliably reflect an individual's usual intake. Also, incomplete collections of 24-h urine samples can introduce bias in measuring daily sodium excretion. Multiple collections and quality control procedures are required to estimate an individual's usual sodium intake reliably.

Homeostatic mechanisms maintain the plasma sodium concentration of healthy individuals within a narrow range. Hyponatraemia and hypernatraemia are typically related to disorders affecting water and electrolyte balances. They are seldom due to inappropriate sodium intake. The Panel considers that there is no biomarker of sodium status that can be used for setting DRVs for sodium in the general population.

Evidence from balance studies on sodium and on the relationship between sodium intake and health outcomes, in particular cardiovascular disease (CVD)-related endpoints and bone health, was reviewed.

Balance studies indicate that adaptation mechanisms enable the maintenance of sodium balance over a wide range of sodium intakes. Recent data from a long-term study of sodium and other electrolytes metabolism suggest that rhythmical variations in the sodium body pools may occur independently from sodium intake. This complicates the interpretation of balance studies and of 24-h urine collections. Overall, the Panel considers that balance studies cannot be used to determine sodium requirements.

The literature on the relationship between sodium intake and selected health outcomes, i.e. blood pressure, cardiovascular disease-related endpoints and bone health, was systematically reviewed. To minimise the risk of bias in the evidence used in the assessment, the review was restricted to randomised controlled trials (RCTs) and prospective studies, studies that excluded participants with pre-existing medical conditions, and studies that used at least one 24-h urinary collection to estimate sodium intake. Risk of bias in eligible studies was assessed using the OHAT-NTP critical appraisal tool. Studies were categorised according to their risk of bias based on a three-tier system (i.e. at low, moderate or high risk of bias).

Eligible studies on bone health provided limited and inconsistent evidence for an association between sodium intake and bone mineral density and could not be used to set DRVs for sodium.

Meta-analyses and modelling of the dose–response between 24-h sodium urinary excretion (UNa) and blood pressure were conducted. Random effects meta-analyses of the 32 eligible RCTs showed significant effects of sodium reduction on systolic blood pressure (SBP) (−3.9 (95% CI −5.1, −2.8) mm Hg; I^2 61.9%, $p < 0.001$) and diastolic blood pressure (DBP) (−2.0 (−2.8, −1.2) mm Hg; I^2 60.6%, $p < 0.001$). Using mixed-effects meta-regression models, mean SBP increased by 5.3 mm Hg (95% CI: 3.6–6.9 mm Hg) and mean DBP increased by 2.6 mm Hg (95% CI: 1.6–3.7 mm Hg) for each 100 mmol (2.3 g)/24 h increase in mean UNa. The Panel considers that there is strong evidence for a positive relationship between UNa and SBP and DBP over the range of mean UNa observed in the studies (between 49 and 209 mmol/24 h (1.3–4.8 g/day)). This is also supported by an eligible prospective observational study that investigated the long-term relationship between UNa and blood pressure levels and by the eligible studies that assessed the relationship between UNa and risk of hypertension (two RCTs and two prospective observational studies).

A small number of prospective observational studies assessing the relationship between UNa and CVD risk was eligible for the assessment: three cohort studies investigated the association between UNa and risk of stroke or coronary heart disease (CHD); three cohort studies investigated the association between UNa and risk of total CVD. Overall, only limited conclusions can be drawn on the relationship between UNa and risk of CVD. The Panel considers that, over the range of UNa observed in these studies:

- There is some evidence for a positive association between UNa and risk of CHD. The positive relationship between UNa and blood pressure levels/incidence of hypertension, which is an established independent risk factor for CHD, supports this association.
- There is some evidence for an inverse association between UNa and risk of stroke. However, the number of eligible studies available investigating this outcome is small and the mechanisms by which UNa could be inversely associated with the risk of stroke are unclear, particularly considering the positive relationship between UNa and blood pressure, which is an established risk factor for stroke.
- There is some evidence for a positive association between UNa and risk of total CVD, which is consistent with the evidence for a positive association between UNa and risk of CHD and the positive relationship between UNa and blood pressure levels/incidence of hypertension.

Overall, the Panel considers that the available evidence cannot be used to determine the sodium requirement in the population; so, an average requirement (AR) and population reference intake (PRI) for sodium cannot be established. Data on the relationship between sodium intake and blood pressure or CVD risks could inform about the levels of sodium intake associated with a reduced risk of chronic diseases.

Balance studies could inform about the levels of sodium intake that are adequate to maintain a null sodium balance. Expert judgement was used to weigh the available evidence and take account of the associated uncertainties by means of a formal expert knowledge elicitation (EKE). The EKE allows a representation of the uncertainty about the quantity (parameter) of interest using a probability distribution.

Integrating the available evidence and associated uncertainties, the Panel considers that a sodium intake of 2.0 g/day represents a level of sodium for which there is sufficient confidence in a reduced risk of CVD in the general adult population. Also, a sodium intake of 2.0 g/day is likely to allow most of the general adult population to maintain physiological sodium balance. Therefore, the Panel considers that 2.0 g sodium/day is a safe and adequate intake for the general EU population of adults (Table 8).

The requirement for the daily accretion rate of sodium in fetal and maternal tissues can be met by the adaptive changes that maintain sodium homeostasis during pregnancy. There is no evidence that the sodium requirement of lactating women differs from the requirement of non-lactating women. So, 2.0 g of sodium/day is a safe and adequate intake for pregnant and lactating women (Table 8).

Sodium intakes that are considered safe and adequate for children are extrapolated from the value for adults, adjusting for their respective energy requirement and including a growth factor, and are as follows: 1.1 g/day for children aged 1–3 years, 1.3 g/day for children aged 4–6 years, 1.7 g/day for children aged 7–10 years and 2.0 g/day for children aged 11–17 years, respectively (Table 8).

For infants aged 7–11 months, an Adequate Intake (AI) of 0.2 g/day is proposed based on upwards extrapolation of the estimated sodium intake in exclusively breast-fed infants aged 0–6 months, on the basis of the energy requirements of the respective age groups (Table 8).

The Panel notes that the mean/median intake of sodium in the European adult populations exceeds the safe and adequate intakes set for sodium. The risk of inadequate (insufficient) intake in European populations is low. Concerns for European populations instead relate to excess intake of sodium. Therefore, in practice, the values proposed can be used to inform the setting of population goals for the reduction in sodium intake.

4.15. Zinc

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3844/full

Zinc has a wide array of vital physiological functions. It has a catalytic role in each of the six classes of enzymes (EFSA NDA Panel, 2014g). The human transcriptome has 2,500 zinc finger proteins, which have a broad intracellular distribution and the activities of which include binding of RNA molecules and involvement in protein–protein interactions. Thus, their biological roles include transcriptional and translational control/modulation and signal transduction.

The majority of dietary zinc is absorbed in the upper small intestine. The luminal contents of the duodenum and jejunum, notably phytate, can have a major impact on the percentage of zinc that is available for absorption. Absorption of zinc by the enterocyte is regulated in response to the quantity of bioavailable zinc ingested. Albumin is the major transporter of zinc in both portal and systemic circulation. Virtually no zinc circulates in a free ionised form, and the majority of total body zinc is in muscle and bone; zinc does not have an identified major storage site. The quantity of zinc secreted into and excreted from the intestinal tract depends on body zinc concentrations, and the quantities of endogenous zinc in the faeces and exogenous zinc absorbed in normal adults are related. The kidneys and integument are minor routes of loss of endogenous zinc.

Plasma/serum zinc concentration and other putative biomarkers of zinc adequacy, deficiency and excess are not useful for estimating DRVs for zinc. Zinc requirements have been estimated by the factorial

approach involving two stages. The first is the estimation of physiological requirements, defined as the minimum quantity of absorbed zinc needed to match losses of endogenous zinc and to meet any additional requirements for absorbed zinc that may be necessary for growth in healthy well-nourished infants and children, and in pregnancy and lactation. The second stage is the determination of the quantity of dietary zinc available for absorption that is needed to meet these physiological requirements. From the published literature, 15 studies were identified that included data on endogenous faecal zinc and total absorbed zinc that enabled an estimation to be made of the physiological zinc requirements of adults. Individual's data from these studies were supplied by the authors. Data were assessed for physiological plausibility and, after careful evaluation, some data were excluded from further calculations. The final numbers of subjects contributing data to the estimate of physiological zinc requirements were 31 males and 54 females, from a total of 10 studies. Dietary phytate intakes were available for some of the included studies, either as mean study values or as individual's data. The range of dietary phytate intakes in the available data was 0–2,080 mg/day. Multiple regression analysis was used to evaluate the possible relationships between physiological requirements and sex, zinc balance (difference between absorbed zinc and total losses of endogenous zinc) and body size. The coefficient of determination (R^2) values for the models with body weight, height, body mass index and body surface area variables were 0.46, 0.42, 0.37 and 0.47, respectively. It was decided to use the equation relating physiological requirement to body weight in further analyses, for reasons of convenience and accuracy of measurement. The equation for physiological requirement was calculated on the basis that physiological requirement is equivalent to total absorbed zinc when absorbed zinc minus total endogenous zinc losses equals zero at a given body weight. For deriving the dietary zinc requirement, a trivariate saturation response model of the relationship between zinc absorption, and dietary zinc and phytate was established using 72 mean datasets (reflecting 650 individual measurements) reported in 18 publications. The R^2 of the fit of this model was 0.81. From this model, the Average Requirement (AR) was determined as the intercept of the total absorbed zinc needed to meet physiological requirements. Estimated ARs and Population Reference Intakes (PRIs) for zinc are provided for phytate intake levels of 300, 600, 900 and 1,200 mg/day, which cover the range of mean/median phytate intakes observed in European populations. ARs range from 6.2 to 10.2 mg/day for women with a reference body weight of 58.5 kg (Table 6) and from 7.5 to 12.7 mg/day for men with a reference body weight of 68.1 kg (Table 4). PRIs for adults were estimated as the zinc requirement of individuals with a body weight at the 97.5th percentile for reference body weights or men and women, respectively, and range from 7.5 to 12.7 mg/day for women (Table 7) and from 9.4 to 16.3 mg/day for men (Table 5).

For infants from seven months of age and children, DRVs for zinc were derived using the factorial approach, taking into account endogenous zinc losses via urine, sweat and integument, faeces and, in adolescent boys and girls, semen and menses, respectively, as well as zinc required for synthesis of new tissue for growth. Urinary and integumental losses were extrapolated based on estimates of adult losses, whereas endogenous faecal zinc losses were estimated by linear regression analysis of endogenous faecal zinc losses versus body weight for the subjects contributing data to the adult estimates, and for infants and young children from two studies from China and the USA. Zinc requirements for growth were taken into account based on the zinc content of new tissue, and by estimating daily weight gains for each age group. Absorption efficiency of zinc from mixed diets was assumed to be 30%. Estimated ARs range from 2.4 mg/day in infants aged 7–11 months to 11.8 mg/day in adolescent boys (Table 4, Table 6). Owing to the absence of reference body weights for infants and children at the 97.5th percentile, and in the absence of knowledge about the variation in requirements, PRIs for infants and children were estimated based on a coefficient of variation (CV) of 10%, and range from 2.9 to 14.2 mg/day (Table 5, Table 7).

The physiological requirements for pregnancy and lactation can be calculated by adding the increases in physiological requirements that are predicted to meet the demands for new tissue primarily of the conceptus, and the replacement of zinc that is secreted in breast milk. For pregnancy, an additional requirement for zinc for the four quarters of pregnancy of about 0.4 mg/day was assumed because of zinc accumulation in the fetus; placental, uterine and mammary tissue; amniotic fluid and maternal blood. The Panel decided not to use the trivariate model to estimate the dietary zinc intake required to meet the additional physiological requirement. Instead, the Panel applied a mean fractional absorption of zinc of 0.3 that has been observed in healthy adults to the physiological requirement of 0.4 mg/day. The additional

requirement for pregnant women was calculated to be 1.3 mg/day (Table 6) and the additional PRI for pregnancy was estimated based on a CV of 10% and was 1.6 mg/day (Table 7).

For lactation, taking into account breast milk zinc concentration, the breast milk volume transferred and the postnatal redistribution of zinc owing to involution of the uterus and reduction of maternal blood volume, the additional physiological requirement calculated over six months of lactation was estimated to be 1.1 mg/day. Assuming that fractional absorption of zinc is increased 1.5-fold in lactation, and applying a fractional absorption of zinc of 0.45 to the additional physiological requirement of 1.1 mg/day, resulted in an additional dietary requirement for lactating women of 2.4 mg/day (Table 6). The additional PRI for lactation, based on a CV of 10%, was 2.9 mg/day (Table 7).

Meat, legumes, eggs, fish, and grains and grain-based products are rich dietary zinc sources. On the basis of data from 12 dietary surveys in nine European Union (EU) countries, zinc intake was assessed using food consumption data from the EFSA Comprehensive Food Consumption Database and zinc composition data from the EFSA nutrient composition database. Average zinc intake ranged from 4.6 to 6.2 mg/day in children aged one to less than three years, from 5.5 to 9.3 mg/day in children aged 3 to < 10 years, from 6.8 to 14.5 mg/day in adolescents (10 to < 18 years) and from 8.0 to 14.0 mg/day in adults. The main food groups contributing to zinc intake were meat and meat products, grains and grain-based products, and milk and dairy products. Published data on phytate intake in the EU are limited and indicate a wide range of dietary phytate intakes.

Table 4: ARs for minerals, males

Age group (years)	Calcium (mg/d)	Age group (years)	Iron (mg/d)	Zinc (mg/d)	
				LPI (mg/d)	
7–11 mo[a]	[b]	7–11 mo[a]	**8**	[c]	**2.4**
1–3	**390**	1–3	**5**	[c]	**3.6**
4–6	**680**	4–6	**5**	[c]	**4.6**
7–10	**680**	7–10	**8**	[c]	**6.2**
11–14	**960**	11–14	**8**	[c]	**8.9**
15–17	**960**	15–17	**8**	[c]	**11.8**
18–24	**860**	≥ 18	**6**	300	**7.5**
≥ 25	**750**			600	**9.3**
				900	**11.0**
				1,200	**12.7**

d, day; LPI, level of phytate intake; mo, months

(a): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(b): an AI was set for infants (see Table 5)
(c): The fractional absorption of zinc considered in setting ARs for children was based on data from mixed diets expected to contain variable quantities of phytate; therefore, no adjustment for phytate intake has been made.

Table 5: PRIs and AIs for minerals, males

Age group (years)	Calcium (mg/d)	Age group (years)	Fluoride (mg/d)	Iodine (µg/d)	Manganese (mg/d)	Molybdenum (µg/d)	Phosphorus (mg/d)	Potassium (mg/d)	Selenium (µg/d)	Iron (mg/d)	Zinc (mg/d)		Age group (years)	Copper (mg/d)	Magnesium (mg/d)
											LPI (mg/d)				
7–11 mo[a]	280	7–11 mo[a]	0.4	70	0.02–0.5[b]	10	160	750	15	**11**	[c]	**2.9**	7–11 mo[a]	0.4	80
1–3	**450**	1–3	0.6	90	0.5	15	250	800	15	**7**	[c]	**4.3**	1–2	0.7	170
4–6	**800**	4–6	1.0	90	1.0	20	440	1,100	20	**7**	[c]	**5.5**	3–9	1.0	230
7–10	**800**	7–10	1.5	90	1.5	30	440	1,800	35	**11**	[c]	**7.4**	10–17	1.3	300
11–14	**1,150**	11–14	2.2	120	2.0	45	640	2,700	55	**11**	[c]	**10.7**			
15–17	**1,150**	15–17	3.2	130	3.0	65	640	3,500	70	**11**	[c]	**14.2**			
18–24	**1,000**	≥ 18	3.4	150	3.0	65	550	3,500	70	**11**	300	**9.4**	≥ 18	1.6	350
≥ 25	**950**										600	**11.7**			
											900	**14.0**			
											1,200	**16.3**			

d, day; LPI, level of phytate intake; mo, months

PRIs are presented **in bold type** and AIs in ordinary type

(a): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(b): In view of the wide range of manganese intakes that appear to be adequate, a range is set for the AI of this age group.
(c): The fractional absorption of zinc considered in setting PRIs for children was based on data from mixed diets expected to contain variable quantities of phytate; therefore, no adjustment for phytate intake has been made.

Table 6: ARs for minerals, females

Age group (years)	Calcium (mg/d)	Age group (years)	Iron (mg/d)	Age group (years)	Zinc (mg/d)	
					LPI (mg/d)	
7–11 mo[a]	(b)	7–11 mo[a]	**8**	7–11 mo[a]	(c)	**2.4**
1–3	**390**	1–3	**5**	1–3	(c)	**3.6**
4–6	**680**	4–6	**5**	4–6	(c)	**4.6**
7–10	**680**	7–11	**8**	7–10	(c)	**6.2**
11–14	**960**	12–14	**7**	11–14	(c)	**8.9**
15–17	**960**	15–17	**7**	15–17	(c)	**9.9**
18–24	**860**	≥ 18		≥ 18	300	**6.2**
≥ 25	**750**	Premenopausal	**7**		600	**7.6**
		Postmenopausal	**6**		900	**8.9**
					1,200	**10.2**
Pregnancy						
18–24	**860**		**7**			**+1.3[d]**
≥ 25	**750**					
Lactation						
18–24	**860**		**7**			**+2.4[d]**
≥ 25	**750**					

d, day; LPI, level of phytate intake; mo, months

(a): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(b): an AI was set for infants (see Table 7)
(c): The fractional absorption of zinc considered in setting ARs for children was based on data from mixed diets expected to contain variable quantities of phytate; therefore, no adjustment for phytate intake has been made.
(d): in addition to the ARs for non-pregnant, non-lactating women

Table 7: PRIs and AIs for minerals, females

Age group (years)	Calcium (mg/d)	Age group (years)	Fluoride (mg/d)	Iodine (µg/d)	Manganese (mg/d)	Molybdenum (µg/d)	Phosphorus (mg/d)	Potassium (mg/d)	Selenium (µg/d)	Zinc (mg/d) LPI (mg/d)	Zinc (mg/d)	Age group (years)	Iron (mg/d)	Age group (years)	Copper (mg/d)	Magnesium (mg/d)
7–11 mo[a]	280	7–11 mo[a]	0.4	70	0.02–0.5[b]	10	160	750	15	[c]	**2.9**	7–11mo[a]	**11**	7–11 mo[a]	0.4	80
1–3	**450**	1–3	0.6	90	0.5	15	250	800	15	[c]	**4.3**	1–3	**7**	1–2	0.7	170
4–6	**800**	4–6	0.9	90	1.0	20	440	1,100	20	[c]	**5.5**	4–6	**7**	3–9	1.0	230
7–10	**800**	7–10	1.4	90	1.5	30	440	1,800	35	[c]	**7.4**	7–11	**11**	10–17	1.1	250
11–14	**1,150**	11–14	2.3	120	2.0	45	640	2,700	55	[c]	**10.7**	12–14	**13**			
15–17	**1,150**	15–17	2.8	130	3.0	65	640	3,500	70	[c]	**11.9**	15–17	**13**			
18–24	**1,000**	≥ 18	2.9	150	3.0	65	550	3,500	70	300	**7.5**	≥ 18		≥ 18	1.3	300
≥ 25	**950**									600	**9.3**	Premenopausal	**16[d]**			
										900	**11.0**	Postmenopausal	**11**			
										1,200	**12.7**					
Pregnancy																
18–24	**1,000**		2.9	200	3.0	65	550	3,500	70		**+1.6[e]**		**16[d]**		1.5	300
≥ 25	**950**															
Lactation																
18–24	**1,000**		2.9	200	3.0	65	550	4,000	85		**+2.9[e]**		**16[d]**		1.5	300
≥ 25	**950**															

d, day; LPI, level of phytate intake; mo, months

PRIs are presented **in bold type** and AIs in ordinary type

(a): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(b): In view of the wide range of manganese intakes that appear to be adequate, a range is set for the AI of this age group.
(c): The fractional absorption of zinc considered in setting PRIs for children was based on data from mixed diets expected to contain variable quantities of phytate; therefore, no adjustment for phytate intake has been made.
(d): The PRI covers the requirement of approximately 95% of premenopausal women.
(e): in addition to the PRIs for non-pregnant, non-lactating women .

Table 8: Safe and adequate intakes for sodium and chloride, males and females

Age group (years)	Chloride [a][b] (g/d)	Sodium [b] (g/d)
7–11 mo	**0.3[c]**	**0.2[c]**
1–3	**1.7**	**1.1**
4–6	**2.0**	**1.3**
7–10	**2.6**	**1.7**
11–17	**3.1**	**2.0**
≥ 18 years[d]	**3.1**	**2.0**

d, day; mo, months

Safe and adequate intakes for sodium: the reference values for sodium are called 'safe' because it takes account of the evidence describing the relationship between sodium intake and CVD risk in the general population and 'adequate' in line with the definition of an AI. The value provides guidance on a level of sodium intake compatible with good health that can inform population goals for sodium. However, the value has limited utility for assessing and planning the diet of individuals. At the individual level, if the usual intake of sodium exceeds this value, it could be associated with an increased risk of cardiovascular diseases, including concurring risk factors such as primary hypertension.

Safe and adequate intakes for chloride: The reference values for chloride are set at values equimolar to the reference values for sodium, under the consideration that the main dietary source of chloride intake is sodium chloride. The reference values for chloride are called 'safe' and 'adequate' consistent with the use made of these terms for sodium.

(a): Derived by multiplying the reference values for sodium (EFSA NDA Panel, 2019) by 35.5/23 and rounded to the nearest 0.1.
(b): Equivalent to: 9 mmol/day for infants 7–11 months, 48 mmol/day for children aged 1–3 years, 57 mmol/day for children aged 4–6 years, 74 mmol/day for children aged 7–10 years, 87 mmol/day for children aged 11–17 years, 87 mmol for adults, including pregnant and lactating women.
(c): Adequate Intake.
(d): Including pregnant and lactating women.

5. Vitamins

5.1. Biotin

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In 1993, the Scientific Committee for Food proposed an Acceptable Range of Intakes of biotin for adults of 15–100 µg/day, based on observed intakes of biotin in European countries, which were considered adequate to meet requirements and prevent deficiency (EFSA NDA Panel, 2014c).

Biotin is a water-soluble vitamin which serves as a co-factor for several carboxylases that play critical roles in the synthesis of fatty acids, the catabolism of branched-chain amino acids and gluconeogenesis. Dietary biotin deficiency is rare.

Free biotin is absorbed nearly completely, while there is a lack of data on the absorption of protein-bound biotin from foods. In the cell, biotin is covalently attached to biotin-dependent carboxylases, from which it can be released by other enzymes, or, alternatively, is catabolised through different pathways. Biotin and its metabolites are excreted in the urine.

The Panel notes that biomarkers sensitive to biotin depletion have been identified. These include urinary biotin excretion and biomarkers of biotin function, such as urinary excretion of 3-hydroxyisovaleric acid (3HIA) and 3HIA-carnitine, activity of propionyl-CoA carboxylase and abundance of biotinylated β-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase in lymphocytes. However, data from the general population are limited so that the variability characteristics of these biomarkers and their ability to discriminate between biotin insufficiency and adequacy are not well known. Dose–response relationships between biotin intakes and these biomarkers have not been established. The Panel considers that data are insufficient to derive an Average Requirement (AR) for biotin from the use of available biomarkers of intake or status for any population group.

Data available on biotin intakes and health consequences are very limited and cannot be used to derive DRVs for biotin.

As the evidence to derive an AR, and thus a Population Reference Intake is considered insufficient, an Adequate Intake (AI) is proposed for all population groups. There is no indication that the AI should differ according to sex. The setting of AIs is based on observed biotin intakes with a mixed diet and the apparent absence of signs of deficiency in the EU, suggesting that current intake levels are adequate. Estimates of the biotin content of foods vary widely partly as a result of natural variation and partly depending on the analytical method used, and this contributes to uncertainty regarding current intake estimates. Estimates of biotin intakes in children, adolescents, adults and older adults were available from five EU countries. In boys and girls (5–12 years), mean/median intakes ranged from 19 to 38 µg/day, while mean/median intakes between 17 and 64 µg/day were reported for adolescent boys and girls (13–19 years). In adult men and women below about 65 years, mean/median intakes ranged from 26 to 50 µg/day, while mean/median intakes between 24 and 43 µg/day were reported for older adult men and women.

The AI for adults is set at 40 µg/day (Table 10: , Table 12). The AI for adults also applies to pregnant women (Table 12). For lactating women, an additional 5 µg/day over and above the AI for adults is proposed, to compensate for biotin losses through breast milk (Table 12). For infants over six months, an AI of 6 µg/day is proposed by extrapolating from the biotin intake of exclusively breastfed infants aged zero to six months, using allometric scaling (body weight to the power of 0.75) and reference body weight for each age group, in order to account for the role of biotin in energy metabolism, and rounding to the nearest unit (Table 10: , Table 12). The AIs for children aged 1–3 and 4–10 years are set at 20 and 25 µg/day, respectively, and for adolescents at 35 µg/day, based on observed intakes in the EU (Table 10: , Table 12).

5.2. Choline

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4484/full

Choline is a quaternary amine (2-hydroxyethyl-N,N,N-trimethylammonium) present in food in free and esterified forms (EFSA NDA Panel, 2016c). The main forms present in foods are phosphatidylcholine (PC, lecithin), which is also the main form present in animal tissues, free choline, phosphocholine (PChol), glycerophosphocholine (GPC) and sphingomyelin (SPM), and minor amounts of cytidine-5-diphosphate-choline (CDP-choline) and acetylcholine. Choline, PChol and GPC are water-soluble choline compounds, whereas PC and SPM are lipid-soluble compounds.

Although choline can be synthesised de novo by the human body, this synthesis may become insufficient, making choline an essential component of the diet. Choline is predominantly provided via the diet. The human body can form choline either de novo by methylation of phosphatidylethanolamine (PE) via the hepatic phosphatidylethanolamine N-methyltransferase (PEMT) pathway, or by hydrolysis of PC formed in the CDP-choline pathway in all cells of the body. The PC formed in the PEMT pathway contains substantial amounts of long-chain polyunsaturated fatty acids, like docosahexaenoic acid and arachidonic acid. Both pathways can be stimulated by dietary choline and the PEMT pathway is sensitive to the presence of oestrogens. Choline is an integral part of some phospholipids, which play an important role in the structure and function of membranes. Choline (as PC) plays an important role in the metabolism and transport of lipids and cholesterol by lipoproteins, and is needed for the assembly and secretion of very low-density lipoproteins by the liver. Choline is a precursor of the neurotransmitter acetylcholine, and of betaine, an osmoregulator to which choline is irreversibly oxidised in the liver and kidney. Via betaine, choline is involved in the folate-dependent one-carbon metabolism. Dietary deficiency of choline can cause fatty liver or hepatic steatosis that can result in non-alcoholic fatty liver disease (NAFLD), and can cause liver and muscle damage. This shows that de novo production can be insufficient.

Dietary free choline is quickly taken up by a carrier-mediated saturable transport system. PC and GPC from the diet or secreted in the bile, and dietary SPM are hydrolysed by phospholipases to liberate choline. Choline and water-soluble choline compounds (PChol and GPC) are rapidly absorbed and appear in plasma predominantly as free choline. Phospholipids (PC and SPM) that have escaped phospholipases enter the lymph incorporated into chylomicrons. The available data do not allow defining the percentage of intestinal absorption of choline in humans, and the total amount of choline in the human body. Non-absorbed choline is a precursor of trimethylamine (TMA) produced in the gut by anaerobic symbiotic microbes. TMA is efficiently absorbed from the gastrointestinal tract and then converted in the liver to trimethylamine-N-oxide (TMAO). Both TMA and TMAO (i.e. total trimethylamine (TTMA)) are eliminated in the urine. Urinary excretion of choline is low in relation to usual dietary intakes, while no human data are available on faecal excretion of choline or choline compounds in relation to dietary intake. Breast milk mainly contains PChol and GPC, besides free choline, PC and SPM, in concentrations depending on the progress of lactation, maternal diet and genotype.

The Panel reviewed possible biomarkers of choline intake and/or status. The Panel considers that the available data do not allow conclusions to be drawn on a dose–response relationship between choline intake or status and plasma choline concentration, and that plasma choline concentrations cannot be used to set DRVs for dietary choline. Plasma concentrations of PC, betaine, dimethylglycine, total homocysteine or TMAO, erythrocyte PC concentration, or urinary betaine and TTMA urinary excretion also cannot be used to set DRVs for dietary choline. The Panel also notes that singlenucleotide polymorphisms in genes coding for enzymes involved in choline metabolism, some of them present with high frequency in the population, can influence the dietary requirement for choline and determine the susceptibility to dietary choline deficiency, but data are insufficient to predict variations in individual choline requirements based on genetic polymorphisms. The Panel considers that the available data on choline intake and health consequences (NAFLD, cardiovascular disease, cancer, birth defects, cognition) cannot be used to set DRVs for dietary choline.

The Panel considers that Average Requirements and Population Reference Intakes for choline cannot be derived for adults, infants and children, and therefore defines Adequate Intakes (AIs). Dietary total choline intake was calculated based on individual food consumption data that were available to the European Food Safety Authority (EFSA) and classified according to EFSA's food classification system, from healthy populations investigated in 12 national surveys undertaken in nine countries of the European Union (EU), between 2000 and 2011. In the absence of food composition data on choline in Europe, composition data on free choline and choline compounds from the US Department of Agriculture were used. The total choline intake mean estimates ranged from 75 to 127 mg/day in infants, from 151 to 210 mg/day in children aged from 1 to < 3 years, from 177 to 304 mg/day in children aged from 3 to < 10 years, and from 244 to 373 mg/day among children aged from 10 to < 18 years. The total choline intake mean estimate was 336 mg/day in pregnant adolescents and 356 mg/day in pregnant women. The total choline intake mean estimates ranged from 269 to 444 mg/day and from 332 to 468 mg/day in women and men, respectively, i.e. for all adults: 269–468 mg/day.

The Panel reviewed 11 choline depletion/repletion studies with similar design. Only one reported the amounts of choline needed to replete depleted subjects who showed signs of organ dysfunction. The Panel concludes that choline depletion/repletion studies do not provide sufficient data to calculate Average Requirements for choline, but may be used to inform data on observed choline intakes to set AIs for choline.

For all adults, the Panel set an AI of 400 mg/day (Table 10: , Table 12). This is based on the midpoint of the range of observed mean intakes in healthy populations in the EU (about 370 mg/day), and in consideration of the results of a depletion–repletion study in which about 70% of the depleted subjects who had developed signs of organ dysfunction were repleted with an intake of about 400 mg/70-kg body weight per day. Although premenopausal women may have a lower requirement for dietary choline (than postmenopausal women or men) in connection with a potential stimulation of the PEMT pathway by oestrogens, and ranges of estimated mean total choline intake in Europe are slightly lower in women than men, the Panel considered it unnecessary to give sex-specific AIs for adults.

For infants aged 7–11 months, the Panel set an AI of 160 mg/day, based on the estimated intake of choline of exclusively breast-fed infants from birth to 6 months, and upwards extrapolation by allometric scaling, taking into account the difference in reference body weight (Table 10: , Table 12).

For all children aged 1–17 years, no data are available that would justify different AIs for boys and girls. The Panel set AIs ranging from 140 mg/day (1–3 years) to 400 mg/day (15–17 years) (Table 10: , Table 12). These were set by downwards extrapolation from the adult AI, by allometric scaling, taking into account the difference in reference body weight and applying growth factors. These AIs are supported by total choline intake mean estimates in the EU.

The Panel considered that, although the available intervention studies on choline supplementation in the second half of pregnancy or in lactating women indicate that pregnant or lactating women may need more choline than non-pregnant non-lactating women, the data are not sufficient to allow an estimate of the additional requirement for dietary choline in pregnant or lactating women (above that of non-pregnant non-lactating women).

For pregnant women, the Panel set an AI of 480 mg/day, calculated by isometric scaling from the AI for non-pregnant women, using the mean gestational increase in body weight (Table 12). For lactating women, the AI for non-lactating women is increased to account for the secretion of choline through breast milk. The Panel set an AI of 520 mg/day, considering an average concentration of choline in mature breast milk of 145 mg/L, and a mean milk transfer during the first 6 months of lactation in exclusively breastfeeding women (0.8 L/day) (Table 12).

5.3. Cobalamin

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Cobalamin is a metal complex with a central cobalt atom bonded to six ligands (EFSA NDA Panel, 2015d). The upper or β-axial ligand varies (R-group: cyano-, hydroxo-, aquo-, methyl- or adenosyl-group), giving rise to the correspondingly named chemical forms of the vitamin. In humans, two reactions are known to require cobalamin as coenzyme. One is the rearrangement of methylmalonyl-coenzyme A (CoA) to succinyl-CoA in propionate metabolism by methylmalonyl-CoA mutase in mitochondria. The other is the cytosolic transmethylation of homocysteine by 5-methyl-tetrahydrofolate to methionine by methionine synthase. The most frequent clinical expression of cobalamin deficiency is megaloblastic anaemia. Independent of megaloblastic anaemia, neurological dysfunction is another clinical feature of cobalamin deficiency. Cobalamin insufficiency is characterised by biochemical abnormalities, such as elevated total homocysteine (tHcy) and/or methylmalonic acid (MMA) concentrations in blood resulting from impaired cobalamin metabolic activity, with no specific clinical symptoms.

Cobalamin absorption consists of several steps, including its release from proteins, its binding by gastric intrinsic factor and the absorption of intrinsic factor-cobalamin complexes through receptormediated endocytosis in the terminal ileum. Fractional absorption of cobalamin is rather variable, depending on the dietary source, the amount of cobalamin ingested, the ability to release cobalamin from food and the proper functioning of the intrinsic factor system. The Panel considers an absorption of 40% as a conservative estimate.

In plasma, cobalamin is bound to the cobalamin-binding proteins transcobalamin (TC) and haptocorrin. HoloTC is the physiologically active form of cobalamin that delivers the vitamin to cells. Intracellular cobalamin concentration is maintained by modulating the expression of holoTC receptor, with an efflux system that shunts the excess cobalamin out of the cells. In contrast, cobalamin accumulates in the liver and kidney. Various studies have indicated losses of 0.1–0.2% of the cobalamin pool per day, regardless of the size of the store. The highest losses of cobalamin occur through the faeces, which include cobalamin secreted in the bile. If the circulating cobalamin exceeds the cobalamin binding capacity of the blood, the excess is excreted in the urine.

Main biomarkers of cobalamin status include blood concentrations of cobalamin, holoTC and the metabolites MMA and tHcy. The sensitivity and specificity of these biomarkers can be affected by factors unrelated to cobalamin status. The limitations of all biomarkers make a combination of biomarkers necessary to assess cobalamin status.

From experimental data in individuals with pernicious anaemia in remission, a daily intake of 1.5–2 µg cobalamin represents a minimum requirement for maintenance of a normal haematological status, associated with low body stores of 1–2 mg. Based on a factorial approach and estimating daily obligatory losses of cobalamin, estimated cobalamin requirement ranges between 4 and 20 µg/day, which reflects the large uncertainties associated with this approach. The Panel considers the approach based on a combination of cobalamin biomarkers of status as the most suitable approach to derive DRVs for cobalamin for adults. The Panel notes the uncertainties with respect to cut-off values for cobalamin insufficiency of these indicators and that an Average Requirement (AR) cannot be determined from the limited data available. There is consistent evidence from observational and intervention studies that a cobalamin intake of 4 µg/day and greater is associated with serum concentrations of holoTC and cobalamin within the reference ranges derived from healthy subjects, together with MMA and tHcy concentrations below the cut-off values for adults, which indicates an adequate cobalamin status. Therefore, the Panel sets an AI for cobalamin at 4 µg/day for adults based on data on different biomarkers of cobalamin status and in consideration of observed intakes, which range between 4.2 and 8.6 µg/day in adults in several EU countries (Table 10: , Table 12).

The Panel considers that there are insufficient data to derive an AR for infants and children. Therefore, AIs are calculated by extrapolation from the AI for adults. Allometric scaling was used on the assumption that cobalamin requirement is related to metabolically active body mass, and growth factors were applied. After rounding, estimated AIs range from 1.5 µg/day in infants aged 7–11 months to 4 µg/day in children aged 15–17 years (Table 10: , Table 12).

For pregnant women, a cobalamin intake of 0.5 µg/day in addition to the AI for non-pregnant women is proposed in consideration of a fetal accumulation of 0.2 µg cobalamin/day and of an absorption of 40%. This addition results in an AI of 4.5 µg/day for pregnant women (Table 12).

For lactating women, an increase in the AI is based on the cobalamin intake required to compensate for the amount of cobalamin secreted in breast milk. Considering a cobalamin concentration of 0.5 µg/L and a mean milk transfer of 0.8 L/day during the first six months of lactation in exclusively breastfeeding women, an average secretion with breast milk of 0.4 µg cobalamin/day is estimated. Taking into account 40% absorption, a mean cobalamin intake of 1.0 µg/day is required to balance the secretion in milk, and results in an AI of 5 µg/day for lactating women (Table 12).

Based on data from 13 dietary surveys in nine European Union countries, average cobalamin intake ranges across countries were 0.8–2.1 µg/day in infants < 1 year, 2.2–4.0 µg/day in children aged 1 to < 3 years, 2.6–5.7 µg/day in children aged 3 to < 10 years, 3.3–6.6 µg/day in children aged 10 to < 18 years and 4.2–8.6 µg/day in adults.

5.4. Folate

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3893/full

Folate is a generic term used for a family of compounds which belong to the group of B-vitamins (EFSA NDA Panel, 2014b). Naturally occurring food folates are a mixture of reduced mono- and polyglutamates, and their chemical structure makes them unstable. In contrast, the synthetic folic acid, which arises in the diet only through ingesting fortified foods or food supplements, is a fully oxidised monoglutamate and the most chemically stable form. Upon ingestion, polyglutamated folate forms are hydrolysed to monoglutamates and actively absorbed by a pH-dependent saturable mechanism in the duodenum and upper jejunum, or by passive diffusion in the ileum if consumed in supraphysiological amounts. Natural food folates have a lower bioavailability than folic acid. In order to take into account these differences, dietary folate equivalents (DFE) have been introduced and defined as 1 µg DFE = 1 µg food folate = 0.6 µg folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a folic acid supplement taken on an empty stomach.

Folates function as cofactors for enzymes involved in one-carbon metabolism. Folate provides one-carbon units for the formation of nucleotides necessary for the synthesis of RNA and DNA. Folate is also fundamental for the normal functioning of the methionine cycle, which is responsible for both the conversion of homocysteine to methionine and the production of the universal methyl donor S-adenosylmethionine (SAM). SAM donates its methyl group to more than 100 methyltransferases for a wide range of substrates such as DNA, hormones, proteins, neurotransmitters and membrane phospholipids, all of which are regulators of important physiological processes. Folate deficiency impairs DNA replication and cell division, which adversely affects rapidly proliferating tissues such as bone marrow and results in the production of unusually large macrocytic cells with poorly differentiated nuclei. The predominant feature of folate deficiency is megaloblastic anaemia.

Serum and red blood cell folate concentrations are sensitive biomarkers of folate intake and status, and the Panel considers that these are suitable primary criteria for deriving DRVs for folate. Although plasma total homocysteine on its own is not suitable for use as a biomarker of folate status, the Panel notes that its relationship with folate can be used to define the blood folate concentrations necessary for plasma total homocysteine to level off. The Panel considers that the previously defined cut-offs for folate adequacy

(serum folate of ≥ 10 nmol/L and red blood cell folate of ≥ 340 nmol/L), based on the inverse and non-linear association of plasma total homocysteine with serum and red blood cell folate concentrations, are suitable criteria for determining the requirement for folate. Homozygosity for the T allele of the methylene-tetrahydrofolate reductase (MTHFR) 677C→T polymorphism, which has a prevalence of up to 24% in some European countries, is associated with low folate status and mostly unfavourable health effects. The Panel considers that this polymorphism should be taken into account when determining the requirement for folate. The Panel has also considered several health outcomes that are possibly associated with folate intake and status, but data are insufficient to establish DRVs.

For healthy adult men and women, an Average Requirement (AR) of 250 µg DFE/day is proposed based on results of one controlled study showing that an intake of 205–257 µg DFE/day for seven weeks after a depletion phase maintains serum folate concentrations above the cut-off for folate adequacy in at least half of the group (Table 9: , Table 11:). These findings are in close agreement with those of two other controlled studies showing that folate intakes of around 200–300 µg/day may be sufficient to maintain serum and red blood cell folate concentrations of ≥ 10 and ≥ 340 nmol/L, respectively. A Population Reference Intake (PRI) of 330 µg DFE/day is derived assuming a coefficient of variation (CV) of 15% to account for the additional variability associated with the higher requirement for folate in individuals with the MTHFR 677TT genotype (Table 10: , Table 12).

For infants aged 7–11 months, an Adequate Intake (AI) of 80 µg DFE/day is derived by extrapolating upwards from the estimated folate intake from breast milk of exclusively breast-fed infants for which folate deficiency has not been observed (Table 10: , Table 12).

For children and adolescents, the ARs for folate are extrapolated from the AR for adults by allometric scaling and the use of growth factors (Table 9: , Table 11:). The PRIs are derived by assuming a CV of 15% and range from 120 µg DFE/day for 1–3 year old children to 330 µg DFE/day for both boys and girls aged 15–17 years (Table 10: , Table 12).

In pregnancy, intakes of 630–680 µg DFE/day administered in a controlled study to pregnant women during their second and third trimesters resulted in concentrations of biomarkers of folate status well above cut-offs for folate adequacy as established in non-pregnant adults. Acknowledging that the database is weaker than that for non-pregnant adults, an AI of 600 µg DFE/day for folate is proposed for pregnancy (Table 12).

For lactating women, an additional requirement of 130 µg DFE/day is derived to compensate for folate losses through breast milk. By adding this additional requirement to account for losses to the AR for non-lactating women, an AR of 380 µg DFE/day is obtained (Table 11:). Assuming a CV of 15%, a PRI of 500 µg DFE/day is established (Table 12).

5.5. Niacin

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Niacin is a generic term for nicotinic acid and nicotinamide, soluble organic compounds that belong to the group of B vitamins (EFSA NDA Panel, 2014h). Niacin is found in a wide range of foods. Main food groups contributing to niacin intakes of adults include meat and meat products, grains and grain-based products and milk and milk products. Depending on the foodstuff, the mean absorption of niacin is from about 23% to about 70%; it is lowest from cereals and highest from animal products. Niacin can be synthesised in the human body from the indispensable amino acid tryptophan. Approximately 60 mg of tryptophan yields 1 mg of niacin defined as 1 mg niacin equivalent (NE). Inadequate iron, riboflavin or vitamin B6 status decreases the conversion of tryptophan to niacin.

In vivo nicotinic acid is converted to nicotinamide, which is a precursor for nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are essential to cells

and involved in many biochemical reactions. Niacin circulates in the plasma as nicotinamide and nicotinic acid. Both forms are transported to cells and tissues, which they enter by diffusion to perform the intracellular functions of niacin. Niacin is trapped within the cell as NAD or NADP.

The major pathway of catabolism of nicotinic acid and nicotinamide is by methylation in the liver to *N*-methyl-nicotinamide (NMN) and subsequent oxidation to *N*-methyl-2-pyridone-carboxamide (2-Pyr) and *N*-methyl-4-pyridone-carboxamide (4-Pyr). In humans, the two major excretion products are NMN and 2-Pyr, which under normal conditions represent about 20–35% and 45–60% of niacin metabolites, respectively. The amount of niacin metabolites excreted depends on the niacin and tryptophan intake. Long-term inadequate intake of tryptophan and niacin results in reduced urinary excretion of niacin metabolites, and can lead to the development of pellagra. Based on experimental studies on niacin deficiency, it is recognised that niacin requirement is strongly dependent on energy intake. No signs of niacin deficiency were observed in subjects on diets containing at least approximately 1 mg NE/MJ (4.4 mg NE/1,000 kcal), while providing no less than 8.4 MJ/day (2,000 kcal/day). Diets providing at least 1.3 mg NE/MJ (5.5 mg NE/1,000 kcal) were sufficient to prevent depletion and maintain niacin body stores, as indicated by a sharp increase in urinary excretion of niacin metabolites above this intake.

The Panel notes that no new scientific data that would necessitate an amendment of the DRVs for niacin have become available since the publication of the Scientific Committee for Food (SCF) report in 1993. The Panel therefore endorses the relationship proposed by SCF (1993) between niacin requirement and energy requirement.

The Panel endorses the Average Requirement (AR) for adults (men and women) of 1.3 mg NE/MJ (about 5.5 mg NE/1,000 kcal) (Table 9: , Table 11:) and the Population Reference Intake (PRI) of 1.6 mg NE/MJ (about 6.6 mg NE/1,000 kcal) adopted by SCF (1993) assuming a coefficient of variation of 10% (Table 10: , Table 12). The Panel considers that there is no evidence that the relationship between niacin requirement and energy requirement for infants aged 7–11 months, children and adolescents differs from that of adults. Therefore, the AR and PRI for adults are applied to these age groups as well. The Panel also considers that, in pregnant and lactating women, there is no evidence that the relationship between niacin requirement and energy requirement differs from that of other adults. Therefore, the AR and PRI for adults are applied to these life stage groups (Table 11: , Table 12). Taking into account the reference energy intake, i.e. the AR for energy for various Physical Activity Levels (PAL values), the intake of NE/MJ is also expressed as mg NE/day. The Panel notes that, as for other nutrient reference values, DRVs for niacin are set under the assumption that intakes of other essential nutrients, particularly iron, riboflavin, vitamin B6 and protein, and energy are adequate.

5.6. Pantothenic acid

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In 1993, the Scientific Committee for Food (SCF) proposed an Acceptable Range of Intakes of pantothenic acid for adults of 3–12 mg/day, based on observed intakes of pantothenic acid in European countries, which were considered adequate to meet requirements and prevent deficiency (EFSA NDA Panel, 2014a).

Pantothenic acid is a water-soluble vitamin, which is a component of coenzyme A (CoA) and acylcarrier proteins. Pantothenic acid is ubiquitous and deficiency is rare. Foods rich in pantothenic acid include meat (products), eggs, nuts, avocados and cruciferous vegetables. The main contributors to pantothenic acid intakes include meat products, bread, milk-based products and vegetables.

Data on pantothenic acid absorption are lacking. Most of the pantothenic acid in tissues is present as CoA, mainly found in mitochondria, with lesser amounts present as acyl-carrier protein and free pantothenic acid. Pantothenic acid is excreted in urine, after hydrolysis of CoA in a multistep reaction.

Urinary excretion of pantothenic acid and, to a lesser extent, pantothenic acid concentration in whole blood or erythrocytes reflect pantothenic acid intake. Data from the general population are limited so that the variability characteristics of these biomarkers and their ability to discriminate between pantothenic acid insufficiency and adequacy are not well known. No cut-off values have been established for these biomarkers. The Panel considers that there are no suitable biomarkers that can be used to derive the Average Requirement (AR) for pantothenic acid.

Data available on pantothenic acid intakes and health consequences are very limited and cannot be used for deriving DRVs for pantothenic acid.

As the evidence to derive an AR and thus a Population Reference Intake is considered insufficient, an Adequate Intake (AI) is proposed for all population groups. There is no indication that the AI should differ according to sex. The setting of AIs is based on observed pantothenic acid intakes with a mixed diet and the apparent absence of signs of deficiency in the EU, suggesting that current intake levels are adequate. Estimates of pantothenic acid intakes in children and adolescents, adults and older adults were available from eight EU countries. In boys and girls (3–12 years), mean/median intakes of 3.0 to 5.7 mg/day were reported, while mean/median intakes of 3.0 to 7.2 mg/day were observed in adolescent boys and girls (11–19 years). In adult men and women below about 65 years, mean/median intakes of 3.2 to 6.3 mg/day were reported, while mean/median intakes were between 2.2 and 6.0 mg/day in older men and women. Data on pantothenic acid intakes in pregnancy were scarce.

The AI for adults is set at 5 mg/day (Table 10: , Table 12). The AI for adults also applies to pregnant women (Table 12). For lactating women, an AI of 7 mg/day is proposed, to compensate for pantothenic acid losses through breast milk (Table 12). For infants over six months, an AI of 3 mg/day is proposed by extrapolating from the pantothenic acid intake of exclusively breast-fed infants aged zero to six months, using allometric scaling (body weight to the power of 0.75) and reference body weight for each age group, in order to account for the role of pantothenic acid in energy metabolism, and rounding to the nearest unit (Table 10: , Table 12). The AIs for children and adolescents are set at 4 and 5 mg/day, respectively, based on observed intakes in the EU (Table 10: , Table 12).

5.7. Riboflavin

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4919/full

Riboflavin or 7,8-dimethyl-10-ribityl-isoalloxazine, is a water-soluble compound naturally present in food of plant and animal origin as free riboflavin and, mainly, as the biologically active derivatives flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (EFSA NDA Panel, 2017a).

Riboflavin is the integral part of the coenzymes FAD and FMN that act as the cofactors of a variety of flavoprotein enzymes such as glutathione reductase or pyridoxamine phosphate oxidase (PPO). FAD and FMN act as proton carriers in redox reactions involved in energy metabolism, metabolic pathways and formation of some vitamins and coenzymes. In particular, riboflavin is involved in the metabolism of niacin and vitamin B6 and FAD is also required by the methylenetetrahydrofolate reductase (MTHFR) in the folate cycle and thereby is involved in homocysteine metabolism. Signs of riboflavin deficiency are unspecific and include sore throat, hyperaemia and oedema of the pharyngeal and oral mucous membranes, cheilosis, glossitis (magenta tongue), and normochromic normocytic anaemia characterised by erythroid hypoplasia and reticulocytopenia. No tolerable upper intake level has been set for riboflavin.

Dietary riboflavin associated with food protein is hydrolysed to free riboflavin and its absorption mainly takes place in the proximal small intestine through carrier-mediated, saturable transport process. The Panel considers an absorption efficiency of dietary riboflavin of 95%. Free riboflavin transported into enterocytes is subjected to phosphorylation to form FMN, subsequently converted to FAD. From the small intestine, riboflavin enters the plasma, where FAD is reported to be the major form. The uptake of riboflavin into the cells of organs such as the liver is facilitated and may require specific carriers. Absorbed riboflavin appears

partly in the plasma, and partly is sequestered by the liver on the first pass through the portal vein from the gut. There is a positive transfer of riboflavin from the pregnant woman to the fetus. Most of the riboflavin in tissues including erythrocytes exists predominantly as FAD and FMN, covalently bound to enzymes. Unbound FAD and FMN are rapidly hydrolysed to free riboflavin that diffuses from cells and is excreted. When riboflavin is absorbed in excess, it is catabolised to numerous metabolites and little is stored in the body tissues. Urine is the main route for elimination of riboflavin.

The Panel reviewed possible biomarkers of riboflavin status and intake, i.e. urinary excretion of riboflavin, erythrocyte glutathione reductase activation coefficient (EGRAC), plasma and erythrocyte riboflavin, FAD and FMN, as well as PPO activity and activation coefficient. The Panel considers that the inflection point in the mean urinary riboflavin excretion curve in relation to riboflavin intake reflects body saturation and can be used to indicate adequate riboflavin status. The Panel also considers that EGRAC is a useful biomarker of riboflavin status and that EGRAC of 1.3 or less indicates adequate riboflavin status in all population groups. However, the Panel considers that the data on the relationship between riboflavin intake and EGRAC cannot be used alone to set DRVs for riboflavin, but can be used in support of data on the inflection in the urinary excretion curve in view of setting DRVs for riboflavin.

The Panel also notes that riboflavin status is modified by physical activity as urinary excretion of riboflavin is (generally) decreased and EGRAC increased when physical activity is increased, suggesting higher utilisation of riboflavin with increased energy expenditure. However, there is a lack of experimental data showing a clear quantitative relationship between riboflavin status biomarkers (urinary excretion of riboflavin and EGRAC) and energy expenditure (or physical activity). In addition, the Panel considers that relationship between riboflavin intake and biomarkers of riboflavin status is also influenced by MTHFR C677T polymorphism, as homozygosity for the T allele can increase the individual requirement for riboflavin, although the extent of this increase cannot be defined. After having reviewed the existing evidence, the Panel concludes that available data on intake of riboflavin and health outcomes cannot be used to derive DRVs for riboflavin.

The Panel notes that new scientific data have become available for adults since the publication of the Scientific Committee for Food (SCF) report in 1993, and considers that updated average requirements (ARs) and population reference intake (PRIs) can be set for adults, children, pregnant and lactating women.

For adults, the Panel considers that an AR of 1.3 mg/day (after rounding) can be determined from the weighted mean of riboflavin intake associated with the inflection point in the mean urinary riboflavin excretion curve in relation to riboflavin intake as reported in four intervention studies in different non-European Union (EU) countries (Table 9: , Table 11:). The Panel considers that the potential effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data presented from the studies considered, thus is accounted for in the assumed the coefficient of variation (CV) applied to set the PRI for riboflavin. A CV of 10% was used to calculate PRIs from the ARs for adults, i.e. 1.6 mg/day after rounding (Table 10: , Table 12), and the same CV was used for all other population groups. The Panel considers that there is no indication of different riboflavin requirement according to sex or between younger and older adults, and sets the same DRV for men and women (without correction per difference in body weight) of all ages.

For all infants aged 7–11 months, in the absence of sufficient data to set an AR, the Panel sets an AI of 0.4 mg/day based on the estimated intake of riboflavin of exclusively breastfed infants from birth to six months, and upward extrapolation by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass), taking into account the difference in reference body weight (Table 10: , Table 12).

For children aged 1–17 years, the Panel sets ARs by downward extrapolation from the AR of adults, by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass), applying growth factors and taking into account the differences in reference body weight. The Panel considers unnecessary to set sex-specific ARs and PRIs for boys and girls of all ages. The Panel sets ARs ranging from 0.5 (children aged 1–3 years) to 1.4 mg/day (children aged 15–17 years) (Table 9: , Table

11:) and PRIs ranging from 0.6 (children aged 1–3 years) to 1.6 mg/day (children aged 15–17 years) (Table 10: , Table 12).

For pregnant women, the Panel considers that data are insufficient to estimate the additional needs for dietary riboflavin during pregnancy based on fetal uptake and riboflavin accretion in the placenta during pregnancy. The Panel sets an AR of 1.5 mg/day, calculated by allometric scaling from the AR for non-pregnant women, considering the mean gestational increase in body weight of 12 kg (Table 11:), and also sets a PRI of 1.9 mg/day (Table 12).

For lactating women, an additional riboflavin requirement of 0.31 mg/day is calculated considering the secretion of riboflavin into milk during lactation (0.291 mg/day), the mean milk transfer during the first six months of lactation in exclusively breastfeeding women (0.8 L/day), and an absorption efficiency of 95%. An AR of 1.7 mg/day is calculated by the Panel, considering the additional requirement above the AR of non-lactating women (Table 11:), and a PRI of 2 mg/day is set for lactating women (Table 12).

Based on data from 13 surveys in nine countries of the EU, riboflavin intake mean estimates ranged across countries from 0.6 to 1.2 mg/day in infants (< 1 year), from 0.9 to 1.4 mg/day in children aged 1 to < 3 years, from 1 to 1.8 mg/day in children aged 3 to < 10 years, and from 1.2 to 2.2 mg/day in children aged 10 to < 18 years. Riboflavin intake mean estimates ranged between 1.4 and 2.2 mg/day in adults.

5.8. Thiamin

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4653/full

Thiamin is a water-soluble vitamin composed of a thiazole and a pyrimidine ring linked by a methylene group. In human tissues, thiamin occurs mostly in phosphorylated forms as thiamine monophosphate (TMP), thiamin diphosphate (TDP, called also thiamin pyrophosphate), thiamine triphosphate (TTP), as well as its non-phosphorylated form ('free thiamin') (EFSA NDA Panel, 2016a). Free thiamin functions as the precursor for TDP, which acts as a coenzyme for enzymes involved in carbohydrate and branchedchain amino acid metabolism, and in energy-yielding reactions. Thiamin deficiency leads to disorders that include several forms of beriberi, with mostly neurological and cardiovascular manifestations.

Thiamin in food exists mainly in phosphorylated forms in animal products, and in free form in foods of plant origin. Upon ingestion, thiamin phosphate esters are hydrolysed in the intestinal lumen by phosphatases. Free thiamin is taken up through the mucosal membrane by a specific saturable transport system. In healthy subjects, thiamin absorption is above 95% at usual intakes. Alcohol and anti-thiamin factors (such as some phenolic compounds, sulfites and thiaminases) can reduce thiamine bioavailability. Thiamin in blood is mainly found in erythrocytes (> 80% of total thiamin in the blood) in the form of TDP and TTP, while low amounts of the vitamin are present in plasma, as free thiamin, TMP and protein-bound TDP. Thiamin in the body is mostly located in the skeletal muscles, heart, brain, liver and kidneys.

Urine is the main route of thiamin excretion, mainly in the form of free thiamin and thiamine metabolites. The Panel notes that 24-h urinary thiamin excretion is related to thiamin intake, particularly to short-term intakes, in thiamin-replete individuals. However, the thiamin intake cannot reliably be estimated from the urinary excretion of the vitamin. The determination of 24-h urinary thiamin excretion is not a reliable marker of thiamin body stores and cannot, on its own, be used as a biomarker of the thiamin status of individuals. Still, in experimental studies where 24-h urinary thiamine excretion is assessed in response to various intakes of the vitamin, a sharp increase in thiamine excretion is considered to be indicative of the saturation of the thiamin body stores.

Measurement of the erythrocyte transketolase activity (ETKA), a TDP-requiring enzyme, is a functional test of thiamin status. The erythrocyte transketolase activity coefficient (aETK, also called 'TDP effect') represents the degree to which ETKA rises in response to addition of TDP. This test can discriminate low ETKA due to thiamin deficiency from a lack of the apoenzyme. A value of aETK < 1.15 is generally

considered to reflect an adequate thiamin status. The concentrations of total thiamin (free thiamin and its phosphate esters) in whole blood, serum and erythrocytes have also been investigated as biomarkers of thiamin status. Erythrocyte TDP concentration was found to have similar performance as the erythrocyte transketolase activation assay for assessment of thiamin status. The Panel notes, however, the lack of established cut-offs for these biomarkers.

The Panel considers that data from depletion–repletion studies in adults on the amount of dietary thiamin intake associated with aETK < 1.15 or with the restoration of normal (baseline) ETKA, without a sharp increase in urinary thiamin excretion, can be used to estimate thiamin requirement. In the absence of new scientific evidence, the Panel endorses the average requirement (AR) of 0.072 mg/MJ (0.3 mg/1,000 kcal) for all adults set by the Scientific Committee for Food (SCF) in 1993 on the basis of one depletion–repletion study in seven healthy males, in which both aETK and urinary excretion of thiamin were measured (Table 9: , Table 11:). Results from other depletion–repletion studies are in agreement with this value. The Panel notes that the AR was based on data on a small number of men, and agrees on the coefficient of variation of 20% used by the SCF to cover uncertainties related to distribution of thiamine requirements in the general population. The Panel endorses the population reference intake (PRI) of 0.1 mg/MJ (0.4 mg/1,000 kcal) set by the SCF for all adults (Table 10: , Table 12). No new evidence has become available that the relationship between thiamin requirement and energy requirement differs between men and women, or between younger and older adults.

The Panel proposes the same AR and PRI as for adults, expressed in mg/MJ, for infants aged 7–11 months, children aged 1 to < 18 years old, and during pregnancy and lactation, under the assumption that the relationship between thiamin requirement and energy requirement is the same in all population groups.

Based on data from 13 dietary surveys in nine countries of the European Union, average thiamine intakes across countries ranged between 0.31 and 0.65 mg/day (0.11–0.21 mg/MJ) among infants (< 1 year old), between 0.58 and 0.98 mg/day (0.12–0.21 mg/MJ) among children aged 1 to < 3 years old, between 0.68 and 1.29 mg/day (0.10–0.21 mg/MJ) among children aged 3 to < 10 years old, between 0.93 and 1.92 mg/day (0.11–0.20 mg/MJ) among children aged 10 to < 18 years old and between 0.88 and 1.99 mg/day (0.11–0.24 mg/MJ) among adults (≥ 18 years old).

5.9. Vitamin A

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Vitamin A is a fat-soluble vitamin obtained from the diet either as preformed vitamin A (mainly retinol and retinyl esters) in foods of animal origin or as provitamin A carotenoids in plant-derived foods (EFSA NDA Panel, 2015e). The term vitamin A comprises all-trans-retinol (also called retinol) and the family of naturally occurring molecules associated with the biological activity of retinol (such as retinal, retinoic acid, retinyl esters), as well as provitamin A carotenoids that are dietary precursors of retinol. The biological value of substances with vitamin A activity is expressed as retinol equivalent (RE). Specific carotenoids/retinol equivalency ratios are defined for provitamin A carotenoids, which account for the less efficient absorption of carotenoids and their bioconversion to retinol. On the basis of available evidence, the Panel decided to maintain the conversion factors proposed by the Scientific Committee for Food (SCF) for the European populations, namely 1 µg RE equals 1 µg of retinol, 6 µg of β-carotene and 12 µg of other provitamin A carotenoids. Vitamin A requirement can be met with any mixture of preformed vitamin A and provitamin A carotenoids that provides an amount of vitamin A equivalent to the reference value in terms of µg RE/day.

Vitamin A is involved in vision as retinal, which plays a central role in the mechanisms of photo-transduction, and in the systemic maintenance of the growth and integrity of cells in body tissues through the action of retinoic acid, which acts as regulator of genomic expression. The most specific spectrum of ocular manifestations. In low-income countries, vitamin A deficiency in young infants and children has been associated with increased infectious morbidity and mortality, including respiratory infection and diarrhoea.

Preformed vitamin A is efficiently absorbed (70–90%). The absorption of β-carotene appears to be highly variable (5–65%), depending on food- and diet-related factors, genetic characteristics and the health status of the subject. The intestine is the primary tissue where dietary provitamin A carotenoids are converted to retinol. Retinol, in the form of retinyl esters, and provitamin A carotenoids enter the body as a component of nascent chylomicrons secreted into the lymphatic system. Most dietary retinol (in chylomicrons and chylomicron remnants) is taken up by the liver, which is the major site of retinol metabolism and storage. Hepatic retinyl esters are hydrolysed to free retinol, and delivered to tissues by retinol-binding protein. The efficiency of storage and catabolism of retinol depends on vitamin A status. Low retinol stores are associated with a reduced efficiency of storage and decreased absolute catabolic rate. The majority of retinol metabolites are excreted in urine, in faeces via bile and to a lesser extent in breath.

Vitamin A status is best expressed in terms of total body store of retinol (i.e. as free retinol and retinyl esters) or, alternatively, as liver concentration of the vitamin. A concentration of 20 µg retinol/g liver (0.07 µmol/g) in adults represents a level assumed to maintain adequate plasma retinol concentration, to prevent clinical signs of deficiency and to provide adequate stores. The Panel considered that this can be used as a target value for establishing the Average Requirement (AR) for vitamin A for all age groups. The relationship between dietary intake of vitamin A and retinol liver stores has been explored with stable isotope dilution methods but available data are considered insufficient to derive an AR. A factorial approach was applied. This approach considered a total body/liver retinol store ratio of 1.25 (i.e. 80% of retinol body stores are in the liver), a liver/body weight ratio of 2.4%, a fractional catabolic rate of retinol of 0.7% per day of total body stores, an efficiency of storage in the whole body of ingested retinol of 50% and the reference body weights for women and men in the EU of 58.5 and 68.1 kg, respectively. On the basis of this approach, ARs of 570 µg RE/day for men (Table 9:) and 490 µg RE/day for women were derived after rounding (Table 11:). Assuming a coefficient of variation (CV) of 15% because of the variability in requirement and the large uncertainties in the dataset, Population Reference Intakes (PRIs) of 750 µg RE/day for men (Table 10:) and 650 µg RE/day for women (Table 12) were set after rounding.

For infants aged 7–11 months and children, the same target concentration of retinol in the liver and the same equation as for adults was used to calculate ARs. Specific values for reference body weight and for liver/body weight ratio were used. There are some indications that retinol catabolic rate may be higher in children than in adults, but data are limited. The Panel decided to apply the value for catabolic rate in adults and correct it on the basis of a growth factor. Estimated ARs range from 190 µg RE/day in infants aged 7–11 months to 580 µg RE/day in boys aged 15–17 years (Table 9: , Table 11:). PRIs for infants and children were estimated based on a CV of 15% and range from 250 to 750 µg RE/day (Table 10: , Table 12).

For pregnant women, the Panel assumed that a total amount of 3,600 µg retinol is accumulated in the fetus over the course of pregnancy. Considering that the accretion mostly occurs in the last months of pregnancy, and assuming an efficiency of storage of 50% for the fetus, an additional daily requirement of 51 µg RE was calculated for the second half of pregnancy. In order to allow for the extra need related to the growth of maternal tissues, the Panel applied this additional requirement to the whole period of pregnancy. Consequently, an AR of 540 µg RE/day was estimated for pregnant women (Table 11:). Considering a CV of 15% and rounding, a PRI of 700 µg RE/day was derived for pregnant women (Table 12).

For lactating women, an increase in the AR was based on the vitamin A intake required to compensate for the loss of retinol in breast milk. Based on an average amount of retinol secreted in breast milk of 424 µg/day and an absorption efficiency of retinol of 80%, an additional vitamin A intake of 530 µg RE/day was considered sufficient to replace these losses. An AR of 1,020 µg RE/day was estimated (Table 11:) and, considering a CV of 15% and rounding down, a PRI of 1,300 µg RE/day was proposed for lactating women (Table 12).

Foods rich in retinol include offal and meat, butter, retinol-enriched margarine, dairy products and eggs, while foods rich in β-carotene include vegetables and fruits, such as sweet potatoes, carrots, pumpkins, dark green leafy vegetables, sweet red peppers, mangoes and melons. On the basis of data from 12 dietary

surveys in nine EU countries, vitamin A intake was assessed using food consumption data from the EFSA Comprehensive Food Consumption Database and vitamin A composition data from the EFSA nutrient composition database. Average vitamin A intake ranged between 409 and 651 µg RE/day in children aged 1 to < 3 years, between 607 and 889 µg RE/day in children aged 3 to < 10 years, between 597 and 1,078 µg RE/day in children aged 10 to < 18 years and between 816 and 1,498 µg RE/day in adults.

5.10. Vitamin B6

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The term vitamin B6, which is used in the current Scientific Opinion, is a generic descriptor for a group of 2-methyl,3-hydroxy,5-hydroxymethylpyridine derivatives. Vitamin B6 includes pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their respective phosphorylated forms, pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP) (EFSA NDA Panel, 2016b). All these derivatives are present in food. The metabolically active forms, PLP and PMP, act as cofactors of enzymes involved in amino acid metabolism, one-carbon reactions, glycogenolysis and gluconeogenesis, haem synthesis, niacin formation, and also in lipid metabolism, neurotransmitter synthesis and hormone action. However, all six vitamin B6 derivatives have vitamin activity as they can be converted in the body to PLP and PMP, through enzyme-mediated reactions. The most typical features of vitamin B6 deficiency, although rare, are hypochromic microcytic anaemia and neurological abnormalities (convulsive seizures, abnormal electroencephalograms).

The bioavailability of PN, PL and PM is similar. The Panel considers that the bioavailability of pyridoxine-5'-β-D-glucoside (PNG) present in some plants is 50% lower than that of PN and, thus that the bioavailability of vitamin B6 from a mixed diet is around 75%. The Panel also considers that the bioavailability of PN from supplements is about 95%. The vitamin B6 derivatives can be converted to each other through enzyme-mediated reactions in the intestine, in the liver and in other tissues. After absorption, vitamin B6 derivatives are transferred via the portal circulation to the liver where they are metabolised, and are released back to the circulation, where PLP and PL, bound to albumin, are the main forms of the total plasma vitamin B6. Vitamin B6 derivatives are distributed to tissues, in which the predominant vitamin B6 derivative is PLP. The average vitamin B6 content of human body is about 15 nmol/g (assumed to be equivalent to 3.7 µg/g tissue). The majority (75–80%) of the total vitamin B6 is located in muscles (PLP bound to muscle glycogen phosphorylase) including heart, about 5–10% is in the liver and smaller amounts of vitamin B6 are contained in plasma, erythrocytes and other organs. Vitamin B6 is excreted mainly through the urine in the form of its catabolic product 4-pyridoxic acid (4-PA). The mechanism (active or passive) of vitamin B6 placental transfer is unclear.

The Panel notes limitations in biomarkers of vitamin B6 intake and status, i.e. plasma PLP concentration, the concentrations of total vitamin B6 in plasma, of PL and PMP in plasma or erythrocytes, of PLP in erythrocytes, and of total vitamin B6 or 4-PA in urine. The Panel also notes limitations in biomarkers of function, i.e. activation coefficients of erythrocyte aspartate aminotransferase and erythrocyte alanine aminotransferase, urinary excretion of tryptophan catabolites after the tryptophan loading test, ratios of tryptophan metabolites in plasma, urinary concentrations of cystathionine and plasma homocysteine concentration after a methionine load, plasma cystathionine concentration, and some immune-related factors.

The Panel considers that the most suitable biomarker for deriving DRVs for vitamin B6 is plasma PLP concentration: although it has some limitations, plasma PLP concentration is the only biomarker that reflects the tissue stores of vitamin B6 (biomarker of status) and has a defined cut-off value for an adequate vitamin B6 status. The Panel considers it suitable to be used for deriving the DRVs for vitamin B6 in children and adults. The Panel notes that mean values below 30 nmol/L are associated with a wide range of metabolic effects including perturbations of amino acid, lipid, and organic acid profiles in plasma. The Panel considers that a plasma PLP concentration of 30 nmol/L, as a population mean, is indicative of an adequate vitamin

B6 status for all age and sex groups. The Panel notes that there is no consistent relationship between plasma PLP concentrations and protein intake, and considers that there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe. Thus, the Panel considers not appropriate to standardise vitamin B6 requirements on protein intake. In view of the limited and/or inconsistent evidence on an association between vitamin B6 intake or plasma PLP concentration and health consequences, the Panel considers that the data available cannot be used for deriving the requirement for vitamin B6.

In the absence of information on the variability in the requirement, a coefficient of variation (CV) of 10% was used to calculate Population Reference Intakes (PRIs) from the Average Requirements (ARs) for all age groups in children and in adults, rounding to the nearest decimal place. When ARs were derived from one group to the other, an allometric scaling was applied on the assumption that vitamin B6 requirement is related to metabolically active body mass.

For adults, the Panel considers that ARs and PRIs for vitamin B6 can be derived from the vitamin B6 intake required to maintain a (mean) concentration of plasma PLP above 30 nmol/L. The Panel considers the inverse prediction examination of a linear regression analysis of plasma PLP concentration vs vitamin B6 intake (from food including supplements, which were adjusted for their difference in bioavailability), which combined data from five references on intervention studies in 44 young women. The Panel also considers data from two small intervention studies supported by results from three large cross-sectional observational studies, all in older adults. The Panel notes that the vitamin B6 intake required to maintain a (mean) concentration of plasma PLP above 30 nmol/L derived from the data in older women (1.3 mg/day) is slightly higher than the result obtained in younger women (1.2 mg/day). As a conservative approach, the Panel sets an AR for all women at 1.3 mg/day (Table 11:) and a PRI at 1.6 mg/day (Table 12). In the absence of reliable data to determine vitamin B6 requirement in men, the Panel sets an AR by an allometric scaling from the AR of women, and taking into account the difference in reference body weight. The Panel sets an AR for men at 1.5 mg/day (Table 9:) and a PRI at 1.7 mg/day (Table 10:).

For infants aged 7–11 months and children aged 1–17 years, the Panel notes the absence of reliable data on which to base vitamin B6 requirements. The Panel also considers unnecessary to give sex-specific DRVs for infants and children up to 14 years of age, but chooses to set different PRIs for boys and girls aged 15–17 years as for adults.

For infants aged 7–11 months, the Panel proposes an Adequate Intake (AI) at 0.3 mg/day, combining the results of two extrapolation approaches based on an allometric scaling, both taking into account the differences in reference body weight. The proposed AI is the average of the results of upwards extrapolation from the estimated intake of vitamin B6 of exclusively breastfed infants from birth to 6 months, and of downwards extrapolation from the ARs for adults applying a growth factor (Table 10: , Table 12).

For children aged 1–17 years, the Panel derives ARs by downwards extrapolation from adult ARs, by an allometric scaling, applying growth factors and taking into account the differences in reference body weight. For children of both sexes aged 1–14 years, the Panel sets ARs ranging between 0.5 and 1.2 mg/day, while for children aged 15–17 years, the Panel derives the same ARs as for adults (Table 9: , Table 11:). PRIs range from 0.6 to 1.4 mg/day for children aged 1–14 years, while for children aged 15–17 years, PRIs are 1.6 mg/day for girls and 1.7 mg/day for boys (Table 10: , Table 12).

For pregnant and lactating women, the AR for non-pregnant non-lactating women is increased to account for the uptake of vitamin B6 by the fetal and maternal tissues, and the losses through breast milk, respectively. For pregnant women, the additional vitamin B6 intake (0.2 mg/day) is estimated, based on the mean gestational weight gain (12 kg) and the average vitamin B6 content of the human tissue (3.7 µg/g tissue), a pregnancy duration of 280 days and the vitamin B6 bioavailability from a mixed diet (75%). The Panel sets an AR for pregnant women at 1.5 mg/day (Table 11:) and a PRI at 1.8 mg/day (Table 12). For lactating women, the additional vitamin B6 intake (0.133 mg/day) is estimated, considering an average concentration of vitamin B6 in breast milk (0.130 mg/L), the mean milk transfer during the first 6 months of lactation in exclusively breastfeeding women (0.8 L/day), and the vitamin B6 bioavailability from a mixed

diet (75%). The Panel sets an AR for lactating women at 1.4 mg/day (Table 11:) and a PRI at 1.7 mg/day (Table 12).

Based on data from 13 surveys in nine countries of the European Union, average total vitamin B6 intake ranges across countries from 0.4 to 0.8 mg/day in infants, from 0.9 to 1.3 mg/day in children aged 1 to < 3 years, from 1 to 1.6 mg/day in children aged 3 to < 10 years, and from 1.5 to 2.3 mg/day in children aged 11 to < 18 years. Average total vitamin B6 intake ranges between 1.4 and 3.1 mg/day in adults.

5.11. Vitamin C

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3418/full

Vitamin C (L-ascorbic acid) is an enzyme cofactor for biochemical reactions catalysed by monooxygenases, dioxygenases and mixed function oxygenases (EFSA NDA Panel, 2013c). Vitamin C plays an important role in the biosynthesis of collagen, is essential for the synthesis of carnitine and catecholamines, and is also involved in the metabolism of cholesterol to bile acids. Vitamin C in aqueous solution readily scavenges reactive oxygen and nitrogen species, and is part of the antioxidant network of the body.

Gastrointestinal absorption is about 80% for an intake of about 100 mg/day. Vitamin C is transported as the free anion ascorbate in plasma, and is distributed to all tissues. Biomarkers of body stores are related to the size and turnover of vitamin C body stores, and to the mass balance of vitamin C in the body. In this Opinion, plasma ascorbate concentration is considered as the primary indicator of body stores. The mass balance of vitamin C in the body is determined from the rate of turnover of the body pool, considering metabolic losses, urinary losses and the quantity of vitamin C required for the replacement of these losses, taking into account absorption efficiency.

Scurvy, characterised by symptoms related to connective tissue defects, occurs in adults at a plasma ascorbate concentration below 10 µmol/L and a body pool less than 300 mg, and can be prevented with an intake of 10 mg vitamin C/day. In vitamin C-depleted men, when vitamin C intake is increased to 60 to 100 mg/day, plasma ascorbate concentrations steeply increase up to a value of about 50 µmol/L, and the body pool rises to 1.0–1.5 g. When vitamin C intake is increased to above 100 mg/day, there is a progressive flattening of the curve until plasma ascorbate reaches a plateau at about 70–80 µmol/L that can be maintained only by chronic ingestion of large doses of vitamin C above 200 mg/day. Plasma ascorbate concentrations above 10 µmol/L but below 50 µmol/L are indicative of a suboptimal status with a risk of insufficiency. A plasma ascorbate concentration of 50 µmol/L is indicative of an adequate status. Urinary excretion of ascorbate is low when plasma ascorbate concentrations are low, but urinary excretion increases sharply for plasma concentrations above about 50 µmol/L, and this is assumed to reflect near-saturation of body pools.

The Average Requirement (AR) for vitamin C in healthy adults was determined from the quantity of vitamin C intake that balances metabolic vitamin C losses and maintains fasting plasma ascorbate concentrations at about 50 µmol/L. Taking a conservative approach and based on the fact that a complete set of data was only available in men, the Panel selected metabolic losses of 50 mg/day, an absorption of 80% and a urinary excretion of 25% of the vitamin C intake. Thus, a mean vitamin C intake of 91 mg/day (rounded to 90 mg/day) was estimated to be required to balance daily losses, and this intake represents the AR (Table 9:). Assuming a coefficient of variation (CV) of 10%, a Population Reference Intake (PRI) of 110 mg/day was derived for healthy men (Table 10:). As no value for metabolic losses was available in women, the AR for women was extrapolated from the AR for men. Extrapolation was done by isometric scaling (linear with body weight), since vitamin C is considered to be distributed throughout the whole body, since the multi-compartment models used to calculate the metabolic losses in men consider an exchange with only one whole body tissue pool, since few sex-related differences could be observed in the pharmacokinetics of vitamin C, and since a main part of the observed differences can be explained by body weight differences between sexes. This calculation led to an AR of 78 mg/day (rounded to 80 mg/day) for women (Table 11:

). Assuming a CV of 10% and rounding to the closest 5, a PRI of 95 mg/day of vitamin C was derived for healthy women (Table 12). Because of a scarcity of data on the influence of ageing, the Panel concluded that there were insufficient data to derive different DRVs for vitamin C for older adults compared to younger adults.

The Panel also considered several health outcomes that may be associated with vitamin C intake. The Panel decided that the available data on the effects of vitamin C intake and/or status on scurvy, blood lipids and blood pressure, common cold, and on chronic disease-related outcomes (cardiovascular disease-related, cancer, vision-related, mortality) could not be used as criteria to derive the requirement for vitamin C.

For infants aged 7–11 months, the Panel decided to retain the PRI of 20 mg/day set by the Scientific Committee for Food (SCF, 1993), as no suitable evidence has emerged since the previous assessment. For children and adolescents, the AR for vitamin C was extrapolated from the ARs for adults taking into account differences in body weight (isometric scaling) (Table 9: , Table 11:). The PRIs were derived by assuming a CV of 10% and range from 20 mg/day for 1 to 3-year old children, to 100 and 90 mg/day for boys and girls aged 15–17 years, respectively (Table 10: , Table 12).

In pregnancy, plasma ascorbate concentration decreases because of haemodilution and active transfer to the fetus. For pregnant women, a vitamin C intake of 10 mg/day in addition to the PRI of non-pregnant women was proposed (Table 12). In lactating women, the amount of vitamin C secreted in breast milk reflects maternal vitamin C intake rather than the infant's requirement. For women exclusively breastfeeding during the first six months post-partum, a vitamin C intake of 60 mg/day, in addition to the PRI of non-lactating women, was proposed to cover vitamin C losses in breast milk (Table 12).

The main contributors to the vitamin C intake of adults are fruits and vegetables and their juices, and potatoes. Data from dietary surveys show that average vitamin C intakes from food only in European countries range from 69 to 130 mg/day in men and from 65 to 138 mg/day in women.

5.12. Vitamin D

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4547/full

Vitamin D is the generic term for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which are formed from their respective provitamins, ergosterol and 7-dehydrocholesterol, following a two step-reaction involving ultraviolet-B (UV-B) irradiation and subsequent thermal isomerisation (EFSA NDA Panel, 2016d). Vitamin D_2 and vitamin D_3 are fat-soluble and present in foods and dietary supplements. Vitamin D3 is also synthesised endogenously in the skin following exposure to UV-B irradiation.

During summer months, or following exposure to artificial UV-B irradiation, the synthesis of vitamin D_3 in the skin may be the main source of vitamin D. Dietary intake of vitamin D is essential in case endogenous synthesis, due to insufficient UV-B exposure, is lacking or insufficient. Factors affecting the synthesis of vitamin D_3 in the skin include latitude, season, ozone layer and clouds (absorbing UV-B irradiation), surface characteristics (reflecting UV-B irradiation), time spent outdoors, use of sunscreen, clothing, skin colour and age. The Panel notes that sun exposure may contribute a considerable and varying amount of vitamin D available to the body and therefore considers that the association between vitamin D intake and status, for the purpose of deriving DRVs for vitamin D, should be assessed under conditions of minimal endogenous vitamin D synthesis. Vitamin D from dietary sources is absorbed throughout the small intestine. The Panel considers that the average vitamin D absorption from a usual diet is about 80% and limited data are available on the effect of the food or supplement matrix on absorption of vitamin D (vitamin D_2 or vitamin D_3).

In the body, within hours of ingestion or synthesis in the skin, vitamin D is either converted into its biologically active metabolite $1,25(OH)_2D$ or delivered to the storage tissues (as either vitamin D or its metabolites). The first step of the conversion occurs in the liver, where vitamin D is hydroxylated to

25(OH)D, while the second step occurs primarily in the kidneys, where 25(OH)D is hydroxylated to $1,25(OH)_2D$. Vitamin D, $1,25(OH)_2D$ and 25(OH)D are transported in the blood bound mainly to the vitamin D-binding protein (DBP). Of the two metabolites of vitamin D, 25(OH)D is the major circulating form, with a longer mean half-life, of about 13–15 days. 25(OH)D is taken up from the blood into many tissues, including in the adipose tissue, muscle and liver for storage.

After its release from DBP to tissues, $1,25(OH)_2D$ exerts, in association with the intracellular vitamin D receptor (VDR), important biological functions throughout the body. In the intestine, it binds to VDR to facilitate calcium and phosphorus absorption. In the kidney, it stimulates the parathyroid hormone (PTH)-dependent tubular reabsorption of calcium. In the bone, PTH and $1,25(OH)_2D$ interact to activate the osteoclasts responsible for bone resorption. In addition, $1,25(OH)_2D$ suppresses the PTH gene expression, inhibits proliferation of parathyroid cells, and is involved in cell differentiation and antiproliferative actions in various cell types. Both 25(OH)D and $1,25(OH)_2D$ are catabolised before elimination and the main route of excretion is via the faeces.

Vitamin D deficiency leads to impaired mineralisation of bone due to an inefficient absorption of dietary calcium and phosphorus, and is associated with an increase in PTH serum concentration. Clinical symptoms of vitamin D deficiency manifest as rickets in children, and osteomalacia in adults.

The Panel reviewed possible biomarkers of vitamin D intake and/or status, namely serum concentration of 25(OH)D, free 25(OH)D, $1,25(OH)_2D$ and PTH concentration, markers of bone formation and bone turnover. In spite of the high variability in 25(OH)D measurements obtained with different analytical methods, the Panel concludes that serum 25(OH)D concentration, which reflects the amount of vitamin D attained from both cutaneous synthesis and dietary sources, can be used as a biomarker of vitamin D status in adult and children populations. Serum 25(OH)D concentration can also be used as a biomarker of vitamin D intake in a population with low exposure to UV-B irradiation.

In consideration of the various biological functions of $1,25(OH)_2D$, the Panel assessed the available evidence on the relationship between serum 25(OH)D concentration and several health outcomes, to evaluate whether they might inform the setting of DRVs for vitamin D. The Panel first considered the available evidence on serum 25(OH)D concentration and musculoskeletal health outcomes, i.e. bone mineral density (BMD)/bone mineral content (BMC) and calcium absorption in adults and infants/ children, risk of osteomalacia, fracture risk, risk of falls/falling, muscle strength/muscle function/ physical performance in adults, and risk of rickets in infants/children. The Panel then reviewed data on the relationship between maternal serum 25(OH)D concentration and health outcomes in pregnancy (risk of pre-eclampsia, of small for gestational age and of preterm birth, and indicators of bone health in infants) and lactation. The Panel took as a starting point the results of the literature search and the conclusions from the most recent report on DRVs for vitamin D by the Institute of Medicine (IOM) that was based on two systematic reviews. The Panel also considered an update of one of these two systematic reviews, as well as two recent reports from DRV-setting bodies. The Panel undertook a separate literature search to identify primary intervention and prospective observational studies in healthy subjects (infants, children and adults, including free-living older adults) that were published after the IOM report until March 2015. As a second step, the Panel considered available evidence on several other non-musculoskeletal health outcomes (e.g. cancer or cardiovascular diseases), based on the reports and reviews mentioned above without undertaking a specific literature search of primary studies. The Panel considers that the available evidence on serum 25(OH)D concentration and musculoskeletal health outcomes and pregnancy-related health outcomes is suitable to set DRVs for vitamin D for (healthy) adults, infants, children, and pregnant women, respectively. However, the Panel considers that there is no evidence for a relationship between serum 25(OH)D concentration and health outcomes of lactating women that may be used to set a DRV for vitamin D, and that the available evidence on non-musculoskeletal health outcomes is insufficient to be used as criterion for setting DRVs for vitamin D.

The Panel notes that data on the relationship between serum 25(OH)D concentration and adverse musculoskeletal or pregnancy-related health outcomes are widely variable. However, taking into account the overall evidence and uncertainties, the Panel considers that, for adults, infants and children, there is

evidence for an increased risk of adverse musculoskeletal health outcomes at serum 25(OH)D concentrations below 50 nmol/L.[14] The Panel also considers that there is evidence for an increased risk of adverse pregnancy-related health outcomes at serum 25(OH)D concentrations below 50 nmol/L.

The Panel assessed the available evidence on the relationship between vitamin D intake and musculoskeletal health outcomes to evaluate whether they might inform the setting of DRVs for vitamin D. The Panel notes that these studies usually do not provide information on the habitual dietary intake of vitamin D, and the extent to which cutaneous vitamin D synthesis has contributed to the vitamin D supply (and thus may have confounded the relationship between vitamin D intake and the reported health outcomes) is not known. The Panel therefore concludes that these studies are not useful as such for setting DRVs for vitamin D, and may only be used to support the outcome of the characterisation of the vitamin D intake–status relationship undertaken by the Panel under conditions of assumed minimal endogenous vitamin D synthesis.

The Panel concludes that a serum 25(OH)D concentration of 50 nmol/L is a suitable target value to set the DRVs for vitamin D, for all age and sex groups (healthy adults, infants, children, pregnant and lactating women). For setting DRVs for vitamin D, the Panel considers the dietary intake of vitamin D necessary to achieve this serum 25(OH)D concentration. As for other nutrients, DRVs for vitamin D are set assuming that intakes of interacting nutrients, such as calcium, are adequate.

EFSA undertook a meta-regression analysis of the relationship between serum 25(OH)D concentration and total vitamin D intake (habitual diet, and fortified foods or supplements using vitamin D_3). Randomised trials conducted in a period of assumed minimal endogenous vitamin D synthesis were identified through a comprehensive literature search and a review undertaken for EFSA by an external contractor (Brouwer-Brolsma et al., 2016). The analysis was performed using summary data from 83 trial arms (35 studies), of which nine were on children (four trials, age range: 2–17 years) and the other arms were on adults (mean age between 22 and 86 years, excluding pregnant or lactating women). Data were extracted for each arm of the individual trials. The meta-regression analysis resulted in two predictive equations of achieved serum 25(OH)D concentrations: one derived from an unadjusted model (including only the natural log of the total intake) and one derived from a model including the natural log of the total intake and adjusted for a number of relevant factors (baseline serum 25(OH)D concentration, latitude, study start year, type of analytical method applied to assess serum 25(OH)D, assessment of compliance) set at their mean values.

The Panel considers that the available evidence does not allow the setting of average requirements (ARs) and population reference intakes (PRIs), and therefore defines adequate intakes (AIs) instead, for all population groups.

For adults, the Panel sets an AI for vitamin D at 15 µg/day[15] (Table 10: , Table 12). This is based on the adjusted model of the meta-regression analysis, and considering that, at this intake, the majority of the adult population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L.

For children aged 1–17 years, the Panel sets an AI for vitamin D for all children at 15 µg/day (Table 10: , Table 12). This is based on the adjusted model of the meta-regression analysis on all trials (adults and children) as well as on a stratified analysis by age group (adults versus children).

For infants aged 7–11 months, the Panel sets an AI for vitamin D at 10 µg/day, considering four recent trials on the effect of vitamin D supplementation on serum 25(OH)D concentration in (mostly) breastfed infants (Table 10: , Table 12).

For pregnant and lactating women, the Panel considers that the AI is the same as for non-pregnant non-lactating women, i.e. 15 µg/day (Table 12).

[14] 2.5 nmol/L = 1 ng/mL

[15] 1 µg = 40 IU of vitamin D; 0.025 µg = 1 IU of vitamin D

The Panel underlines that the meta-regression analysis on adults and children was done on data collected under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may even be zero.

5.13. Vitamin E as a-tocopherol

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Vitamin E is a fat-soluble vitamin (EFSA NDA Panel, 2015f). Previously, the term vitamin E was used as the generic term for four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), which are organic compounds that possess antioxidant activity to a different degree. Factors have been used to convert food contents of tocopherols and tocotrienols to α-tocopherol equivalents. In this Opinion, based on the available evidence and in line with other authoritative bodies, the Panel considers vitamin E as being α-tocopherol only. Its naturally occurring form is RRR-α-tocopherol. Commercially available forms of α-tocopherol include RRR-α-tocopherol, a synthetic form that contains in equal proportions the eight stereoisomers of α-tocopherol (RRR-, RRS-, RSR-, RSS- and their enantiomers SSS-, SSR-, SRS-, SRR-) and is called all-rac-α-tocopherol, and their esterified forms.

Efficient α-tocopherol absorption requires the presence of fat. The Panel considered that the average α-tocopherol absorption from a usual diet is about 75%. This is based on the means observed in two balance studies and in a kinetic study using a multi-compartmental model of α-tocopherol metabolism. After its intestinal absorption, α-tocopherol is incorporated into chylomicrons and transported to the liver. There, the α-tocopherol transfer protein (α-TTP), which preferentially binds α-tocopherol rather than other tocopherols or tocotrienols, is responsible for its incorporation into nascent very lowdensity lipoproteins to be secreted by the liver into the circulation and distributed to body tissues. α-Tocopherol not bound to α-TTP is catabolised in the liver (to 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman, i.e. α-CEHC) by hepatic ω-hydroxylase, which catabolises tocopherols and has a stronger activity towards tocopherols other than α-tocopherol. Because of differences in activities of α-TTP and ω-hydroxylase towards α-tocopherol and other tocopherols, α-tocopherol predominantly accumulates in body tissues, whereas other tocopherols are preferentially catabolised in the liver.

Blood α-tocopherol concentrations are maintained by the preferential binding of α-tocopherol by α-TTP. Among chemically synthesised α-tocopherol forms, only 2R-α-tocopherol stereoisomers (i.e. RRR-, RRS-, RSR-, RSS-) were found to meet human requirements for the vitamin, because the 2Sstereoisomers (i.e. SSS-, SSR-, SRS-, SRR-) present in all-rac-α-tocopherol possess low affinity for α-TTP and are rapidly metabolised in the liver. Currently, only RRR-α-tocopherol is considered to be the physiologically active vitamer.

α-Tocopherol is part of the antioxidant defence system and is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and plasma lipoproteins. Primary α-tocopherol deficiency, a result of mutations in the α-TTP gene, is associated with neurological symptoms, including ataxia. Symptomatic α-tocopherol deficiency in individuals without any disease and who consume diets 'low' in α-tocopherol has not been reported.

The Panel considers that there is, at present, insufficient data on markers of α-tocopherol intake/status/function (e.g. plasma/serum α-tocopherol concentration, hydrogen peroxide-induced haemolysis, urinary α-CEHC excretion, markers of oxidative damage) to derive the requirement for α-tocopherol. The Panel notes the lack of convergence of the values that would be derived from the use of data on markers of α-tocopherol intake/status or on α-tocopherol kinetics and body pools. The Panel considers that available data on markers of α-tocopherol intake/status/function, on α-tocopherol kinetics and body pools, on the relationship between PUFA intake and α-tocopherol intake/requirement can be used neither on their own nor in combination to derive the requirement for α-tocopherol in adults. The Panel considers that data on the relationship between vitamin E (unspecified form) or α-tocopherol intake and

health consequences are inconsistent or limited and cannot be used to derive the requirement for α-tocopherol. The Panel also considers that there are no data that can be used to derive the requirement for α-tocopherol for infants or children.

The Panel considers that Average Requirements (ARs) and Population Reference Intakes (PRIs) cannot be set for α-tocopherol. Therefore, the Panel proposes to set Adequate Intakes (AIs) for α-tocopherol for all population groups.

For adults and children, the AIs are based on observed dietary intakes in healthy populations with no apparent α-tocopherol deficiency and such intakes were estimated by EFSA using the EFSA Comprehensive European Food Consumption Database and the EFSA Food Composition Database. This intake assessment considered 13 dietary surveys in nine countries of the European Union (EU) (Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the United Kingdom). As most food composition databases in EU countries contain values for vitamin E as α-tocopherol equivalents (α-TEs) and only two countries (Finland and Sweden) considered in the intake assessment by EFSA have vitamin E values in their food composition databases as α-tocopherol values, dietary intakes of both α-tocopherol and α-TE were estimated by EFSA for males and females for all included countries. The Panel noted the uncertainties in the available food composition and consumption data and dietary assessment methods, the contribution of average α-tocopherol intakes to average α-TE intakes in the nine EU countries considered, as well as the specific methodological uncertainties of the EFSA intake estimates for α-tocopherol. The Panel considered the range of average EFSA intake estimates for α-tocopherol as well as the range of average EFSA intake estimates for α-TEs, and combined the approximate mid-points of both ranges of average EFSA intake estimates to set AIs for α-tocopherol for children and adults, after rounding.

For adults, an AI for α-tocopherol is set at 13 mg/day for men (Table 10:) and 11 mg/day for women (Table 12). For children aged 1 to < 3 years, an AI for α-tocopherol is set at 6 mg/day for both sexes (Table 10: , Table 12). For children aged 3 to < 10 years, an AI for α-tocopherol is set at 9 mg/day for both sexes (Table 10: , Table 12). For children aged 10 to < 18 years, an AI for α-tocopherol is set at 13 mg/day for boys (Table 10:) and 11 mg/day for girls (Table 12).

For infants aged 7–11 months, an AI for α-tocopherol of 5 mg/day is extrapolated upwards from the estimated α-tocopherol intake in exclusively breast-fed infants aged 0–6 months, using allometric scaling (assuming that the requirement for this vitamin is related to metabolically active body mass) and rounding to the closest unit (Table 10: , Table 12).

The Panel considers that the available data do not indicate an additional dietary α-tocopherol requirement during pregnancy or during lactation, and that a full compensation of the transitory secretion of α-tocopherol in breast milk is not justified for the derivation of DRVs for α-tocopherol for lactating women. The Panel therefore considers that the AI for pregnant or lactating women is the same (11 mg/day of α-tocopherol) as for non-pregnant non-lactating women (Table 12).

5.14. Vitamin K

Full scientific opinion: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4780/full

Vitamin K represents a family of fat-soluble compounds with the common chemical structure of 3-substituted 2-methyl-1,4-napthoquinone. It naturally occurs in food as phylloquinone (vitamin K_1) and menaquinones (vitamin K_2) (EFSA NDA Panel, 2017b). Phylloquinone has a phytyl side chain and is the primary dietary form of vitamin K in Europe: it is mainly found in dark green leafy vegetables (e.g. spinach, lettuce and other salad plants) and Brassica. Menaquinones are a group of compounds with an unsaturated side chain from 4 to 13 isoprenyl units (vitamin K_2 or MK-n) and are found mainly in animal products such as meat, cheese and eggs. Apart from MK-4 that is formed via metabolic conversion of phylloquinone during its absorption in the intestinal mucosa and in other organs, menaquinones are produced by bacteria capable

of food fermentation and specific anaerobic bacteria of the colon microbiota. In this Opinion, the Panel considers vitamin K to comprise both phylloquinone and menaquinones.

Vitamin K acts as a cofactor of c-glutamyl carboxylase (GGCX) that catalyses the carboxylation of glutamic acid (Glu) residues into c-carboxyglutamic acid (Gla) residues in vitamin K-dependent proteins (Gla-proteins), which convert them into their active forms. These Gla-proteins are involved in different physiological processes, including blood coagulation or bone mineralisation. MK-7 may have a greater bioactivity compared to phylloquinone in stimulating c-carboxylation, but the available data are insufficient to set different activity coefficients for phylloquinone and menaquinones.

In adults, vitamin K deficiency is clinically characterised by a bleeding tendency in relation to a low activity of blood coagulation factors, resulting in an increase in prothrombin time (PT) or partial thromboplastin time (or activated partial thromboplastin time). Symptomatic vitamin K deficiency and impairment of normal haemostatic control in healthy adults may take more than 2–3 weeks to develop at a 'low' phylloquinone intake (i.e. < 10 µg/day). Exclusively breastfed infants are susceptible to bleeding, due to the low vitamin K content of human milk and their small body pool of vitamin K. Administration of phylloquinone at a pharmacological dose, either orally or by intramuscular injection, is usual practice for prevention of haemorrhagic disease in newborn infants. No tolerable upper intake level has been set for vitamin K by the Scientific Committee on Food (SCF).

Phylloquinone is absorbed in the intestine in the presence of dietary fat. Studies on absorption of phylloquinone in healthy adults show widely variable results. The data for absorption of some dietary menaquinones (MK-4, MK-7 or MK-9) in comparison with phylloquinone are also limited. Absorption of menaquinones produced by gut bacteria in the distal intestine remains uncertain, and therefore their contribution to vitamin K status is unclear. The Panel considers that it is not possible to estimate precisely an average absorption of phylloquinone, menaquinones, and thus vitamin K from the diet.

After intestinal absorption, phylloquinone and individual menaquinones are transported into the blood by lipoproteins. The clearance of MK-7 and MK-9 from serum/plasma is slower than for phylloquinone. Vitamin K accumulates primarily in the liver, but is also present in bones and other tissues and has a fast turnover in the body. The liver contains widely variable concentrations of phylloquinone and menaquinones, which are catabolised to the same metabolites and excreted in bile and urine. Phylloquinone crosses the placenta in small quantities, while for menaquinones, this is unclear.

PT is the only vitamin K biomarker for which a change (increase) has been associated with vitamin K deficiency. Possible changes in the other biomarkers (concentration/activity of coagulation factors, of the undercarboxylated forms of vitamin-K dependent proteins, or of vitamin K in blood; urinary concentration of Gla residues or of the 5C- and 7C-metabolites) according to phylloquinone intake are difficult to interpret, as no cut-off value to define adequate vitamin K status is available. There is no biomarker for which a dose–response relationship with phylloquinone intake has been established. Studies investigating the relationship between biomarkers and intake of different individual menaquinones often used doses that are much higher than the limited observed intake data of these individual menaquinones available in Europe. There is no reference level for c-carboxylation that can be considered as 'optimal' related to functions controlled by vitamin K status and the dietary intakes of phylloquinone or menaquinones required for maximal or 'optimal' urinary Gla excretion have not been determined. Thus, the Panel concludes that none of these biomarkers is suitable by itself to assess vitamin K adequacy. The Panel also concludes that data are insufficient for deriving the requirement for vitamin K according to sex or for 'younger' and 'older' adults.

The Panel notes the uncertainties in the food composition data and available consumption data related to phylloquinone, individual menaquinones or vitamin K. The Panel concludes that available data on intake of phylloquinone or menaquinones and health outcomes in healthy subjects cannot be used to derive DRVs for vitamin K. Data on vitamin K biomarkers and health outcomes with no quantitative data on vitamin K intake were not considered. The Panel considers a total body pool of phylloquinone of about 0.55 µg/kg body weight in healthy adults at steady state not to be associated with signs of vitamin K deficiency and to

be a desirable body pool size for phylloquinone. The Panel notes that available data do not allow the estimation of the daily dietary intake of phylloquinone required to balance total phylloquinone losses through urine and bile and to maintain an adequate body pool of phylloquinone. There is no data on the total body pool of menaquinones.

The Panel considers that average requirements and population reference intakes for vitamin K cannot be derived for adults, infants and children, and therefore sets adequate intakes (AIs). The Panel considers that available evidence on intake, absorption, function and content in the body or organs of menaquinones is insufficient, and thus sets AIs for phylloquinone only. Having assessed additional evidence available since 1993 related to biomarkers, intake data and the factorial approach, the Panel concludes that all possible approaches investigated to set DRVs for vitamin K are associated with considerable uncertainties and that the available scientific evidence is insufficient to update the previous reference value. Therefore, the Panel maintains the reference value proposed by the SCF in 1993. Thus, an AI of 1 µg phylloquinone/kg body weight per day is set for all age and sex population groups.

For adults, the Panel considers the respective reference body weights of men and women and after rounding up, sets the same AI of 70 µg phylloquinone/day (Table 10: , Table 12). The Panel notes that the proposed AI in adults is close to the median phylloquinone intake of 76 µg/day in the 2012 German National Nutrition Survey II that used updated phylloquinone composition data. The Panel considers that there is no evidence of different vitamin K absorption and different losses according to age in adults; thus sets the same AI for 'younger' and 'older' adults.

For infants and children, the Panel considers that the requirement for growth would be covered by an intake of 1 µg phylloquinone/kg body weight per day. Considering the respective reference body weights, and after rounding up, AIs for phylloquinone are set at 10 µg/day for infants aged 7–11 months, and between 12 µg/day for children aged 1–3 years and 65 µg/day for children aged 15–17 years (Table 10: , Table 12).

For pregnant women, taking into account the mean gestational increase in body weight and the reference body weight of non-pregnant women, the AI is the same as that for non-pregnant women obtained after rounding (Table 12). For lactating women, the Panel considers that the AI of 1 µg/kg body weight per day of phylloquinone set for non-lactating women covers the small excretion of vitamin K in breast milk. Thus, the AI for pregnant or lactating women is set at 70 µg phylloquinone/day (Table 12).

Table 9: ARs for vitamins, males

Age group (years)	Folate (µg DFE/day) [a]	Niacin (mg NE/MJ) [b]	Riboflavin (mg/d)	Thiamin (mg/MJ)	Vitamin A (µg/d) [c]	Vitamin B6 (mg/d)	Vitamin C (mg/d)
7–11 mo[d]		**1.3**		**0.072**	**190**		
1–3	**90**	**1.3**	**0.5**	**0.072**	**205**	**0.5**	**15**
4–6	**110**	**1.3**	**0.6**	**0.072**	**245**	**0.6**	**25**
7–10	**160**	**1.3**	**0.8**	**0.072**	**320**	**0.9**	**40**
11–14	**210**	**1.3**	**1.1**	**0.072**	**480**	**1.2**	**60**
15–17	**250**	**1.3**	**1.4**	**0.072**	**580**	**1.5**	**85**
≥ 18	**250**	**1.3**	**1.3**	**0.072**	**570**	**1.5**	**90**

d, day; MJ, megajoule; mo, months

(a): DFE: dietary folate equivalents. For combined intakes of food folate and folic acid, DFEs can be computed as follows: µg DFE = µg food folate + (1.7 x µg folic acid)
(b): NE: niacin equivalent (1 mg niacin = 1 niacin equivalent = 60 mg dietary tryptophan)
(c): RE: retinol equivalent, 1 µg RE equals 1 µg of retinol, 6 µg of β-carotene and 12 µg of other provitamin A carotenoids
(d): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)

Table 10: PRIs and AIs for vitamins, males

Age group (years)	α-Tocopherol (mg/d)	Age group (years)	Biotin (µg/d)	Choline (mg/d)	Cobalamin (µg/d)	Folate (µg DFE/d) (a)	Niacin (mg NE/MJ) (b)	Pantothenic acid (mg/day)	Riboflavin (mg/d)	Thiamin (mg/MJ)	Vitamin A (µg/d) (c)	Vitamin B6 (mg/d)	Vitamin C (mg/d)	Vitamin D (µg/d) (e)	Vitamin K (µg/d) (g)
7–11 mo(d)	5	7–11 mo(d)	6	160	1.5	80	**1.6**	3	0.4	**0.1**	**250**	0.3	**20**	10	10
1–2	6	1–3	20	140	1.5	**120**	**1.6**	4	**0.6**	**0.1**	**250**	**0.6**	**20**	15(f)	12
3–9	9	4–6	25	170	1.5	**140**	**1.6**	4	**0.7**	**0.1**	**300**	**0.7**	**30**	15(f)	20
		7–10	25	250	2.5	**200**	**1.6**	4	**1.0**	**0.1**	**400**	**1.0**	**45**	15(f)	30
10–17	13	11–14	35	340	3.5	**270**	**1.6**	5	**1.4**	**0.1**	**600**	**1.4**	**70**	15(f)	45
		15–17	35	400	4.0	**330**	**1.6**	5	**1.6**	**0.1**	**750**	**1.7**	**100**	15(f)	65
≥ 18	13	≥ 18	40	400	4.0	**330**	**1.6**	5	**1.6**	**0.1**	**750**	**1.7**	**110**	15(f)	70

d, day; MJ, megajoule; mo, months

PRIs are presented **in bold type** and AIs in ordinary type

(a): DFE: dietary folate equivalents. For combined intakes of food folate and folic acid, DFEs can be computed as follows: µg DFE = µg food folate + (1.7 x µg folic acid)
(b): NE: niacin equivalent (1 mg niacin = 1 niacin equivalent = 60 mg dietary tryptophan)
(c): RE: retinol equivalent, 1 µg RE equals 1 µg of retinol, 6 µg of β-carotene and 12 µg of other provitamin A carotenoids
(d): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)
(e): for conversion between µg and International Units (IU) of vitamin D intake: 1 µg = 40 IU and 0.025 µg = 1 IU
(f): under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may be even zero.
(g): based on phylloquinone only

Table 11: ARs for vitamins, females

Age group (years)	Folate (µg DFE/day) [a]	Niacin (mg NE/MJ) [b]	Riboflavin (mg/d)	Thiamin (mg/MJ)	Vitamin A (µg/d) [c]	Vitamin B6 (mg/d)	Vitamin C (mg/d)
7–11 mo[d]	-	**1.3**	**-**	**0.072**	**190**	-	**-**
1–3	**90**	**1.3**	**0.5**	**0.072**	**205**	**0.5**	**15**
4–6	**110**	**1.3**	**0.6**	**0.072**	**245**	**0.6**	**25**
7–10	**160**	**1.3**	**0.8**	**0.072**	**320**	**0.9**	**40**
11–14	**210**	**1.3**	**1.1**	**0.072**	**480**	**1.2**	**60**
15–17	**250**	**1.3**	**1.4**	**0.072**	**490**	**1.3**	**75**
≥ 18	**250**	**1.3**	**1.3**	**0.072**	**490**	**1.3**	**80**
Pregnancy							
	-	**1.3**	**1.5**	**0.072**	**540**	**1.5**	**-**
Lactation							
	380	**1.3**	**1.7**	**0.072**	**1,020**	**1.4**	**140**

d, day; MJ, megajoule; mo, months

(a): DFE: dietary folate equivalents. For combined intakes of food folate and folic acid, DFEs can be computed as follows: µg DFE = µg food folate + (1.7 x µg folic acid)
(b): NE: niacin equivalent (1 mg niacin = 1 niacin equivalent = 60 mg dietary tryptophan)
(c): RE: retinol equivalent, 1 µg RE equals 1 µg of retinol, 6 µg of β-carotene and 12 µg of other provitamin A carotenoids
(d): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)

Table 12: PRIs and AIs for vitamins, females

Age group (years)	α-Tocopherol (mg/d)	Age group (years)	Biotin (µg/d)	Choline (mg/d)	Cobalamin (µg/d)	Folate (µg DFE/d) (a)	Niacin (mg NE/MJ) (b)	Pantothenic acid (mg/day)	Riboflavin (mg/d)	Thiamin (mg/MJ)	Vitamin A (µg/d) (c)	Vitamin B6 (mg/d)	Vitamin C (mg/d)	Vitamin D (µg/d) (e)	Vitamin K (µg/d) (g)
7–11 mo(d)	5	7–11 mo(d)	6	160	1.5	80	**1.6**	3	0.4	**0.1**	**250**	0.3	**20**	10	10
1–2	6	1–3	20	140	1.5	**120**	**1.6**	4	**0.6**	**0.1**	**250**	**0.6**	**20**	15(f)	12
3–9	9	4–6	25	170	1.5	**140**	**1.6**	4	**0.7**	**0.1**	**300**	**0.7**	**30**	15(f)	20
		7–10	25	250	2.5	**200**	**1.6**	4	**1.0**	**0.1**	**400**	**1.0**	**45**	15(f)	30
10–17	11	11–14	35	340	3.5	**270**	**1.6**	5	**1.4**	**0.1**	**600**	**1.4**	**70**	15(f)	45
		15–17	35	400	4.0	**330**	**1.6**	5	**1.6**	**0.1**	**650**	**1.6**	**90**	15(f)	65
≥ 18	11	≥ 18	40	400	4.0	**330**	**1.6**	5	**1.6**	**0.1**	**650**	**1.6**	**95**	15(f)	70
Pregnancy															
	11		40	480	4.5	600	**1.6**	5	**1.9**	**0.1**	**700**	**1.8**	**105**	15(f)	70
Lactation															
	11		45	520	5.0	**500**	**1.6**	7	**2.0**	**0.1**	**1,300**	**1.7**	**155**	15(f)	70

d, day; MJ, megajoule; mo, months

PRIs are presented **in bold type** and AIs in ordinary type

(a): DFE: dietary folate equivalents. For combined intakes of food folate and folic acid, DFEs can be computed as follows: µg DFE = µg food folate + (1.7 x µg folic acid)

(b): NE: niacin equivalent (1 mg niacin = 1 niacin equivalent = 60 mg dietary tryptophan)

(c): RE: retinol equivalent, 1 µg RE equals 1 µg of retinol, 6 µg of β-carotene and 12 µg of other provitamin A carotenoids

(d): i.e. the second half of the first year of life (from the beginning of the 7th month to the 1st birthday)

(e): for conversion between µg and International Units (IU) of vitamin D intake: 1 µg = 40 IU and 0.025 µg = 1 IU

(f): under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may be even zero.

(g): based on phylloquinone only

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Abbreviations

1,25(OH)$_2$D	1,25-dihydroxy-vitamin D
25(OH)D	25-hydroxy-vitamin D
2-Pyr	*N*-methyl-2-pyridone-carboxamide
3HIA	3-hydroxyisovaleric acid
4-Pyr	*N*-methyl-4-pyridone-carboxamide
α-CEHC	2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman
αETK	erythrocyte transketolase activity coefficient
α-TE	α-tocopherol equivalents
α-TTP	α-tocopherol transfer protein
AI	Adequate Intake
ALA	alpha-linolenic acid
ALAP	as low as possible
AR	Average Requirement
ARA	arachidonic acid
ATP	adenosine triphosphate
BMI	body mass index
CDP-choline	cytidine-5-diphosphate-choline
CVD	cardiovascular disease
CHD	coronary heart disease
CLA	conjugated linoleic acids
CoA	coenzyme A
CrIII	chromium III
CV	coefficient of variation
DFE	dietary folate equivalents
DHA	docosahexaenoic acid
DLW	doubly labelled water
DRVs	Dietary Reference Values
E%	percentage of energy intake
EFA	essential fatty acids
EGRAC	erythrocyte glutathione reductase activation coefficient
EPA	eicosapentaenoic acid
ETKA	erythrocyte transketolase activity
EU	European Union
FAD	flavin adenine dinucleotide
FAO	Food and Agriculture Organization of the United Nations
FMN	flavin mononucleotide
FOS	fructo-oligosaccharides
GPC	glycerophosphocholine
GGCX	C-glutamyl carboxylase
Gla	C-carboxyglutamic acid
Glu	glutamic acid
GOS	galactooligosaccharides
GPC	glycerophosphocholine
GPxs	glutathione peroxidases
HDL	high density lipoprotein
IU	international unit
LA	linoleic acid
LCPUFA	long-chain polyunsaturated fatty acids
LDL	low density lipoprotein
LPI	level of phytate intake
LTI	Lower Threshold Intake
MJ	megajoule
MK	menaquinones
MMA	methylmalonic acid
MTHFR	methylene-tetrahydrofolate reductase
MUFA	monounsaturated fatty acids

NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NE	niacin equivalent
NMN	*N*-methyl-nicotinamide
NSP	nonstarch polysaccharides
PAL	physical activity level
PC	phosphatidylcholine
PChol	phosphocholine
PD-CAAS	protein digestibility-corrected amino acid score
PE	phosphatidylethanolamine
PEMT	phosphatidylethanolamine N-methyltransferase
PL	pyridoxal
PLP	pyridoxal 5'-phosphate
PMP	pyridoxamine 5'-phosphate
PN	pyridoxine
PNG	pyridoxine-5'-β-D-glucoside
PNP	pyridoxine 5'-phosphate
PPO	pyridoxamine phosphate oxidase
PRI	Population Reference Intake
PT	prothrombin time
PTH	parathyroid hormone
PUFA	polyunsaturated fatty acids
RE	retinol equivalent
REE	resting energy expenditure
RI	Reference Intake ranges for macronutrients
SAM	S-adenosylmethionine
SCF	Scientific Committee for Food
SD	standard deviation
SEPP1	selenoprotein P
SFA	saturated fatty acids
SPM	sphingomyelin
TC	transcobalamin
TDP	thiamin diphosphate
TEE	total energy expenditure
TFA	*trans* fatty acids
TG	triglycerides
tHcy	total homocysteine
TMA	trimethylamine
TMAO	trimethylamine-N-oxide
TMP	thiamine monophosphate
TPN	total parenteral nutrition
TTMA	total trimethylamine
TTP	thiamine triphosphate
UI	urinary iodine
UL	Tolerable Upper Intake Level
UNU	United Nations University
UV-B	ultraviolet B
WHO	World Health Organization

Appendix A – Concise tables on data used by the NDA Panel to set DRVs, by nutrient and population group

Table 13: Concise table on data used to set DRVs for adults

	Values	Criteria used to set DRVs	Type of studies
Energy, macronutrients and water			
Energy	AR	Factorial approach based on estimates of resting energy expenditure (REE), derived from predictive equations based on anthropometric measures, plus the energy needed for various levels of physical activity (PAL)	Metabolic studies assessing REE by direct or indirect calorimetry
Glycaemic carbohydrates	RI	Amount of energy to be provided when reference intakes for protein and fat have been met Practical considerations (e.g. current levels of intake[(a)], achievable dietary patterns) Amount compatible with the improvement of metabolic risk factors for chronic disease	Food consumption surveys Intervention studies
Dietary fibre	AI	Bowel function	Intervention studies, observational studies
Total fat	RI	Practical considerations (e.g. current levels of intake[(a)], achievable dietary patterns) No overt signs of EFA or micronutrient deficiency No adverse effects on blood lipids	Food consumption surveys Intervention studies
LA	AI	Observed intake data[(a)]	Food consumption surveys
ALA	AI	Observed intake data[(a)]	Food consumption surveys
EPA + DHA	AI	Prevention of cardiovascular diseases	Intervention studies, observational studies (prospective)
Protein	AR, PRI	Null nitrogen balance	Nitrogen balance studies
Water	AI	Observed intake data[(a)] and intake required to achieve a urine osmolarity of 500 mosm/L, considering a median potential renal solute load	Food consumption surveys
Minerals			
Calcium	AR, PRI	Young adults, 18-24 years: Intermediate value between AR for children aged 11–17 years and AR for adults ≥ 25 years Older adults, ≥ 25 years: Null balance	Balance studies
Chloride	Safe and adequate intake	Derived from the reference values for sodium on an equimolar basis, under the consideration that the main dietary source of chloride intake is sodium chloride	
Copper	AI	Observed intake data[(b)] Null balance	Food consumption surveys Balance studies

	Values	Criteria used to set DRVs	Type of studies
Fluoride	AI	Prevention of caries; extrapolated from data in children (isometric scaling)	
Iodine	AI	Prevention of goitre; extrapolated from data in children (daily urinary volume)	
Iron	AR, PRI	Factorial approach; data on iron turnover and total obligatory iron losses	Isotope studies Factorial model for bioavailability derived from cross-sectional studies
Magnesium	AI	Observed intake data[(b)]	Food consumption surveys
Manganese	AI	Observed intake data[(a)] Null balance	Food consumption surveys Balance studies
Molybdenum	AI	Observed intake data[(a)] Null balance	Food consumption surveys Balance studies
Phosphorus	AI	Derived from the PRI for calcium (molar calcium to phosphorus ratio of 1.4:1)	Studies on bone mineral content (calcium to phosphorus ratio)
Potassium	AI	Lower blood pressure levels and reduced risk of stroke	Intervention studies, observational studies (prospective)
Selenium	AI	Plasma selenoprotein P concentration	Intervention studies
Sodium	Safe and adequate intake	Lower blood pressure levels and reduced risk of CVD Null balance	Intervention studies (meta-regression), observational studies (prospective) Balance studies
Zinc	AR, PRI	Null balance, taking into account the inhibitory effect of phytate on absorption	Compartmental modelling and faecal isotope dilution studies; zinc absorption–intestinal balance studies
Vitamins			
Biotin	AI	Observed intake data[(a)]	Food consumption surveys
Choline	AI	Observed intake data[(c)] Correction of deficiency symptoms	Food consumption surveys Depletion–repletion studies
Cobalamin	AI	Serum/plasma holotranscobalamin, methylmalonic acid, total homocysteine and cobalamin Observed intake data[(b)]	Intervention studies, observational studies (cross-sectional) Food consumption surveys
Folate	AR, PRI	Serum folate and red blood cell folate	Controlled feeding studies, depletion–repletion studies
Niacin	AR, PRI	Urinary excretion of niacin metabolites, i.e. *N*-methyl-nicotinamide and *N*-methyl-2-pyridone-carboxamide	Depletion–repletion studies
Pantothenic acid	AI	Observed intake data[(a)]	Food consumption surveys
Riboflavin	AR, PRI	Urinary riboflavin excretion	Intervention studies
Thiamin	AR, PRI	Erythrocyte transketolase activity, urinary thiamin excretion	Depletion–repletion studies
Vitamin A	AR, PRI	Adequate retinol liver stores; factorial approach considering the fractional catabolic rate of body retinol and its efficiency of storage	Stable isotope studies of retinol metabolism and model-based compartmental analyses
Vitamin B6	AR, PRI	Plasma pyridoxal 5'-phosphate concentration	Intervention studies, including depletion–repletion studies, in women. Extrapolation for men

	Values	Criteria used to set DRVs	Type of studies
Vitamin C	AR, PRI	Balance of vitamin C losses and adequate body pool (assessed by fasting plasma ascorbate concentration)	Studies in men. Extrapolation for women
Vitamin D	AI	Serum 25-hydroxy-vitamin D and musculoskeletal health outcomes	Intervention studies (meta-regression), observational studies (prospective)
Vitamin E as α-tocopherol	AI	Observed intake data(b)	Food consumption surveys
Vitamin K (phylloquinone only)	AI		Value set by SCF (1993)

AI, Adequate Intake; ALA, Alpha-linolenic acid; AR, Average Requirement; DHA, docosahexaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; LA, linoleic acid; PAL, level of physical activity; PRI, Population Reference Intake; REE, resting energy expenditure; RI, Reference Intake ranges for macronutrients

(a): Dietary intakes were compiled from published reports of national food consumption surveys in European countries.

(b): Dietary intakes were calculated based on individual food consumption data from the EFSA Comprehensive European Food Consumption Database[16] (EFSA, 2011) and nutrient composition data derived from the EFSA Nutrient Composition Database.[17]

(c): Dietary intakes were calculated based on individual food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011); in the absence of food composition data on choline in Europe, composition data on free choline and choline compounds from the National Nutrient Database for Standard Reference from the US Department of Agriculture Department of Agriculture (release 26; issued in November 2013) (USDA, 2013) were used.

(d): The 2012 German National Nutrition Survey II (DGE, 2012), which used recent phylloquinone composition data, was used.

[16] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[17] https://www.efsa.europa.eu/en/data/food-composition

Table 14: Concise table on data used to set DRVs for infants (7–11 months)

	Values	Criteria used to set DRVs	Type of studies
Energy, macronutrients and water			
Energy	AR	Factorial approach based on estimates of total energy expenditure (TEE), derived from predictive equations based on anthropometric measures, plus estimates of energy deposition based on protein and fat gains	Metabolic studies assessing TEE by DLW method Studies on body composition (infants)
Total fat	RI	Gradual reduction (on E% basis) from the intake of exclusively breastfed infants aged 0–6 months	
LA	AI	Same AI (on E% basis) as for children and adolescents	
ALA	AI	Same AI (on E% basis) as for children and adolescents	
DHA	AI	Visual function	Intervention studies
Protein	AR, PRI	Factorial approach; requirements for maintenance + growth, corrected for efficiency of protein utilisation	Nitrogen balance studies Studies on whole-body potassium deposition
Water	AI	Observed intake data[a]	Food consumption surveys
Minerals			
Calcium	AI	Extrapolation from intake of exclusively breastfed infants[b] (isometric scaling)	
Chloride	AI	Derived from the reference values for sodium on an equimolar basis, under the consideration that the main dietary source of chloride intake is sodium chloride	
Copper	AI	Observed intake data[c] Extrapolation from intake of exclusively breastfed infants[b] (allometric scaling)	Food consumption surveys Studies on composition of mature milk from healthy mothers
Fluoride	AI	Prevention of caries; extrapolated from data in older children (isometric scaling)	
Iodine	AI	Prevention of goitre; extrapolated from data in older children (based on daily urinary volume)	
Iron	AR, PRI	Factorial approach	Isotope studies Factorial model for bioavailability derived from cross-sectional studies
Magnesium	AI	Observed intake data[c]	Food consumption surveys
Manganese	AI	Extrapolation from intake of exclusively breastfed infants[b] (isometric scaling) Observed intake data[a] Extrapolation from the adult AI (isometric scaling)	Food consumption surveys
Molybdenum	AI	Extrapolation from the adult AI (isometric scaling)	
Phosphorus	AI	Derived from the AI for calcium (molar calcium to phosphorus ratio of 1.4:1)	
Potassium	AI	Extrapolation from the adult AI (isometric scaling + growth factor)	

	Values	Criteria used to set DRVs	Type of studies
Selenium	AI	Extrapolation from intake of exclusively breastfed infants(b) (isometric scaling)	
Sodium	AI	Extrapolation from intake of exclusively breastfed infants(b) (scaling based on the difference in ARs for energy of infants aged 3 months and 9 months)	
Zinc	AR, PRI	Factorial approach; losses extrapolated from adult + requirement for growth	
Vitamins			
Biotin	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Choline	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Cobalamin	AI	Extrapolation from the adult AI (allometric scaling + growth factor)	
Folate	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Niacin	AR, PRI	Same AR and PRI as for adults, expressed per energy unit (mg NE/MJ)	
Pantothenic acid	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Riboflavin	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Thiamin	AR, PRI	Same AR and PRI as for adults, expressed per energy unit (mg NE/MJ)	
Vitamin A	AR, PRI	Adequate retinol liver stores; factorial approach using adult equation with age-specific values	
Vitamin B6	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling) Extrapolation from the adult AR (allometric scaling + growth factor)	Studies on composition of mature milk from healthy mothers
Vitamin C	AR, PRI	Arbitrarily set at a value three times higher than the amount known to prevent scurvy	Value set by SCF (1993)
Vitamin D	AI	Serum 25-hydroxy-vitamin D	Intervention studies
Vitamin E as α-tocopherol	AI	Extrapolation from intake of exclusively breastfed infants(b) (allometric scaling)	Studies on composition of mature milk from healthy mothers
Vitamin K (phylloquinone only)	AI	Extrapolation from the adult AI (isometric scaling)	

AI, Adequate Intake; ALA, Alpha-linolenic acid; AR, Average Requirement; DHA, docosahexaenoic acid; DLW, doubly labelled water; E%, percentage of energy intake; LA, linoleic acid; PRI, Population Reference Intake; RI, Reference Intake ranges for macronutrients; TEE, total energy expenditure

(a): Dietary intakes were compiled from published reports of national food consumption surveys in European countries. No European data were available on manganese intake in infants; data collected in Canada were used.

(b): Nutrient intake of exclusively breastfed infants aged zero to six months was calculated by multiplying the average concentration of the nutrient of interest in mature breast milk by an average breast milk intake of 0.8 L/day over the first six months of lactation (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009).

(c): Dietary intakes were calculated based on individual food consumption data from the EFSA Comprehensive European Food Consumption Database[18] (EFSA, 2011) and nutrient composition data derived from the EFSA Nutrient Composition Database.[19]

[18] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[19] https://www.efsa.europa.eu/en/data/food-composition

Table 15: Concise table on data used to set DRVs for children (1–17 years)

	Values	Criteria used to set DRVs	Type of studies
Energy, macronutrients and water			
Energy	AR	Factorial approach; resting energy expenditure (REE) derived from predictive equations + energy needed for physical activity	Metabolic studies assessing REE by direct or indirect calorimetry
Glycaemic carbohydrates	RI	Amount of energy to be provided when reference intakes for protein and fat intake have been met Practical considerations (e.g. current levels of intake[a], achievable dietary patterns) Amount compatible with the improvement of metabolic risk factors for chronic disease	Food consumption surveys Intervention studies
Dietary fibre	AI	Extrapolation from the AI for adults (adjusting for energy intake) Compatibility with normal growth and development	
Total fat	RI	Children ≤ 3 years: gradual reduction (on E% basis) from the intake of exclusively breastfed infants aged 0–6 months Children > 3 years: same RI as for adults	
LA	AI	Observed intake data[a]	Food consumption surveys
ALA	AI	Observed intake data[a]	Food consumption surveys
DHA	AI	1 year of age: visual function	Intervention studies
EPA + DHA	AI	2–17 years: dietary advice consistent with advice (food and nutrient based dietary guidelines, respectively) for the adult population	
Protein	AR, PRI	Factorial approach; requirements for maintenance + growth, corrected for efficiency of protein utilisation	Nitrogen balance studies Studies on whole-body potassium deposition
Water	AI	Observed intake data[a], corrected for a desirable water-energy relationship of 1 mL/kcal and inter-individual variation	Food consumption surveys
Minerals			
Calcium	AR, PRI	Factorial approach; calcium accretion in bone + replacement of obligatory losses, correcting for absorption efficiency	Observational studies (bone mineral content) Isotope studies Controlled feeding studies
Chloride	Safe and adequate intake	Derived from the reference values for sodium on an equimolar basis, under the consideration that the main dietary source of chloride intake is sodium chloride	
Copper	AI	Observed intake data[b]	Food consumption surveys
Fluoride	AI	Prevention of caries	Observational studies (prospective), intervention studies
Iodine	AI	Prevention of goitre	Observational study (cross-sectional)
Iron	AR, PRI	Factorial approach	Isotope studies Factorial model for bioavailability derived from cross-sectional studies
Magnesium	AI	Observed intake[b]	Food consumption surveys

	Values	Criteria used to set DRVs	Type of studies
Manganese	AI	Extrapolation from adult AI (isometric scaling)	
Molybdenum	AI	Extrapolation from adult AI (isometric scaling)	
Phosphorus	AI	Derived from the PRI for calcium (molar calcium to phosphorus ratio of 1.4:1)	Studies on bone mineral content (calcium to phosphorus ratio)
Potassium	AI	Extrapolation from adult AI (isometric scaling + growth factors)	
Selenium	AI	Extrapolation from adult AI (isometric scaling + growth factors)	
Sodium	Safe and adequate intake	Extrapolation from adult value (scaling based on the differences in AR for energy + growth factors)	
Zinc	AR, PRI	Factorial approach; losses extrapolated from adult + requirement for growth	
Vitamins			
Biotin	AI	Observed intake data[(a)]	Food consumption surveys
Choline	AI	Extrapolation from adult AI (allometric scaling + growth factors) Observed intake data[(c)]	
Cobalamin	AI	Extrapolation from adult AI (allometric scaling + growth factors)	
Folate	AR, PRI	Extrapolation from adult AR (allometric scaling + growth factors)	
Niacin	AR, PRI	Same AR and PRI as for adults, expressed as mg NE/MJ	
Pantothenic acid	AI	Observed intake data[(a)]	Food consumption surveys
Riboflavin	AR, PRI	Extrapolation from adult AR (allometric scaling + growth factors)	
Thiamin	AR, PRI	Same AR and PRI as for adults, expressed as mg/MJ	
Vitamin A	AR, PRI	Adequate retinol liver stores; factorial approach using adult equation with age-specific values	
Vitamin B6	AR, PRI	Extrapolation from adult AR (allometric scaling + growth factors)	
Vitamin C	AR, PRI	Extrapolation from adult AR (isometric scaling)	
Vitamin D	AI	Serum 25(OH)D) and musculoskeletal health outcomes	Intervention studies (meta-regression), observational studies (prospective)
Vitamin E as α-tocopherol	AI	Observed intake data[(b)]	Food consumption surveys
Vitamin K (phylloquinone only)	AI	Extrapolation from adult AI (isometric scaling)	

AI, Adequate Intake; ALA, Alpha-linolenic acid; AR, Average Requirement; DHA, docosahexaenoic acid; E%, percentage of energy intake; EPA, eicosapentaenoic acid; LA, linoleic acid; MJ, megajoule; PRI, Population Reference Intake; REE, resting energy expenditure; RI, Reference Intake ranges for macronutrients

(a): Dietary intakes were compiled from published reports of national food consumption surveys in European countries.

(b): Dietary intakes were calculated based on individual food consumption data from the EFSA Comprehensive European Food Consumption Database[20] (EFSA, 2011) and nutrient composition data derived from the EFSA Nutrient Composition Database.[21]

(c): Dietary intakes were calculated based on individual food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011); in the absence of food composition data on choline in Europe, composition data on free choline and choline compounds from the National Nutrient Database for Standard Reference from the US Department of Agriculture Department of Agriculture (release 26; issued in November 2013) (USDA, 2013) were used.

[20] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[21] https://www.efsa.europa.eu/en/data/food-composition

Table 16: Concise table on data used to set DRVs for pregnant women

	Values	Criteria used to set DRVs	Type of studies
Energy, macronutrients and water			
Energy	AR	AR for non-pregnant women + amount of energy related to the increase in body mass, estimated using the cumulative increment in TEE estimated with the DLW technique plus the energy deposited as protein and fat	Metabolic studies assessing TEE by DLW method Observational studies of body composition during pregnancy
Total fat	RI	Same RI as for other adults	
LA	AI	Same AI (on E% basis) as for other adults	
ALA	AI	Same AI (on E% basis) as for other adults	
EPA + DHA	AI	AI of DHA and EPA for other adults + additional supply of DHA to compensate for oxidative losses and accumulation in the fetus	
Protein	AR, PRI	Factorial approach; requirement for maintenance + protein deposition in fetal and maternal tissues, correcting for efficiency of protein utilisation	Nitrogen balance studies Studies on whole-body potassium deposition
Water	AI	AI for non-pregnant women + increase based on energy intake during pregnancy and a desirable water-energy relationship of 1 mL/kcal	
Minerals			
Calcium	AR, PRI	Same AR and PRI as for non-pregnant women (adaptive physiological changes)	
Chloride	Safe and adequate intake	Derived from the reference values for sodium on an equimolar basis, under the consideration that the main dietary source of chloride intake is sodium chloride	
Copper	AI	AI for non-pregnant women + fetal and maternal accumulation, correcting for absorption efficiency	Studies on body composition (newborn, maternal tissues)
Fluoride	AI	Same AI as for non-pregnant women	
Iodine	AI	AI for non-pregnant women + needs for increased maternal thyroid hormone production and iodine uptake by the fetus, placenta and amniotic fluid	Studies in pregnant women on thyroid hormone substitution therapy Studies on body composition (newborn, maternal tissues)
Iron	AR, PRI	Same AR and PRI as for non-pregnant women (adaptive physiological changes)	
Magnesium	AI	Same AI as for non-pregnant women is applied (small increase in magnesium requirement covered by adaptive physiological mechanisms and increase in energy intake)	Studies on body composition (newborn, maternal tissues)
Manganese	AI	Same AI as for non-pregnant women is applied (homeostatic control)	
Molybdenum	AI	Same AI as for non-pregnant women (insufficient data)	

	Values	Criteria used to set DRVs	Type of studies
Phosphorus	AI	Same AI as for non-pregnant women (adaptive physiological changes)	
Potassium	AI	Same AI as for non-pregnant women (adaptive physiological changes)	
Selenium	AI	Same AI as for non-pregnant women (adaptive physiological changes)	
Sodium	Safe and adequate intake	Same safe and adequate intake as for non-pregnant women (adaptive physiological changes)	
Zinc	AR, PRI	AR for non-pregnant women + fetal and maternal accumulation, correcting for absorption efficiency	Studies on body composition (newborn, maternal tissues)
Vitamins			
Biotin	AI	Same AI as for non-pregnant women (insufficient data)	
Choline	AI	Extrapolation from the AI for non-pregnant women (isometric scaling)	
Cobalamin	AI	AI for non-pregnant women + fetal accumulation, correcting for absorption efficiency	Studies on body composition (newborns)
Folate	AI	Serum folate and RBC folate	Controlled feeding study
Niacin	AR, PRI	Same AR and PRI (expressed as mg NE/MJ) as for non-pregnant women	
Pantothenic acid	AI	Same AI as for non-pregnant women (insufficient data)	
Riboflavin	AR, PRI	Extrapolation from the AI for non-pregnant women (allometric scaling)	
Thiamin	AR, PRI	Same AR and PRI (expressed as mg/MJ) as for non-pregnant women	
Vitamin A	AR, PRI	AR for non-pregnant women + fetal and maternal tissues accumulation, correcting for retinol efficiency of storage	Studies on body composition (newborns)
Vitamin B6	AR, PRI	AR for non-pregnant women + fetal and maternal accumulation, correcting from bioavailability from a mixed diet	Studies on body composition
Vitamin C	AR, PRI	AR for non-pregnant women + need of the fetus	Value set by WHO/FAO (2004)
Vitamin D	AI	Same AI as for non-pregnant women (no data to suggest a different target value for 25-hydroxyvitamin D concentration)	
Vitamin E as α-tocopherol	AI	Same AI as for non-pregnant women (no evidence for an increased requirement)	
Vitamin K (phylloquinone only)	AI	Extrapolation from the AI for non-pregnant women (isometric scaling)	

AI, Adequate Intake; ALA, Alpha-linolenic acid; AR, Average Requirement; DHA, docosahexaenoic acid; DLW, doubly labelled water; E%, percentage of energy intake; EPA, eicosapentaenoic acid; LA, linoleic acid; MJ, megajoule; PRI, Population Reference Intake; REE, resting energy expenditure; RI, Reference Intake ranges for macronutrients; TEE, total energy expenditure

Table 17: Concise table on data used to set DRVs for lactating women

	Values	Criteria used to set DRVs	Type of studies
Energy, macronutrients and water			
Energy	AR	AR for non-lactating women + energy requirement for milk production (considering milk energy density and energetic efficiency), corrected for energy mobilisation from maternal tissues	Observational studies on the rate of body mass loss after delivery
Total fat	RI	Same RI as for other adults	
LA	AI	Same AI (on E% basis) as for other adults	
ALA	AI	Same AI (on E% basis) as for other adults	
EPA + DHA	AI	AI of DHA and EPA for other adults + additional supply of DHA to compensate for oxidative losses and accumulation in the infant	
Protein	AR, PRI	Factorial approach; requirement for maintenance + requirement for milk production estimated from milk protein output and the efficiency of dietary protein utilisation	
Water	AI	AI for non-lactating women + amount lost through breast milk	
Minerals			
Calcium	AR, PRI	Same AR and PRI as for non-lactating women (adaptive physiological changes)	
Chloride	Safe and adequate intake	Derived from the reference values for sodium on an equimolar basis, under the consideration that the main dietary source of chloride intake is sodium chloride	
Copper	AI	AI for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Fluoride	AI	Same AI as for non-pregnant women	
Iodine	AI	AI for non-lactating women + partial compensation of the amount secreted in breast milk[(a)] (assuming adequate iodine status before pregnancy)	Studies on composition of mature milk from healthy mothers
Iron	AR, PRI	Same AR and PRI as for non-pregnant premenopausal women (corresponds to basal losses + amount secreted in breast milk[(a)], assuming no menstruation)	Studies on composition of mature milk from healthy mothers Isotope studies
Magnesium	AI	Same AI as for non-lactating women (adaptive physiological changes)	
Manganese	AI	Same AI as for non-lactating women (small amount secreted in breastmilk)	Studies on composition of mature milk from healthy mothers
Molybdenum	AI	Same AI as for non-lactating women (insufficient data)	
Phosphorus	AI	Same AI as for non-lactating women (adaptive physiological changes)	
Potassium	AI	AI for non-lactating women + amount secreted in breast milk[(a)]	Studies on composition of mature milk from healthy mothers

	Values	Criteria used to set DRVs	Type of studies
Selenium	AI	AI for non-lactating women + amount secreted in breast milk[(a)]	Studies on composition of mature milk from healthy mothers
Sodium	Safe and adequate intake	Same safe and adequate intake as for non-lactating women	
Zinc	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Vitamins			
Biotin	AI	AI for non-lactating women + amount secreted in breast milk[(a)]	Studies on composition of mature milk from healthy mothers
Choline	AI	AI for non-lactating women + amount secreted in breast milk[(a)]	Studies on composition of mature milk from healthy mothers
Cobalamin	AI	AI for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Folate	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Niacin	AR, PRI	Same AR and PRI (expressed as mg NE/MJ) as for non-lactating women	
Pantothenic acid	AI	AI for non-lactating women + amount secreted in breast milk[(a)]	Studies on composition of mature milk from healthy mothers
Riboflavin	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Thiamin	AR, PRI	Same AR and PRI (expressed as mg/MJ) as for non-lactating women	
Vitamin A	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Vitamin B6	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting from bioavailability from a mixed diet	Studies on composition of mature milk from healthy mothers
Vitamin C	AR, PRI	AR for non-lactating women + amount secreted in breast milk[(a)], correcting for absorption efficiency	Studies on composition of mature milk from healthy mothers
Vitamin D	AI	Same AI as for non-lactating women (compensation of loss in breast milk not justified)	
Vitamin E as α-tocopherol	AI	Same AI as for non-lactating women (compensation of loss in breast milk not justified)	
Vitamin K (phylloquinone only)	AI	Same AI as for non-lactating women (covers the small excretion in breast milk)	

AI, Adequate Intake; ALA, Alpha-linolenic acid; AR, Average Requirement; DHA, docosahexaenoic acid; E%, percentage of energy intake; EPA, eicosapentaenoic acid; LA, linoleic acid; MJ, megajoule; PRI, Population Reference Intake; RI, Reference Intake ranges for macronutrients; TEE, total energy expenditure

(a): Nutrient secretion was calculated by multiplying the average concentration of the nutrient of interest in mature breast milk by an average breast milk secretion of 0.8 L/day over the first six months of lactation (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009).

Appendix B – Scaling

In isometric scaling the AI or AR for the population group X under consideration is derived by multiplication of the known and sex-specific AI or AR of group Y with the quotient between the typical weight of group X and the weight of group Y (Table 18):

Isometric scaling:

$$AI\ (or\ AR)_X = AI\ (or\ AR)_Y \times (weight_X/weight_Y)$$

Allometric scaling reflects that the metabolic rate of an organism is an exponential function of body mass (weight). In allometric scaling the AI or AR for the population group X under consideration is derived by multiplication of the known and sex-specific AI or AR of group Y with the quotient between the typical weight of group X and the weight of group Y (Table 18), to the power of 0.75:

Allometric scaling:

$$AI\ (or\ AR)_X = AI\ (or\ AR)_Y \times (weight_X/weight_Y)^{0.75}$$

When scaling down from an adult AI or AR to children, corrections for growth requirements have to be considered in order to account for the nutrient amount required to be incorporated into newly formed tissue. An age specific growth factor may be added based on the proportional increase in protein requirements for growth (Table 19):

Allometric scaling and growth factor:

$$AI\ (or\ AR)_X = AI\ (or\ AR)_Y \times (weight_X/weight_Y)^{0.75} \times (1 + growth\ factor)$$

Isometric scaling and growth factor:

$$AI\ (or\ AR)_X = AI\ (or\ AR)_Y \times (weight_X/weight_Y) \times (1 + growth\ factor)$$

Table 18: Reference body weights for children and adults used for scaling

Population group	Age taken as reference	Reference weight (kg)			References
		Males[a]	Females[a]	Males and Females[b]	
0–6 mo	3 mo	6.4	5.8	6.1	(WHO Multicentre Growth Reference Study Group, 2006)
7–11 mo	9 mo	8.9	8.2	8.6	(WHO Multicentre Growth Reference Study Group, 2006)
1–3 yrs	2 yrs	12.2	11.5	11.9	(WHO Multicentre Growth Reference Study Group, 2006)
4–6 yrs	5 yrs	19.2	18.7	19.0	(van Buuren et al., 2012)
7–10 yrs	8.5 yrs	29.0	28.4	28.7	(van Buuren et al., 2012)
11–14 yrs	12.5 yrs	44.0	45.1	44.6	(van Buuren et al., 2012)
15–17 yrs	16 yrs	64.1	56.4	60.3	(van Buuren et al., 2012)
≥ 18 yrs	n.a.	68.1[c]	58.5[c]	63.3	(EFSA NDA Panel, 2013b)
Pregnant women	n.a.	-	70.5	-	(EFSA NDA Panel, 2013b)

mo, months; yrs, years

(a): The median weight-for-age at the age taken as reference was used as reference weight

(b): Mean of the values for males and females

(c): Derived from measured body heights of men and women aged 18–79 years in 13 EU Member States and assuming a body mass index of 22 kg/m^2

Table 19: Growth factors

Age (yrs)	Maintenance requirement (g protein/kg per day) [a] (A)	Growth requirement (g protein/kg per day) [a] (B)	Average Requirement for protein (g/kg per day) [a] (A+B)	Calculated growth factor (B/A)	Growth factor per age group [b]
Boys					
0.5	0.66	0.46	1.12	0.70	7–11 mo: 0.57
1	0.66	0.29	0.95	0.44	
1	0.66	0.29	0.95	0.44	1–3 yrs: 0.25
2	0.66	0.13	0.79	0.20	
3	0.66	0.07	0.73	0.11	
4	0.66	0.03	0.69	0.05	4–6 yrs: 0.06
5	0.66	0.03	0.69	0.05	
6	0.66	0.06	0.72	0.09	
7	0.66	0.08	0.74	0.12	7–10 yrs: 0.13
8	0.66	0.09	0.75	0.14	
9	0.66	0.09	0.75	0.14	
10	0.66	0.09	0.75	0.14	
11	0.66	0.09	0.75	0.14	11–14 yrs: 0.11
12	0.66	0.08	0.74	0.12	
13	0.66	0.07	0.73	0.11	
14	0.66	0.06	0.72	0.09	
15	0.66	0.06	0.72	0.09	15–17 yrs: 0.08
16	0.66	0.05	0.71	0.08	
17	0.66	0.04	0.70	0.06	
Girls					
0.5	0.66	0.46	1.12	0.70	7–11 mo: 0.57
1	0.66	0.29	0.95	0.44	
1	0.66	0.29	0.95	0.44	1–3 yrs: 0.25
2	0.66	0.13	0.79	0.20	
3	0.66	0.07	0.73	0.11	
4	0.66	0.03	0.69	0.05	4–6 yrs: 0.06
5	0.66	0.03	0.69	0.05	
6	0.66	0.06	0.72	0.09	
7	0.66	0.08	0.74	0.12	7–10 yrs: 0.13
8	0.66	0.09	0.75	0.14	
9	0.66	0.09	0.75	0.14	
10	0.66	0.09	0.75	0.14	
11	0.66	0.07	0.73	0.11	11–14 yrs: 0.08
12	0.66	0.06	0.72	0.09	
13	0.66	0.05	0.71	0.08	
14	0.66	0.04	0.7	0.06	
15	0.66	0.03	0.69	0.05	15–17 yrs: 0.03
16	0.66	0.02	0.68	0.03	
17	0.66	0.01	0.67	0.02	

mo, months; yrs, years

(a): EFSA NDA Panel (2012).
(b): The value for each age group corresponds to the mean of values for the years included.

Growth factors were calculated as the proportional increase in protein requirement for growth relative to the maintenance requirement at the different ages (EFSA NDA Panel, 2012). The value for each age group corresponds to the mean of values for the years included (EFSA NDA Panel, 2014e).