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SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for biotin[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) derived Dietary Reference Values (DRVs) for biotin. 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. Data on biomarkers of biotin intake or status are insufficient to be used in determining the requirement for biotin. Data available on biotin intakes and health consequences are very limited and cannot be used for deriving DRVs for biotin. As there is insufficient evidence available to derive an Average Requirement and a Population Reference Intake, an Adequate Intake (AI) is proposed. 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. The AI for adults is set at 40 µg/day. The AI for adults also applies to pregnant women. For lactating women, an additional 5 µg biotin/day over and above the AI for adults is proposed, to compensate for biotin losses through breast milk. 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 and reference body weight for each age group, in order to account for the role of biotin in energy metabolism. 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.

KEY WORDS

biotin, Dietary Reference Value, Adequate Intake

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values (DRVs) for the European population, including biotin.

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.

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. The AI for adults also applies to pregnant women. 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. 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. 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.

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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[4]. 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 the 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[5], 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 the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;

[4] 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.

[5] 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.

- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;
- Protein;
- Dietary fibre.

Following on from the first part of the task, the 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, the 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).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on the nutrient and energy intakes for the European Community but was unable to define a specific physiological requirement of biotin for human health (SCF, 1993). The SCF noted that average intakes in adults in the European Community were about 28–42 µg/day, but that individuals consumed 15–100 µg/day. The SCF proposed an Acceptable Range of Intakes of biotin for adults of 15–100 µg/day, which was considered adequate to meet requirements and prevent deficiency. The SCF considered that there was no information on which to base additional requirements for biotin in pregnancy or lactation. The SCF did not set reference values for infants and children.

2. Definition/category

2.1. Chemistry

Biotin is a bicyclic water-soluble vitamin that comprises an ureido ring fused with a tetrahydrothiophene ring, functionalised with a valeric acid side chain (Mock, 2014). It has a molecular mass of 244.31 Da. The only form found in nature that is biologically active is the isomer D(+)-biotin (Mock, 2014).

Biotin in food and human tissues may occur in the free form or bound to protein. Microbiological assays and avidin-binding assays have most frequently been used to quantify biotin in foods and biological fluids. Assays need to account for interferences of biotin analogues and metabolites, as well as the need for prior acid or enzymatic hydrolysis necessary to measure protein-bound biotin (Mock and Malik, 1992; Lahely et al., 1999), which affect their specificity and sensitivity. Thus, both natural variation and analytical aspects may account for the variability of reported biotin concentrations in foods or body fluids and it is important to establish how biotin was quantified when comparing studies (SCF, 2001).

High-performance liquid chromatography (HPLC)/avidin-binding assay is considered the most accurate and sensitive assay for quantification of biotin (IOM, 1998; SCF, 2001; Staggs et al., 2004).

2.2. Function, physiology and metabolism

Biotin is a co-factor for the enzymes acetyl-CoA carboxylase (ACC), propionyl-CoA carboxylase (PCC), β-methylcrotonyl-CoA carboxylase (MCC) and pyruvate carboxylase (PC), which play critical roles in the synthesis of fatty acids, the catabolism of branched-chain amino acids and gluconeogenesis (Zempleni and Mock, 1999c). Humans cannot synthesise biotin and depend on its dietary intake.

At usual intakes, free biotin is absorbed nearly completely through a saturable carrier-mediated process. Absorption efficiency of free biotin is also high upon ingestion of large doses (up to 20 mg/day) as a result of passive diffusion (Zempleni and Mock, 1999b). Protein-bound biotin requires to be released by biotinidase before absorption. The proportion of free biotin versus protein-bound biotin varies among foods; the majority of biotin in meats and cereals is bound to protein (Zempleni and Mock, 1999c). There is a lack of data on the level of absorption of protein-bound biotin from foods. Avidin, a protein found in raw egg white, has a very high affinity for biotin and prevents its absorption in the small intestine. Faecal excretion of biotin has been observed to be three to six times higher than intakes, owing to the production of large amounts of biotin by the intestinal

microbiota; however, the extent to which biotin is absorbed from the large intestine and contributes to biotin requirements is uncertain (SCF, 2001).

Once in plasma, biotin is transported as free biotin (81 %), as well as covalently or reversibly bound to plasma proteins (12 % and 7 %, respectively) (Mock, 2014). Biotin uptake into the liver and peripheral tissues occurs via a specific sodium-dependent, carrier-mediated process and by diffusion. In the cell, biotin is covalently attached to biotin-dependent carboxylases. Holocarboxylases can be degraded to biocytin or biotin-containing oligopeptides from which biotin can be released by biotinidase and re-used in the metabolism. The biotin that is not incorporated into carboxylase enzymes is catabolised through different pathways to form bisnorbiotin, tetranorbiotin and related intermediates, by β-oxidation of the valeric side-chain, or biotin sulphoxides and sulphone, by oxidation of the sulphur present in its heterocyclic ring (Mock, 2014). Biotin and its metabolites are excreted in the urine (Mock NI et al., 1997; Zempleni et al., 1997b). Biliary excretion of biotin and its metabolites is thought to be quantitatively negligible (Zempleni and Mock, 1999a), as indicated by data in rats and pigs (Zempleni et al., 1997a). Biotin metabolites do not have vitamin activity.

Placental transport of biotin involves an active mechanism (Grassl, 1992; Wang et al., 1999; Mock, 2014); from the second trimester, biotin concentrations have been observed to be 3- to 17-fold higher in the plasma of fetuses than in plasma of their mothers (Mantagos et al., 1998).

The concentration of biotin in human milk has been observed to vary significantly among subjects, over the course of the day and as a function of time *post partum* (Mock et al., 1992; Mock DM et al., 1997b). Mean concentrations of biotin in mature human milk measured by microbiological assays typically range between about 4 and 6 µg/L (data from Finland, the UK, Japan and the USA, up to one year of lactation) (Goldsmith et al., 1982; Ford et al., 1983; Salmenpera et al., 1985; Hirano et al., 1992; Sakurai et al., 2005) (Appendix A). Using an HPLC/avidin-binding assay, Mock DM et al. (1997b) reported mean biotin concentrations in mature milk of around 7 µg/L in a cohort of 15 healthy breastfeeding women in the USA who were not consuming daily supplements containing more than 4 µg biotin (about 10 % of daily intake).

Dietary biotin deficiency is rare and is characterised by fine scaly dermatitis, hair loss, conjunctivitis, ataxia and delayed child development (Zempleni and Mock, 1999c). Cases of biotin deficiency have been observed in patients receiving long-term total parenteral nutrition without biotin supplementation and in patients with biotinidase deficiency, as well as in people who had consumed large amounts of raw eggs (Zempleni and Mock, 1999c). Biotin deficiency during pregnancy has been shown to be teratogenic in several species, including mice, hamsters, chicken and turkeys (Said, 1999; Zempleni and Mock, 2000; Mock, 2005), but no data are available in humans indicative of an association between biotin deficiency in pregnancy and an increased incidence of fetal malformations.

The SCF could not derive a Tolerable Upper Intake Level (UL) and stated that available evidence indicates that observed levels of intake of biotin from all sources do not represent a health risk for the general population (SCF, 2001).

Although biotin and pantothenic acid have been shown to share common carrier-mediated uptake mechanisms *in vitro* (Said, 2009), the nutritional implications of this interaction are not known.

2.3. Biomarkers

Mock and his collaborators have studied urinary and blood biomarkers of biotin status by providing healthy men and women with a biotin-depleted diet based on raw egg white, to induce asymptomatic biotin insufficiency. Between 7 and 11 subjects were involved in each study and depletion lasted for three to four weeks. The 24-hour urinary excretion of biotin was found to decrease significantly over the depletion period (Mock NI et al., 1997; Mock et al., 2002a), while plasma concentration of biotin

was not significantly affected (Mock NI et al., 1997). Biotin insufficiency was also shown to affect markers of the activities of biotin-dependent carboxylase enzymes and related metabolic pathways. The 24-hour urinary excretion of 3-hydroxyisovaleric acid (3HIA) and 3HIA-carnitine, as well as fasting plasma 3HIA-carnitine concentration, were found to increase significantly in male and female subjects over the depletion period, indicating a reduced activity of MCC (Mock NI et al., 1997; Mock et al., 2002a; Horvath et al., 2010b; Horvath et al., 2010a; Stratton et al., 2011). Pooling of data from two sources (Mock NI et al., 1997; Mock et al., 2002a) indicated that urinary excretion of 3HIA or biotin did not differ between sexes, either before egg white feeding or at day 21 of depletion (Mock et al., 2002a). Urinary excretion of 3HIA and 3HIA-carnitine was also shown to significantly increase in response to an oral challenge of leucine, an amino acid whose degradation requires MCC (Mock et al., 2002a; Mock et al., 2011). A decrease in PCC activity in lymphocytes and, more recently, an increase in the ratios of acylcarnitines in urine, arising from acyl-CoA substrates and their products, which reflect disturbances in biotin-dependent carboxylase activities, were also observed in response to induced biotin insufficiency (Stratton et al., 2006; Bogusiewicz et al., 2012).

In a randomised cross-over study in 16 healthy non-smoking men and women (21–45 years), possible markers of biotin status were assessed. Each subject followed three intervention phases of three weeks each interrupted by wash-out periods of two weeks, i.e. biotin "depletion" (using an egg white diet), "sufficiency" (habitual diet supplemented with 30 µg/day of biotin) and "supplementation" (habitual diet supplemented with 600 µg/day of biotin) (Eng et al., 2013). Significant differences in the amounts of biotinylated MCC and PCC in lymphocytes were observed between the three interventions. Urinary excretion of biotin did not differ between the "biotin-deficient" and "biotin-sufficient" interventions. Average urinary excretion of 3HIA was twice as high during the "biotin-deficient" intervention as during the two other interventions. However, for 8 of the 16 subjects, the urinary excretion of 3HIA did not increase during biotin "depletion" compared with the other intervention periods. The amount of mRNAs coding for biotin-dependent carboxylases, biotin transporters and holocarboxylase synthetase in lymphocytes was not different among the interventions.

Smoking (Sealey et al., 2004) and the use of some anticonvulsant drugs (Mock and Dyken, 1997; Mock et al., 1998) were also found to increase urinary excretion of 3HIA and to increase ratios of urinary biotin catabolites to biotin, indicating increased catabolism of biotin.

The Panel notes that biomarkers sensitive to biotin depletion have been identified in adults. These include urinary biotin excretion and biomarkers of biotin function, such as urinary excretion of 3HIA and 3HIA-carnitine, and PCC activity and abundance of biotinylated MCC and PCC 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.

3. Dietary sources and intake data

3.1. Dietary sources

Staggs et al. (2004) compared the biotin content of 87 foods measured by HPLC/avidin-binding assay to values published from earlier analyses, mostly using bioassays. This study confirmed previous assessments that meat, fish, poultry, egg, some cheeses and some vegetables are rich dietary sources of biotin. The HPLC/avidin-binding assay showed that the richest sources of biotin are liver (416 ng/g wet weight), eggs (214 ng/g wet weight), mushrooms (22 ng/g wet weight) and some cheeses (15–30 ng/g wet weight), while smaller amounts are found in lean meat, fruit, cereals and bread (1–10 ng/g wet weight). Most previously published values were higher than those measured by the HPLC/avidin-binding assay. Differences may relate to the natural variation of the biotin content of foods

(depending on, for instance, growing conditions, season, geographic origin, processing), as well as to differences in the specificity and sensitivity of the HPLC/avidin-binding assay and bioassays inherent to these methodologies (see Section 2.1).

Currently, D-biotin may be added to foods[6] and food supplements.[7] The biotin content of infant and follow-on formulae is regulated.[8]

3.2. Dietary intakes

Estimates of biotin intakes in children and adolescents, adults and older adults from five EU countries (Austria, Germany, Hungary, Ireland and Latvia, data collected between 2003 and 2010) are provided in Appendices B, C and D, respectively. Values were calculated from individual consumption data collected from dietary history, three-/four-day dietary records, or 24-hour recall, combined with analytical data from food composition tables. Dietary intake data are prone to reporting errors and there is a varying degree of under-reporting in different surveys (Merten et al., 2011). Although the differences in methodologies have an impact on the accuracy of between-country comparisons, the data presented give an overview of the biotin intake in a number of European countries.

In young children aged 1–4 years, median intakes ranged from 19 to 28 µg/day. A median biotin intake of 19 µg/day was observed in Irish boys and girls (IUNA, online-a). Median biotin intakes of 25 µg/day and 28 µg/day were observed in German girls and boys, respectively (DGE, 2012).

In boys and girls aged 5–12 years, mean/median intakes ranged from 19 to 38 µg/day. Median intakes ranged from 19 to 24 µg/day in Ireland (boys and girls, 5–12 years), and were reported to be 36 µg/day in girls and 38 µg/day in boys in Germany (6–11 years), while mean intakes ranged from 30 to 35 µg/day in Austria (boys and girls, 7–12 years).

In adolescent boys and girls aged 13–19 years, mean/median intakes ranged from 17 to 64 µg/day. Median intakes ranged from 17 to 27 µg/day in Ireland (boys and girls, 13–19 years) and were reported to be 36 µg/day in girls and 45 µg/day in boys (15–19 years, determined by 24-hour recall) or 50 µg/day in girls and 64 µg/day in boys (12–17 years, using dietary history over four weeks) in Germany. Mean intakes ranged from 31 to 47 µg/day in Austria (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. Data from Germany and Ireland indicated median intakes between 40 and 48 µg/day in men and between 29 and 42 µg/day in women, while mean intakes were observed to range between 33 and 50 µg/day in men and 26 and 43 µg/day in women in Hungary and Austria, and between 34 and 45 µg/day for both sexes in Latvia.

In older adult men and women, mean/median intakes ranged from 24 to 43 µg/day. Median intakes between 36 and 43 µg/day in men and 32 and 39 µg/day in women were observed in Germany and Ireland and mean intakes of 29 to 34 µg/day in men and 24 to 34 µg/day in women were reported in Hungary and Austria.

Data on biotin intakes in pregnancy are scarce. Using a 24-hour recall, mean intakes of 49 µg/day and 48 µg/day were reported in 87 Viennese and 426 Austrian pregnant women, respectively (Elmadfa et al., 2004; Elmadfa et al., 2009). Some intake estimates are also available from an observational study conducted in the UK, in which mean intakes of biotin in a population of 123 pregnant women were

[6] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26.

[7] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51.

[8] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p. 1.

18 ± 7 µg/day (range 5–37 µg/day), using a food frequency questionnaire and 19 ± 10 µg/day (range 5–79 µg/day), using 24-hour recall (Mouratidou et al., 2006).

The Panel notes that estimates of the biotin content of foods vary widely partly as a result of natural variation and partly depending on the analytical method used (see Sections 2.1 and 3.1), and that this contributes to uncertainty regarding current intake estimates.

4. Overview of Dietary Reference Values and recommendations

Several national and international authorities have proposed reference values or recommendations for biotin intakes. There has been consensus so far that evidence is lacking to establish an Average Requirement (AR) for biotin. Rather, Adequate or Acceptable Ranges of Intakes have been proposed (Table 1). The Nordic countries did not set a reference value for biotin (NNR, 2012).

4.1. Adults

The SCF (1993), the UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) and the German-speaking countries (D-A-CH, 2013) set Acceptable Ranges of Intakes, and the French Food Safety Agency (Afssa, 2001) set Adequate Intakes (AIs), based on data from dietary intake surveys, considering the absence of deficiency at observed intakes. The US Institute of Medicine (IOM, 1998) and the World Health Organization / Food and Agriculture Organization of the United Nations (WHO/FAO, 2004) proposed AIs derived from upward extrapolation of the AI for infants using allometric scaling (body weight to the power of 0.75 and reference body weights).

4.2. Infants and children

The German-speaking countries (D-A-CH, 2013), WHO/FAO (2004), Afssa (2001) and IOM (1998) proposed AIs for infants based on estimated biotin intakes with human milk in exclusively breastfed infants. WHO/FAO (2004) and IOM (1998) derived AIs for children and adolescents from upward extrapolation of the AI for infants using allometric scaling (body weight to the power of 0.75 and reference body weights), while Afssa (2001) scaled down from the AI for adults using height squared as the basis for extrapolation.

4.3. Pregnancy and lactation

Although an increase in urinary excretion of 3HIA and a decrease in urinary excretion of biotin have been observed in some pregnant women (Mock and Stadler, 1997; Mock DM et al., 1997a), data have been considered insufficient to derive a specific reference value for pregnant women (IOM, 1998; Afssa, 2001; WHO/FAO, 2004). The AI set for adults was considered to be sufficient to cover the period of pregnancy.

An additional intake of 5 µg/day has generally been proposed for lactating women to cover biotin losses due to breastfeeding, considering the amount of biotin that would be excreted by women breastfeeding exclusively (IOM, 1998; Afssa, 2001; WHO/FAO, 2004).

Table 1: Overview of Dietary Reference Values for biotin

	D-A-CH (2013)	WHO/FAO (2004)	Afssa (2001)	IOM (1998)	SCF (1993)	DH (1991)
Infants						
Age (months)	4–<12	7–12	0–12	7–12		
AI (µg/day)	5–10 [(a)]	6	6	6	–	–
Children and adolescents						
Age (years)	1–<4	1–3	1–3	1–3		
AI (µg/day)	10–15 [(a)]	8	12	8	–	–
Age (years)	4–<7	4–6	4–6	4–8		
AI (µg/day)	10–15 [(a)]	12	20	12	–	–
Age (years)	7–<10	7–9	7–9	9–13		
AI (µg/day)	15–20 [(a)]	20	25	20	–	–
Age (years)	10–<13	10–18	10–12	14–18		
AI (µg/day)	20–30 [(a)]	25	35	25	–	–
Age (years)	13–<19		13–15			
AI (µg/day)	25–35 [(a)]	–	45	–	–	–
Age (years)			16–19			
AI (µg/day)	–	–	50	–	–	–
Adults						
Age (years)	≥ 19	≥ 19	> 19	≥ 19	≥ 19	≥ 19
AI (µg/day)	30–60 [(a)]	30	50	30	15–100 [(a)]	10–200 [(a)]
Age (years)			≥ 75			
AI (µg/day)	–	–	60	–	–	–
Pregnancy						
AI (µg/day)	30–60 [(a)]	30	50	30	15–100 [(a)]	–
Lactation						
AI (µg/day)	30–60 [(a)]	35	55	35	15–100 [(a)]	–

(a): Acceptable Range of Intakes.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of biotin requirement

The Panel considers that data are insufficient to derive the AR for biotin from the use of available biomarkers of intake or status for any population group.

5.2. Biotin intake and health consequences

Data examining the relationship between biotin intake and health outcomes are scarce.

A comprehensive search of the literature published between January 1990 and July 2012 was performed as preparatory work to this assessment, to identify relevant health outcomes upon which DRVs for biotin may potentially be based (Eeuwijk et al., 2012). Three cross-sectional studies were retrieved, which investigated associations between biotin intakes and genome damage (Fenech et al., 2005) or blood pressure (Schutte et al., 2003b, 2003a).

The Panel considers that the data available from these studies are very limited and cannot be used to derive DRVs for biotin.

5.3. Specific considerations for pregnancy and lactation

The Panel notes that urinary excretion of 3HIA has been observed to be higher in pregnant women than in non-pregnant women, while results on urinary biotin excretion were inconsistent (Mock and Stadler, 1997; Mock DM et al., 1997a; Shibata et al., 2013). However, biotin intake levels were not reported in these studies and the relevance of these findings with respect to biotin status in pregnancy is unclear. Another study (Mock et al., 2002b) was of insufficient duration (i.e. two weeks) to allow conclusions with respect to changes in biomarkers of biotin status (urinary 3HIA and biotin excretions) in unsupplemented pregnant women.

No data on an association between biotin insufficiency in pregnancy and increased incidence of fetal malformations in humans are available (see Section 2.2).

Assuming an average breast milk biotin concentration of 5 µg/L (see Section 2.2) and 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), the Panel notes that mean biotin secretion in milk is 4 µg/day in fully breastfeeding women.

6. Data on which to base Dietary Reference Values

The Panel considers that the available data are insufficient to derive ARs and PRIs for biotin, and therefore proposes to set an AI for all population groups. The setting of an AI for biotin 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. There is no indication that the AI should differ according to sex.

6.1. Adults

The Panel chooses the approximate midpoint of the observed mean/median intakes (Appendices C and D) to set an AI for biotin at 40 µg/day for adults of all ages.

6.2. Infants, children and adolescents

Assuming an average breast milk biotin concentration of 5 µg/L (see Section 2.2) and an average breast milk intake of infants aged 0–6 months of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009), the estimated biotin intake of infants between zero and six months is 4 µg/day. The AI for infants over six months of age can be derived by extrapolation from this figure, using allometric scaling (body weight to the power of 0.75) and reference body weight of each age group[9] (WHO Multicentre Growth Reference Study Group, 2006), in order to account for the role of biotin in energy metabolism, and rounding to the nearest unit. The AI for infants aged 7–11 months is set at 6 µg/day.

The Panel sets an AI for biotin of 20 µg/day for young children (1–3 years), based on observed median intakes in this age group. In consideration of the AI set for infants in the second half year of life, a value at the lower end of the range of observed intakes was chosen. The Panel sets an AI of 25 µg/day for children (4–10 years) and 35 µg/day for adolescents (11–17 years) (Table 2), based on the approximate midpoint of the observed mean/median intakes of each age group (Appendix B).

[9] Mean of body weight-for-age at 50th percentile of male and female infants aged three and nine months.

6.3. Pregnancy and lactation

The Panel considers that data are insufficient to derive a specific AI for biotin in pregnancy. The Panel considers that the AI for adults of 40 µg/day also applies to pregnant women.

Considering average biotin losses through breast milk of 4 µg/day during lactation (see Section 5.3) and rounding up, the Panel proposes to increase the AI for lactating women to 45 µg/day.

CONCLUSIONS

The Panel concludes that there is insufficient evidence to derive an Average Requirement (AR) and a Population Reference Intake (PRI) for biotin. Suitable data on biotin intake or status and health outcomes were not available for the setting of DRVs for biotin. Thus, the Panel proposes an Adequate Intake (AI) for adults based on observed intakes in the EU. It was considered unnecessary to give sex-specific values. The Panel proposes that the AI for adults also applies to pregnant women. For lactating women, an increment in the adult AI is proposed, in order to compensate for biotin losses through secretion of breast milk. An AI is also proposed for infants aged 7–11 months based on extrapolation from the estimated intake of infants aged zero to six months using allometric scaling, and for children and adolescents based on observed intakes in the EU.

Table 2: Summary of Adequate Intakes for biotin

Age	Adequate Intake (µg/day)
7–11 months	6
1–3 years	20
4–10 years	25
11–17 years	35
≥ 18 years [(a)]	40
Lactation	45

(a): Including pregnancy.

RECOMMENDATIONS FOR RESEARCH

The Panel recommends to review analytical data for biotin in food composition tables, to reflect the most reliable quantification methods. Dietary biotin intakes should be reassessed accordingly, in order to ascertain current AIs for biotin.

The Panel recommends further research on the dose-response relationships between biotin intake and functional biomarkers (e.g. markers of activities of biotin-dependent carboxylases) to characterise an adequate biotin status and to allow the derivation of the requirement for biotin.

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APPENDICES

Appendix A. Biotin concentration of human milk from healthy mothers of term infants

Reference	Number of women (number of samples)	Country	Total maternal intake (µg/day), mean (range)	Plasma concentration (ng/L)	Stage of lactation	Biotin concentration (µg/L)		Method of analysis
						Mean ± SD	Range	
Sakurai et al. (2005)	(6)	Japan	Not reported [a]	Not reported	6–10 days	2.8 ± 2.7	n.a.	Microbiological assay (*Lactobacillus arabinosus)*
	(6)				11–20 days	5.9 ± 4.0		
	(34)				21–89 days	5.8 ± 2.4		
	(34)				90–180 days	4.8 ± 1.7		
	(34)				181–365 days	4.2 ± 1.7		
	(60)				Summer	4.7 ± 2.2		
	(69)				Winter	5.2 ± 2.3		
	(129)				Overall	5.0 ± 2.3		
Mock DM et al. (1997b)	15	USA	Dietary supplement containing < 4 µg (~10 % of daily intake)	Not reported	8 days	~ 2.0	n.a.	HPLC/avidin-binding assay
					36 days	~ 6.8		
Hirano et al. (1992)	38 (102)	Japan	Not reported [b]	Not reported	0–5 days	0.8 ± 0.6	n.a.	Microbiological assay (*Lactobacillus plantarum*, ATCC 8014)
					6–14 days	1.8 ± 1.4		
					15–24 days	5.2 ± 2.1		
Salmenpera et al. (1985)	200	Finland	Not reported [c]	250 [d]	0 months	n.d.	n.a.	Microbiological assay (*Lactobacillus plantarum*, ATCC 8014)
	n.a.			330 [d]	2 months	4.5 [d]	n.d.–27	
	116			330 [d]	6 months	4.5 [d]	n.d.–18	
	36			330 [d]	9 months	4.5 [d]	1.8–10	
Ford et al. (1983)	35	UK	Not reported [b]	Not reported	1–5 days	0.21	0.02–0.83	'Standard microbiological methods'
					6–15 days	2.21	0.05–8.0	
					16–244 days	5.30	0.60–12.0	
Goldsmith et al. (1982)	84	USA	Not reported [b]	Not reported	3–8 days	0.7 ± 0.9	0.0–7.2	Microbiological assay (*Lactobacillus plantarum* ATCC 8014*)*
	67				10–14 days	3.0 ± 2.2	0.1–11.8	
	64				30–47 days	4.7 ± 2.2	0.5–14.1	

(a): Not taking supplements.
(b): No indication of supplementation.
(c): No supplementation during lactation or pregnancy.
(d): Geometric mean.
n.a., not available; n.d., non-detectable.

Appendix B. Biotin intake among children and adolescents in European countries

Country	Reference	Dietary assessment method (year of survey) [(a)]	Age (years)	n	Mean (µg/day)	SD	Median (µg/day)	P5–P95
Boys								
Austria	Elmadfa et al. (2009)	Seven-day record (2003)	7–9	n.a.	34	n.a.	n.a.	n.a.
			10–12	n.a.	35	n.a.	n.a.	n.a.
			13–15	n.a.	34	n.a.	n.a.	n.a.
		Three-day record (2007–2008)	7–9	148	33	n.a.	n.a.	n.a.
			10–12	155	32	n.a.	n.a.	n.a.
			13–15	86	33	n.a.	n.a.	n.a.
		24-hour recall (2004) (Berufsschüler/ allgemeinbildende höhere Schulen-Schüler) (from Vienna)	14–19	35/47	35/47	n.a.	n.a.	n.a.
Germany	DGE (2008)	Three-day record (2001–2002)	1–< 4	242	n.a.	n.a.	30.9	n.a.
	DGE (2008)	Three-day record (2001–2002)	4–< 5	242	n.a.	n.a.	28.2	n.a.
	Mensink et al. (2007)	Three-day record (2006)	6–11	626	48.2	n.a.	38.2	22.2–119.4
	Mensink et al. (2007)	Dietary history (over the last four weeks) (2006)	12–17	622	96.7	n.a.	63.5	27.9–265.6
	DGE (2012)	Two non-consecutive 24-hour recalls (2005–2006)	15–19	506	n.a.	n.a.	45.0	n.a.
Ireland	IUNA (online-b)	Seven-day record (2003–2004)	5–8	145	26.0	23.8	19.7	9.2–65.4
	IUNA (online-b)	Seven-day record (2003–2004)	9–12	148	27.8	22.0	24.2	9.9–57.7
	IUNA (online-c)	Seven-day record (2005–2006)	13–14	95	34.4	34.1	25.0	11.0–119.5
	IUNA (online-c)	Seven-day record (2005–2006)	15–17	129	40.5	41.5	27.0	14.0–131.5
Girls								
Austria	Elmadfa et al. (2009)	Seven-day record (2003)	7–9	n.a.	30	n.a.	n.a.	n.a.
			10–12	n.a.	33	n.a.	n.a.	n.a.
			13–15	n.a.	31	n.a.	n.a.	n.a.
		Three-day record (2007–2008)	7–9	175	31	n.a.	n.a.	n.a.
			10–12	152	29	n.a.	n.a.	n.a.
			13–15	64	27	n.a.	n.a.	n.a.
		24-hour recall (2004) (Berufsschüler/ allgemeinbildende höhere Schulen-Schüler) (from Vienna)	14–19	28/39	28/39	n.a.	n.a.	n.a.

Country	Reference	Dietary assessment method (year of survey) [(a)]	Age (years)	n	Mean (µg/day)	SD	Median (µg/day)	P5–P95
Germany	DGE (2008)	Three-day record (2001–2002)	1–< 4	246	n.a.	n.a.	25.4	n.a.
	DGE (2008)	Three-day record (2001–2002)	4–< 5	246	n.a.	n.a.	29.3	n.a.
	Mensink et al. (2007)	Three-day record (2006)	6–11	608	44.7	n.a.	35.7	17.9–106.5
	Mensink et al. (2007)	Dietary history (over the last four weeks) (2006)	12–17	650	84.4	n.a.	49.5	22.8–266.8
	DGE (2012)	Two non-consecutive 24-hour recalls (2005–2006)	15–19	536	n.a.	n.a.	36.0	n.a.
Ireland	IUNA (online-b)	Seven-day record (2003–2004)	5–8	151	23.3	26.5	19.1	9.9–52.2
	IUNA (online-b)	Seven-day record (2003–2004)	9–12	150	24.4	24.3	19.6	9.1–61.5
	IUNA (online-c)	Seven-day record (2005–2006)	13–14	93	27.5	50.1	16.5	7.7–79.1
	IUNA (online-c)	Seven-day record (2005–2006)	15–17	124	24.6	20.4	20.5	6.9–66.0
Both sexes								
Ireland	IUNA (online-a)	Four-day weighed dietary record (2010–2011)	1–4	500	22.9	16.3	19.3	9.9–53.4

(a): Supplements excluded.
n.a., not available; P5, 5th percentile; P95, 95th percentile.

Appendix C. Biotin intake among adults aged ~ 19–65 years in European countries

Country	Reference	Dietary assessment method (year of survey) [(a)]	Age (years)	n	Mean (µg/day)	SD	Median	P5–P95
Men								
Austria	Elmadfa et al. (2009)	24-hour recall	18–25	93	50	n.a.	n.a.	n.a.
			25–51	541	44	n.a.	n.a.	n.a.
			51–64	144	42	n.a.	n.a.	n.a.
Germany	DGE (2012)	Two non-consecutive 24-hour recalls (2005–2006)	19–24	469	n.a.	n.a.	46	n.a.
			25–34	614	n.a.	n.a.	48	n.a.
			35–50	1 946	n.a.	n.a.	48	n.a.
			51–64	1 460	n.a.	n.a.	47	n.a.
Hungary	Zajkas et al. (2007)	Three-day record (2003–2004)	18–34	136	32.5	8.4	n.a.	n.a.
			35–59	199	32.6	9.3	n.a.	n.a.
Ireland	IUNA (2011)	Four-day record (2008–2010)	18–64	634	42	18	40	19-72
Women								
Austria	Elmadfa et al. (2009)	24-hour recall	18–25	187	41			
			25–51	959	42			
			51–64	199	43			
Germany	DGE (2012)	Two non-consecutive 24-hour recalls (2005–2006)	19–24	486	n.a.	n.a.	39	n.a.
			25–34	852	n.a.	n.a.	42	n.a.
			35–50	2 648	n.a.	n.a.	41	n.a.
			51–64	1 740	n.a.	n.a.	41	n.a.
Hungary	Zajkas et al. (2007)	Three-day record (2003–2004)	18–34	176	26.9	8.6	n.a.	n.a.
			35–59	295	26.2	8.0	n.a.	n.a.
Ireland	IUNA (2011)	Four-day record (2008–2010)	18–64	640	32	17	29	13-58
Both sexes								
Latvia	Joffe et al. (2009)	Two non-consecutive 24-hour dietary recalls + food frequency questionnaire (2008)	17–26	378	34.3	n.a.	n.a.	n.a.
			27–36	206	36.3	n.a.	n.a.	n.a.
			37–46	272	35.0	n.a.	n.a.	n.a.
			47–56	304	34.8	n.a.	n.a.	n.a.
			57–64	217	45.4	n.a.	n.a.	n.a.

(a): Supplements excluded.
n.a., not available; P5, 5th percentile; P95, 95th percentile.

Appendix D. Biotin intake among adults aged ~ 65 years and over in European countries

Country	Reference	Dietary assessment method (year of survey) [(a)]	Age (years)	n	Mean (µg/day)	SD	Median (µg/day)	P5–P95
Men								
Austria	Elmadfa et al. (2009)	Three-day record (2007–2008)	≥ 55	121	34	n.a.	n.a.	n.a.
Germany	DGE (2012)	Two non-consecutive 24-hour recalls (2005-2006)	65–80	1 165	n.a.	n.a.	43	n.a.
Hungary	Zajkas et al. (2007)	Three-day record (2003–2004)	≥ 60	138	29.1	8.5	n.a.	n.a.
Ireland	IUNA (2011)	Four-day record (2008–2010)	≥ 65	106	40	19	36	17–68
Women								
Austria	Elmadfa et al. (2009)	Three-day record (2007–2008)	≥ 55	302	34	n.a.	n.a.	n.a.
Germany	DGE (2012)	Two non-consecutive 24-hour recalls (2005–2006)	65–80	1 331	n.a.	n.a.	39	n.a.
Hungary	Zajkas et al. (2007)	Three-day record (2003–2004)	≥ 60	235	24.5	7.8	n.a.	n.a.
Ireland	IUNA (2011)	Four-day record (2008–2010)	≥ 65	120	37	28	32	18–61

(a): Supplements excluded.
n.a., not available; P5, 5th percentile; P95, 95th percentile.

ABBREVIATIONS

ACC	acetyl-CoA carboxylase
Afssa	Agence française de sécurité sanitaire des aliments
AI	Adequate Intake
AR	Average Requirement
CoA	coenzyme A
COMA	Committee on Medical Aspects of Food Policy
D-A-CH	Deutschland- Austria- Confoederatio Helvetica
DGE	Deutsche Gesellschaft für Ernährung
DRV	Dietary Reference Value
DH	Department of Health
DRV	Dietary Reference Value
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
3HIA	3-hydroxyisovaleric acid
HPLC	high-performance liquid chromatography
IOM	US Institute of Medicine of the National Academy of Sciences
IUNA	Irish Universities Nutrition Alliance
IUPAC	International Union of Pure and Applied Chemistry
MCC	β-methylcrotonyl-CoA carboxylase
mRNA	messenger ribonucleic acid
NNR	Nordic Nutrition Recommendations
PC	pyruvate carboxylase
PCC	propionyl-CoA carboxylase
SCF	Scientific Committee for Food

SD	standard deviation
UL	Tolerable Upper Intake Level
UNU	United Nations University
WHO	World Health Organization