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Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Biotin

(expressed on 26 September 2001)

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FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Biotin is a heterocyclic compound, an imidazolidone ring joined to a tetrahydrothiophene ring. The latter possesses a valeric acid side chain. Of eight theoretically possible stereoisomers only D(+)-biotin occurs in nature and binds to and activates four carboxylases found in humans. Its molecular weight is 244.3.

Biotin is soluble in water, insoluble in organic solvents, stable at pH 5 to 8 against air, light and heat. Oxidation of the sulphur atom and shortening of the valeric acid side chain result in loss of vitamin activity.

Biotin cannot be synthesised by mammals, therefore, in humans it must be acquired from exogenous sources. It is still controversial if and how much biotin synthesised by intestinal bacteria can contribute to the body's requirement for biotin.

Quantification of biotin in foods and body fluids for nutritional studies has been done by three basic methods: bioassays, avidin-binding assays or fluorescent derivative assays. As these assays do not have the same specificity the results show major discrepancies and it is important to establish how biotin concentrations were determined when comparing different studies. The avidin-binding assay of biotin and its metabolites after separation by high-performance liquid chromatography is considered as one of the best currently available methods (Mock, 1996; Mock *et al.*, 1993; 1995; 1997).

2. NUTRITIONAL BACKGROUND

2.1 Biotin in food

Biotin requirements must be met by dietary uptake. The contribution of bacterial biotin synthesis in the gut has never been quantified.

Biotin occurs in many foods in very variable amounts. The variability of contents reported is due both to natural variation and methodological problems. Rich sources are liver, kidney, egg yolk, some vegetables like soybeans, nuts, spinach, mushrooms and lentils (20 to 100

µg/100 g edible portion). Lean meat, fruit, cereals and bread contain 1 to 20 µg/100 g. Biotin in vegetables, green plants and fruits occurs in water-extractable forms, whereas it occurs in firmly bound complexes in yeast and animal products. There are no reliable data on the bioavailability of biotin from different foods. Biotinidase, present in the intestinal mucosa and in pancreatic juice of mammals can cleave biotin linked to lysine (biocytin) and to oligopeptide. It may play an important role in the uptake of dietary biotin in humans after proteolysis (Wolf, 1995).

Biotin concentrations in human milk vary significantly during any 24-hour period and increase 5- to 30-fold during the progression from colostrum to transitional milk and eventually mature milk (Mock *et al.*, 1992a; Salmenperä *et al.*, 1985) and are then 20- to 50-fold higher than the plasma concentrations of normal women (Mock *et al.*, 1992b). More than 95% of total biotin was found in the skim fraction of milk, of which more than 95% was free as opposed to reversibly or covalently bound biotin. At day 8 postpartum bisnorbiotin and biotinsulfoxide accounted for more than 50% of total biotin (3.9 ng/ml) in the skim fraction. Thereafter only true biotin rises significantly (mean value at day 30 to 40 is 7 ng/ml) (Mock *et al.*, 1997a).

2.2 Biotin intake estimates

Dietary biotin intake of children and adults in different populations ranges between 17 and 60 µg per day (Heseker *et al.*, 1994; Helbich, 1997; Food and Nutrition Board, 1998; Mensink and Ströbel, 1999; Expert Group on Vitamins and Minerals, UK Food Standards Agency, 2001; IUNA 2001). Data are scarce because in most dietary intake surveys biotin has not been assessed.

The estimated mean dietary biotin intakes in Germany, UK and Ireland between 1986 and 2000 are given in Table 1.

Table 1. Mean and 97.5 percentile biotin intake (µg/day) from food and supplements in three EU countries (adults >16 y)

Country	Type of survey	n	Method	Supplements*	Mean	97.5%
Germany[a]	individual (M/F)	1988	7-day record	-	36.8	86.2
Germany[b]	individual (M)	1268	record+interview	-	52.9	53.7
	individual (F)	1540	record+interview	-	42.5	43.1
UK[c]	individual (M)	1087	7-day record	-	38.9	69.7
(16-64 yr)	individual (M)	1087	7-day record	+	39.1	71.4
	individual (F)	1110	7-day record	-	28.3	56.3
	individual (F)	1110	7-day record	+	28.7	58.1
Ireland[d]	individual (M)	662	7-day record	-	40.4	74.9
(18-64 yr)	individual (M)	662	7-day record	+	42.8	91.8
	individual (F)	717	7-day record	-	29.8	53.9
	individual (F)	717	7-day record	+	34.1	103.3

* + data included supplements; - data excluded supplements.
[a] Heseker *et al.* (1994) [b] Mensink and Ströbel (1999)
[c] EVM (2001) [d] IUNA (2001)

Dietary supplements containing biotin were taken on a regular basis by 4 to 5% of the German subgroups in the MONICA study 1994/1995. Whereas the median content of supplements was 10 µg biotin, maximal doses up to 5000 µg were reported (Schellhorn *et al.*,

1998). Biotin containing supplements were consumed by 1 to 2% of the adult UK population. Biotin intakes from supplements ranged from 0.1 to 130 µg/day (EVM 2001).

In 145 elderly persons (aged 68 to 90 years) mean biotin intakes per day, assessed by seven-days weighed or estimated records were 19.5 µg (range 7.6 to 37) in women and 23.5 µg (range 9.9 to 59) in men (Bailey *et al.*, 1997).

2.3 Biotin absorption and metabolism

Biotin is actively transported by different carrier systems.

Biotin absorption in the intestinal brush-border membrane proceeds via a structurally specific, temperature dependent carrier against a concentration gradient. Transport is electroneutral and coupled to sodium. This transport is saturable. At high intakes of biotin in pharmacologic doses simple diffusion predominates. Exit of biotin from the enterocyte across the basolateral membrane is also carrier-mediated, but independent of sodium and electrogenic, and does not accumulate biotin against a concentration gradient.

An electroneutral sodium-dependent transport system for biotin has also been reported for human renal brush border membrane vesicles, resulting in transfer from the lumen of the renal tubule into the blood.

A specific transporter for biotin together with sodium was demonstrated in human lymphocytes. This transport can be stimulated by mitogens apparently as a consequence of an increased number of biotin transporters in proliferating lymphocytes (Zempleni and Mock, 2000a).

Specific transport systems for biotin from the mother to the foetus have been reported, with little evidence of accumulation on the foetal side (Mock, 1996). A sodium dependent multivitamin transporter, which transports pantothenate, lipoate and biotin in an electrogenic process has been identified in human placenta and is also but to a lesser extent expressed in human kidney, liver, pancreas, heart, brain, lung and skeletal muscle (Wang *et al.*, 1999).

Renal clearance of biotin in normal children and adults is 0.4 times the creatinine clearance.

In humans the ratio of free biotin between cerebrospinal fluid and ultrafiltrates of plasma was found to be 0.85 ± 0.50 (Mock, 1989).

From studies with 6 healthy adults given biotin in pharmacologic doses, either orally (512, 2000 or 20,000 µg) or intravenously (4500 µg) it was concluded that oral biotin was completely absorbed. 50% of an oral dose was recovered in urine within 24 hours as biotin plus biotin metabolites. Intravenous administration resulted in a larger percentage excreted as intact biotin. The higher biotin concentration in plasma after intravenous dosage may have exceeded the capacity for renal tubular reabsorption (Zempleni and Mock, 1999).

The elimination half life time from plasma of a single oral biotin dose of 600 µg was calculated to be 110 minutes in 15 healthy volunteers (Bitsch *et al.*, 1989).

Acute and short-term (14 days) ingestion of 1200 µg biotin daily by 15 healthy adults increased serum biotin from 60 ng/L (range 34 to 89 ng/L) to a mean of 3,738 ng/L after one day and to 5,521 ng/L after 14 days. Bisnorbiotin (basal level 46 ng/L) and biotin sulfoxide

(basal level mean 3.7 ng/L, not detectable in 9 of 15 subjects) increased substantially both after one day (24 fold and 46 fold, respectively compared with pretreatment) and after 14 days (2.5 fold and 2.3 fold compared with day 1). The ratio of bisnorbiotin plus biotin sulfoxide to the total of avidin-binding substances did not change after 14 days. The excretion of biotin, bisnorbiotin and biotin sulfoxide in the urine likewise increased, 324 fold, 85 fold and 114 fold, respectively, providing indirect proof of biotin catabolism in human tissues (Mock and Heird, 1997; Mock and Mock, 1997).

Biotin absorption is reduced by ingestion of raw eggs which contain avidin, a protein resistant to proteases which binds 4 moles biotin per mole of avidin thereby making it unavailable. Prolonged heating at 100°C denatures avidin and sets biotin free.

Biotin is degraded in the human body by β-oxidation of the valeric acid side chain to bisnorbiotin and bisnorbiotinmethylketone and by oxidation of the sulphur in the thiophene ring to biotin-d,l-sulfoxide and biotin sulfone, which are excreted into the urine, and also found in plasma (Mock *et al.*, 1993; Zempleni and Mock, 1999). These metabolites are inactive as vitamin. Biotin accounts for only approximately 50% of avidin binding substances in plasma and urine.

Biotin catabolism is increased by anticonvulsant drug treatment, by alcohol consumption and during pregnancy (Mock and Dyken, 1997; Zempleni and Mock, 2000b).

2.4 Functions of biotin

In man biotin is an essential co-factor for four carboxylases which catalyse the incorporation of bicarbonate into a substrate and are involved in gluconeogenesis and provision of intermediates into the citric acid cycle (pyruvate carboxylase, PC, EC 6.4.1.1), fatty acid synthesis (acetyl-CoA carboxylase, ACC, EC 6.4.1.2), leucine catabolism (3-methylcrotonyl-CoA carboxylase, MCC, EC 6.4.1.4.), and propionate catabolism (propionyl-CoA carboxylase, PCC, EC 6.4.1.3). The propionate to be carboxylated has various sources: catabolism of valine, isoleucine, threonine, methionine, the side chain of cholesterol, odd-numbered saturated fatty acids, and metabolism of intestinal bacteria.

Biotin is attached to the carboxylases via an amide bond formed between the carboxyl group of the valeric acid side chain and the epsilon-amino group of a specific lysine in the apocarboxylases by holocarboxylase synthetase (EC 6.3.1.10), driven by hydrolysis of ATP.

In the turnover of cellular protein catalysed by lysosomal proteases the holocarboxylases release biocytin, which is biotin linked to lysine, or biotin bound to oligopeptides. For the cleavage of the amide bond between biotin and lysine a specific hydrolase, biotinidase (EC 3.5.1.12) is needed, present in many tissues. The highest activity is found in serum, liver, kidney and adrenal gland. Serum biotinidase is produced in the liver. Impaired hepatic function is accompanied by decreased biotinidase activity in serum (Grier *et al.*, 1989). Biotinidase activity is very low in human brain and cerebrospinal fluid.

Biotinidase is able both to recycle biotin bound to carboxylases and to cleave biotin bound to dietary proteins. Apart from this important function of biotinidase in providing biotin for intermediary metabolism, a function of this enzyme, is the transfer of biotin to nucleophilic acceptor proteins such as histones, thereby affecting gene expression (Hymes and Wolf, 1996) and e.g. embryological development (Bender, 1999; Zempleni and Mock, 2000b). Biotin is

essential for cell proliferation. Its proliferative effect in immune cells can become of clinical relevance in biotin deficiency (Zempleni and Mock, 2001).

2.5 Biotin requirement

Biotin requirement cannot be accurately estimated. For infants the amount provided by breastmilk is considered to be adequate. For children and adults either the usual dietary intake or an extrapolation from the intake of exclusively breastfed infants is the basis for setting estimated adequate intakes (FNB, 1998).

The Scientific Committee on Food of the European Commission has defined a biotin reference value for adults of 15 to 100 µg per day (31st Report of the SCF, 1993).

Age-related adequate intakes of biotin have been estimated (Food and Nutrition Board,1998; D-A-CH Referenzwerte, 2000) and are summarised in Table 2.

Table 2. Adequate intakes of biotin (µg/day)

Age	**0-4 m**	**4-12 m**	**1-4 yr**	**4-7 yr**	**7-10 yr**	**10-13 yr**	**13-15 yr**	**>15 yr**
D-A-CH (2000)	5	5-10	10-15	10-15	15-20	20-30	25-35	30-60
Age	**0-6 m**	**6-12 m**	**1-3 yr**	**4-8 yr**	**9-13 yr**		**14-18 yr**	**>18 yr**
FNB (1998)	5	6	8	12	20		25	30

2.6 Biotin nutritional status

Mock *et al.* (1997) determined serum true biotin concentrations in normal adults to be 60 ± 14.9 ng/L (range 34 to 89 ng/L), bisnorbiotin 46 ± 33 ng/L (range 5 to 145), biotin sulfoxide 3.7 ± 8 ng/L (range 0 to 31). Most (81%) of these avidin-binding substances are free, approximately 12% are covalently bound to plasma proteins and 7% are reversibly bound (Mock and Malik, 1992). The urinary excretion of biotin in normal adults ranges between 18 and 79 nmol/24 h (4.4 µg to 19.3 µg/24 h) plus approximately the same amount of biotin analogues.

The biotin status of an individual cannot be well assessed by determination of the biotin plasma concentrations. This was shown in 10 healthy subjects made biotin deficient by 20 days consumption of egg white containing enough avidin to bind more than 7 times the (normal) dietary biotin intake. The mean serum biotin level did not decrease significantly, and only five subjects had serum biotin concentrations below the lower limit of normal on day 20. In contrast, biotin and bisnorbiotin excretion into the urine decreased significantly from day 3 onwards and was less than the lower limit of normal in 8 of 10 subjects by day 14. The most sensitive indicator was an increase of the amount of 3-hydroxyisovaleric acid excreted per 24 h, which was significant at day 3, and greater than the upper limit of normal (112 ± 38 µmol/24 h) by day 10 in all subjects. Increased 3-hydroxyisovaleric acid excretion is the consequence of decreased activity of 3-methylcrotonyl-CoA carboxylase because of insufficient availability of its prosthetic group biotin (Mock *et al.*, 1997). In these experimental subjects no clinical signs of overt biotin deficiency evolved.

There are no systematic studies to show if a deficient biotin status can be recognised by increased excretion of other organic acids as a consequence of impaired activity of propionyl-CoA carboxylase (3-hydroxypropionic acid, methylcitrate), which might also lead to an

increase in the proportion of odd-numbered fatty acids in plasma lipids. However, as intestinal bacteria produce an unpredictable amount of propionic acid and as odd-numbered fatty acids are contained in variable amounts in dietary fat it can be expected that these measurements would be unreliable in assessing biotin status (Bender, 1999). Measurement of the activities of carboxylases in blood leukocytes might constitute another potential indicator of biotin status. In children with severe protein-energy malnutrition propionyl-CoA carboxylase activity in blood lymphocytes was significantly reduced in all and pyruvate carboxylase activity in some compared to normal controls and both increased in response to biotin administration. The plasma biotin concentration in these patients did not correlate with carboxylase activity (Velázquez *et al.*, 1995).

2.7 Biotin deficiency

2.7.1 Dietary biotin deficiency

Overt dietary biotin deficiency is rare. It does not occur in breastfed infants. It has been observed in association with total parenteral nutrition without biotin and in chronic feeding of raw egg white and it has been seen in an infant fed an amino acid formula and hypoallergenic rice presumably containing no biotin (Higuchi *et al.*, 1996). This latter case especially argues that biotin is not provided by intestinal bacteria in sufficient amounts.

Clinical symptoms of biotin deficiency are alopecia and cutaneous abnormalities such as seborrhoeic dermatitis, periorificial erythema, and fungal infection. In adults effects on the central nervous system are expressed as depression, lethargy, muscular pain, hyperesthesia and paresthesia. Symptoms take months to years to become apparent. Biocytin excretion is normal during symptomatic biotin deficiency.

Biotin-deficient infants become symptomatic within a shorter time (3 to 6 months) and show - in addition to hair loss and skin rash- hypotonia, lethargy and developmental delay.

Subclinical biotin deficiency in patients on anticonvulsant therapy (Mock and Dyken, 1997), in patients undergoing chronic haemodialysis (Yatzidis *et al.*, 1984), in alcoholics, patients with gastric disease, or inflammatory bowel disease is a matter of some concern (Mock, 1996; Zempleni and Mock, 2000b). A considerable proportion of pregnant women show increased excretion of 3-hydroxyisovalerate in both early and late gestation with a decrease in urinary biotin excretion (Mock *et al.*, 1997b). 3-Hydroxyisovalerate excretion was reduced by 300 µg of biotin per day over two weeks (Zempleni and Mock, 2000b). Subclinical maternal biotin deficiency was shown to be teratogenic in several species (chicken, turkey, mouse, rat, hamster) (Mock, 1996; Zempleni and Mock, 2000b).

2.7.2 Biotinidase and holocarboxylase synthetase deficiencies

Both genetic defects in holocarboxylase synthetase (Burri *et al.*, 1981) and biotinidase (Wolf *et al.*, 1983) result in multiple carboxylase deficiency with a typical pattern of organic acids in urine (and serum) and a wide spectrum of clinical symptoms, ranging from asymptomatic to neonatal death.

Treatment of biotinidase deficiency requires at least physiologic doses of biotin, but treatment is empirical in most cases with 10 mg of biotin given per day.

Holocarboxylase synthetase deficiency requires higher therapeutic doses of biotin, up to 100 mg/day in individual cases, dependent on the severity of the enzyme defect (Baumgartner and Suormala, 1997).

3. HAZARD IDENTIFICATION

Single or repeated doses of biotin (total dose of 50 and 100 mg/Kg of body weight by subcutaneous injection) given to rats resulted in production of irregularities of the oestrus cycle (Paul *et al.*, 1973a and b) and resorption of foetuses and placentae in pregnant rats (Paul *et al.*, 1973b) accompanied by decreased uterine weight, reduced glycogen and protein in the uterus and reduced protein in the liver. Estrogen treatment prevented loss of pregnancy and normalised these organ parameters (Paul and Duttagupta, 1975). However, these studies cannot be regarded as conclusive for human dietary biotin uptake because of the route of administration and the extreme dosage selected (corresponding to 10^5 times the adequate human daily intake).

The administration of oral biotin in doses up to 100 mg per day to patients with holocarboxylase synthetase and with biotinidase deficiency did not result in adverse effects, although the metabolic defect may have prevented or masked toxicity. The prenatal administration of 10 mg biotin orally per day during the third trimester of pregnancy in pregnant women at risk of carrying a foetus with holocarboxylase synthetase deficiency have not resulted in apparent adverse effects (Baumgartner and Suormala, 1997; Packman *et al.*, 1982; Roth *et al.*, 1982). However, systematic studies on biotin effects in healthy humans have not been undertaken.

In a recent publication, however, biotin supplementation of 750 µg per day for 14 days to five healthy adults resulted in significant decreases of mitogen-stimulated proliferation of peripheral blood mononuclear cells in all subjects, and a significantly reduced release of interleukin-1β and interleukin-2 in four of five subjects. This was not due to relative changes in differentiation of individual subsets of peripheral blood mononuclear cells nor to inhibition of cellular pantothenic acid uptake via the multivitamin transporter by high biotin levels. The significance of these findings is not known (Zempleni *et al.*, 2001).

4. DOSE RESPONSE ASSESSMENT AND DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

Due to the lack of systematic oral intake dose-response studies of biotin a quantitative risk assessment can not be carried out and it is not possible to derive a numerical UL for biotin.

5. CHARACTERISATION OF RISK

The risk of human toxicity from the usual dietary intake of biotin and from biotin supplements, such as are described in Table 1, appears to be low according to available data. There are insufficient data to draw any conclusions concerning the safety of very high-level supplements.

Although no numerical UL can be established, existing evidence that is available from observational studies indicates that current levels of intake of biotin from all sources do not represent a health risk for the general population.

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