EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF BIOTIN

The attached review of biotin is a revised version of the paper presented to the Expert Group on Vitamins and Minerals at the meeting on 9 February 2001 and in October 2001.

The following annexes are also included:

Annex 1	Tables referred to in the review
Annex 2	Intakes of biotin from food and supplements in the UK
Annex 3	Summary table of selected nutrition related information and existing guidance on intakes

Expert Group on Vitamins and Minerals Secretariat
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GLOSSARY

ACC	Acetyl-CoA carboxylase
AI	Adequate Intake (US)
CoA	Co-enzyme A
COMA	The Committee on Medical Aspects of Food Policy (UK)
CNS	Central nervous system
DH	Department of Health (UK)
DRV	Dietary Reference Value
FNB	Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients Food and Nutrition board, Institute of Medicine (US)
HCS	holocarboxylase synthetase
3-HIA	3-hydroxyvaleric acid
HPLC	High Performance Liquid Chromatography
HSDB	Hazardous Substances Data Bank (US)
i.v.	intravenous
MCC	β-Methylcrotonyl-CoA
MCD	Multiple carboxylase deficiency
NTHANES	National Health and Nutrition Examination Survey (US)
OAA	oxaloacetic acid
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
PKC	Protein kinase C
s.c.	subcutaneous
SIDS	Sudden Infant Death Syndrome
TPN	Total parenteral nutrition
USPDI	United States Practitioners Dispensing Information
NRC	National Research Council (US)
RNI	Reference nutrient intake (UK)
UL	Tolerable Upper Intake (US)

BIOTIN

Figure 1. Structure of biotin

Chemistry and nomenclature

1. Name: biotin; coenzyme R, vitamin H; (3aS-(3aα,4b,6α))- hexahydro-2-oxo- 1H-thieno(3,4-d)imadaz-ole-4-pentanoic acid

CASNR:	58-85-5
Molecular formula:	$C_{10}H_{16}N_2O_3S$
Molecular weight:	244.31
Colour/Form:	colourless, crystalline
Melting point:	232 ^oC (decomposes)
Solubility:	slightly soluble in chloroform; slightly soluble in water (22 mg/100 ml at 25^oC); salts are quite soluble; sodium salt is highly soluble
Stability:	aqueous solutions are stable at 100 ^oC; dry substance is thermostable and photostable; unstable in strong acid and alkaline solutions

2. Biotin is a bicyclic compound (Fig 1). One ring contains a ureido (-N-CO-N-) group and the other, a tetrahydrothiophene ring, contains a sulphur atom and has a valeric acid side-chain. There are eight stereoisomers of biotin, but the *d*-(+)-form, generally referred to simply as biotin or D-biotin, is the only naturally occurring isomer that is enzymically active (Mock 1998).

Natural occurrence

3. Mammals do not synthesise biotin and consequently must derive it from other sources. The ultimate source of biotin appears to be from *de novo* synthesis by bacteria, primitive eukaryotic organisms including yeasts, moulds and algae, and some plant species (Mock 1998).

Occurrence in food, food fortifications and supplements and licensed products

Foods

4. Biotin is widely distributed in natural foodstuffs. However, the absolute content of even the richest sources is low compared to that of the other water-soluble vitamins. Foods relatively rich in biotin include egg yolk, liver, kidney, muscle and organ meats, and some vegetables (Mock, 1998 and references therein). Other sources include brewer's yeast, whole grains, breads, fish, nuts and dairy products. Liver contains ~ 1000 μg/kg

whereas fruits and most other meats contain ~ 10 μg/kg (FNB[1] 2000). Some food processing and preservation methods, such as milling or canning, can result in a reduced biotin content (Bonjour 1984).

Food fortifications and supplements

5. Biotin is commercially available as the crystalline D-isomer (as cited by HSDB 2000) and is also found in brewer's yeast. It is included in most nutritionally complete dietary supplements (Said and Mock 1999), infant milk formulas and other baby foods and various dietetic products (Roche 2000). Dietary supplements available in the UK contain up to 150 μg/tablet (OTC 2000).

6. Tolaymat and Mock (1989) reported that biotin may be present in some over-the -counter vitamin and nutritional supplements that are not labelled accordingly. Amounts of the vitamin, deemed by the authors to be nutritionally significant (total biotin >0.2 μg/tablet), were found in 3 of 18 products where the biotin content was either unspecified or labelled as present in only trace amounts. It was noted that these three products contained extracts of liver and/or yeast. The authors suggested that biotin intake may be under-estimated in subjects receiving nutritional supplements containing either or both of these extracts.

Licensed medicinal products for oral use

7. Three products containing biotin may be sold in supermarkets and other retail outlets, without the supervision of a pharmacist, for use in nutrient deficiency or where there are increased requirements for vitamins. All contain a range of other nutrients. The highest daily dose authorised is 150 micrograms. Nine products can only be sold in pharmacies. Their licensed uses include the prevention and treatment of nutrient deficiency, convalescence, supplementation of special diets and malabsorption. All contain a range of other nutrients. The highest daily dose authorised is 500 micrograms.

[1] Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients Food and Nutrition board, Institute of Medicine.

Intake and exposure

8. In the UK, the average intake of biotin from all sources (food and supplements) is ~39 μg (range 15-70 μg) and ~29 μg (range 10-58 μg) in adult males and adult females, respectively (Gregory *et al* 1990 – see annex 2). The average intake from food alone differs from the total intake by < 1.5 %. Gregory *et al* (1990) commented that most individuals derive very little biotin from supplements.

9. The US Department of Agriculture Continuing Survey of Food Intakes by Individuals, The Third National Health and Nutrition Examination Survey (NTHANES III) and the Boston Nutritional Status Survey do not report biotin intake. Using data from NTHANES II, estimated biotin intake in young women was 40 ± 27 μg/day (as cited by FNB 2000). The 1986 National Health Interview Survey indicated that ~ 17% of adults in the US took supplements containing biotin (as cited by FNB 2000).

10. The concentration of biotin in mature (21+ days postpartum) human milk varies considerably but has been estimated to be in the order of ~ 6 μg/l (FNB 2000 and references therein).

Recommended amounts

11. There have been no formal studies to determine biotin requirement in humans. Signs of biotin deficiency, observed in patients receiving total parenteral nutrition (TPN) for prolonged periods following major resection of the gut, are reported to resolve following provision of ~100 μg biotin per day (Mock *et al* 1985).

12. The average biotin intake in the UK does not result in deficiency. This indicates that the average requirement must lie below this value. Due to insufficient data, COMA (DH 1991) was unable to set Dietary Reference Values (DRVs) for biotin. However, it agreed that intakes between 10-200 μg/day were both safe and adequate.

13. Adequate intake (AI)[2] values for biotin for all age groups have been set by the US FNB (2000) (Table 1). The AI for adults (30 μg/day) was based upon limited assessments of intake and extrapolation of the available intake data from infants. The AI for biotin was estimated to be 5μg (0.7 μg/kg) per day in infants aged 0-6 months. This was derived from a mean milk consumption by this age group of 0.78 l/day and an estimated biotin concentration in mature (21+ days postpartum) milk of 6 μg/l.

14. The biotin nutritional status of vegans and lactovegetarians is not thought to be impaired (Lombard and Mock 1989).

Analysis of tissue levels and assessment of biotin status

[2] An Adequate Intake is set instead of a Recommended Dietary Allowance if sufficient scientific evidence is not available to calculate an Estimated Average Requirement (the amount estimated to meet the requirements of 50% of the population). An AI is derived from observed average intakes which are obviously more than adequate to prevent deficiency.

15. Biotin in body fluids and tissues have been measured most frequently by microbial bioassay or avidin-binding assay. Reported plasma values range from ~ 500 pmol/l to >10,000 pmol/l (~100-2500 ng/l). However, an unresolved problem in biotin analysis is the disagreement among the various microbial bioassays and the avidin-binding methods concerning the true concentration of biotin in human plasma (reviewed by Mock 1996 and 1998).

16. Microbial bioassays (and in particular, bacterial assays) can suffer from interference by unrelated substances and from a variable growth response to different biotin analogues. Furthermore, microbial bioassays can give differing results depending on whether biotin is present in the free or protein-bound form. Prior acid or enzymatic hydrolysis may be required before protein-bound biotin becomes bioavailable to the organism. There also may be the added complication that acid hydrolysis itself can lead to a degree of biotin destruction (reviewed by Mock 1996 and 1998).

17. There are several variations of the competitive avidin-binding assay that employ a variety of different reporter systems. Generally, the assay measures the ability of biotin to compete with radio-labelled biotin for binding to avidin (isotope dilution), or compete with biotin linked to a solid phase for binding to avidin coupled to a reporter molecule, or prevent inhibition by avidin of a biotinylated enzyme. It should be noted that avidin-binding assays detect avidin-binding substances other than biotin, including vitamin-active biotin analogues and vitamin-inactive biotin metabolites. These substances can be separated chromatographically prior to their determination. However, due to differences in their affinity for avidin, the "detectability" of the different avidin-binding substances may vary and can be underestimated if not accounted for by the use of authentic standards (Mock 1996 and Mock 1998 and references therein). In human serum, biotin accounts for ~50% of the total avidin-binding substances present (Mock *et al* 1993, Mock *et al* 1995).

18. Correct evaluation of the biotin status of an individual requires a specific and sensitive analytical method. Metabolites of biotin must be distinguished from biotin itself since only the latter is biologically active as a vitamin. Furthermore, methods used for the assessment of biotin status in individuals with biotinidase deficiency are required to distinguish between biotin and biocytin. For the purposes of assessing the absorption and bioavailability of biotin, it is necessary to employ methods that determine both biotin and its metabolites. To date, many of the studies reported in the literature have not employed such methods.

19. Plasma biotin is not necessarily a good indicator of biotin deficiency. A decreased plasma biotin level was not found in 50% of individuals maintained on a raw egg diet and in some overt cases of biotin deficiency. However, a decreased urinary excretion of biotin and its metabolite, bisnorbiotin, is thought to be an early and sensitive indicator of biotin deficiency (Mock 1999 and references therein, FNB 2000 and references therein).

20. Deficiency of biotin also causes a reduction in the activities of the biotin-dependent carboxylase enzymes (see paragraphs 50 & 64). Reduction in β-methylcrotonyl-CoA carboxylase activity results in an increased production and urinary excretion of 3-hydroxyisovaleric acid (3-HIA) and 3-methylcrotonylglycine via an alternative metabolic route. Consequently, measurement of 3-HIA has been considered as a possible

early and sensitive indicator of biotin deficiency (Mock, 1999). Normal values for urinary 3-hydroxyisovaleric in humans were reported as 112 ± 38 [standard deviation] μmol/day (range 77-195 μmol/day) but these levels increased to 272 ± 92 μmol/day following maintenance on a raw egg white diet for 10 days (FNB, 2000 and references therein).

21. Reduction in propionyl-CoA carboxylase leads to an increased excretion of 3-hydroxypropionic acid and 3-methylcitric acid in urine (Mock 1996 and references therein). However, the sensitivity and clinical usefulness of the measurement of accumulation of odd-chain fatty acids in plasma resulting from biotin-deficiency-related impairment of propionyl-CoA carboxylase activity have yet to be determined (FNB 2000).

Bioavailability

22. There is considerable uncertainty regarding the factors that affect the bioavailability of biotin. Dietary biotin exists in the free form and, more often, in a form that is covalently bound to protein. Protein-bound biotin undergoes digestion by gastrointestinal proteases and peptidases to form biocytin (ε-N-biotinyl-L-lysine) and biotin-containing short peptides.

23. Mammalian growth studies have shown that biocytin, on a molar basis, is as bioactive as biotin. However, studies in rats have shown that conversion to the free biotin form is necessary for efficient biotin absorption and optimum bioavailability (Said *et al* 1993). It has been suggested the enzyme biotinidase, present in pancreatic juice and the intestinal mucosa, may be responsible for the biotin release from biotinyl oligopeptides (Mock 1998 and references therein). The bioavailability of protein-bound biotin is reduced in individuals exhibiting biotinidase deficiency, possibly due to an impaired digestion of protein-bound biotin, inadequate renal reabsorption or both. Doses of free biotin, in the range estimated to represent a typical dietary intake, prevent the symptoms associated with biotinidase deficiency (Theone and Wolf *et al* 1983).

24. Most biotin in meat and cereal is protein-bound but that present in cereal appears to be less bioavailable. Avidin, a protein found in raw egg white, binds biotin very avidly in the small intestine and prevents its absorption (Mock 1996 and references therein).

25. The USPDI (1994) states that ~50% biotin is absorbed (data source was not supplied). Zempleni and Mock (1999*b*) estimated the bioavailability of crystalline biotin in healthy individuals to be ~25-60%, based upon the data of Bitsch *et al* (1989) and Clevidence *et al* (1988) (studies described in paragraph 49) and the amounts of biotin recovered in urine following single oral doses of 75-900 μg. However, these estimates did not account for the formation of biotin metabolites and, therefore, were likely to be underestimates. Zempleni and Mock (1999*b*) went on to suggest that biotin is nearly completely absorbed, even at pharmacological doses. These workers specifically quantified biotin and its metabolites (using HPLC/avidin-binding assay) in urine collected over 24 hours following oral administration of 0.5, 2 or 22 mg of biotin to healthy individuals. The authors assumed that the biotin metabolites present in urine originated from metabolism in the tissues (see paragraph 46) and, not unreasonably, that biliary excretion of biotin in humans was as minor a component as it is in rats. Bioavailability was calculated relative

to the recovery of the sum of biotin and its metabolites excreted in urine following intravenous administration of 4.5 mg biotin. Absolute recovery of biotin and metabolites following intravenous administration was ~50%. On the basis that this recovery represented 100% bioavailability, the bioavailability of the 2 and 22 mg oral doses was also found to be ~100%. However, for unexplained reasons the recovery of the 0.5 mg dose yielded a bioavailability of 200%.

26. The bacteria present in the large intestine synthesise large amounts of biotin. However, whether substantial (nutritionally significant) amounts of biotin are derived from this source remains controversial (see paragraph 37).

Interactions

Alcohol

27. A substantial proportion of alcoholics are found to have reduced plasma biotin concentrations. Studies in rats suggest that this, at least in part, is due to an alcohol-related inhibition of the intestinal carrier-mediated transport of biotin (Said *et al* 1990).

Anticonvulsant therapy drugs

28. Patients receiving long-term anticonvulsant therapy (phenobarbitone, phenytoin, carbamazepine and primidone) are known to have reduced plasma biotin levels (Krause *et al* 1982 *a* & *b*, 1985). This may be attributed to drug-related inhibition of biotin transport in the intestine (Said *et al* 1989), acceleration of biotin catabolism in the tissues (Mock and Dyken 1997, Mock *et al*, 1998) and/or displacement of biotin from biotinidase (Chuahan and Dakshinamurti 1988).

Peroxisome proliferators, steroid hormones

29. Biotin transformation to bisnorbiotin is found to be accelerated in rats pretreated with peroxisome proliferators (clofibrate and di(2-ethylhexyl)phthalate) and steroid hormones (dexamethasone and dehydroepiandrosterone) (Wang *et al* 1997). The human relevance of the effect of peroxisome proliferators in rats is questionable. However, there is some evidence to suggest that pregnancy in humans may cause marginal biotin deficiency (see paragraphs 54 & 60) and this may be partially related to steroid hormone status.

Pantothenic acid

30. *In vitro* kinetic studies suggest that biotin and pantothenic acid share a common carrier-mediated uptake mechanism in both the small and the large intestine (Said 1999*a*, Said *et al* 1998). Prasad *et al* (1999) recently cloned a Na+-dependent multivitamin transporter from rabbit intestine that induced the uptake of biotin, pantothenate and lipoate, when expressed in mammalian cells. A similar transporter was cloned from human Caco-2 cells[3] and was identical to that expressed in a human choriocarcinoma cell line. Both transporters catalysed Na^+-dependent uptake of biotin, pantothenate and lipoate. A shared

[3] Caco-2 cells are a human-derived colonic carcinoma cell line. Under specific culture conditions, these cells demonstrate many of the structural and functional properties of mature small intestine absorptive cells and, as a consequence, they are used as an *in vitro* model of enterocytic function.

uptake mechanism for biotin and pantothenic acid may also exist in heart tissue (Beinlich *et al* 1990), placenta (Grassl 1992, Prasad *et al* 1997) and the blood-brain barrier (Spector and Mock 1987) but not in brain microvessel endothelial cells (Shi *et al* 1993). There are no known physiological or nutritional implications of the interaction between biotin and pantothenic acid.

Other antagonists

31. A number of other compounds antagonise the actions of biotin *in vitro*. Among them are biotin sulphone, desthiobiotin and certain imidazolidone carboxylic acids (Marcus and Coulston 1996).

Absorption, distribution, metabolism and excretion

Absorption from the small intestine

32. The uptake of biotin from the small intestine occurs by both a carrier-mediated process and by passive diffusion. The carrier-mediated process has a low K_m, is saturable and predominates at physiological biotin concentrations.

33. The biotin carrier is located in the brush-border membrane of the intestinal epithelial cells. The process is driven by an electron-neutral Na^+ gradient and is temperature-dependent and pH-dependent. The carrier has high structural specificity, requiring an intact ureido ring and possibly a free carboxyl group on the valeric acid side-chain (Reviewed by Mock 1996 and 1998).

34. Studies of the kinetics of biotin uptake, using membrane preparations from gut epithelial cells, have indicated both developmental and regio-differences in the ability of the small intestine to absorb biotin. In rats, absorptive ability increases with maturation. The major site of carrier-mediated biotin transport shifts from the ileum to the jejunum. Studies using preparations from adult human and rat have shown that there are differences in V_{max}, but not in K_m, along the small intestine (duodenum>jejunum>ileum) suggesting a higher carrier density proximally (Said and Redha 1987 & 1988, Said *et al* 1988*b*, reviewed by Said 1999*a*).

35. Studies in human-derived cultured intestinal Caco-2 cells and intact rats have suggested that biotin uptake may be regulated by the availability of the vitamin in the culture medium or diet and/or body stores (Said *et al* 1989, Ma *et al* 1994). Transport of biotin in brush-border membrane preparations from biotin-deficient rats was greater than in those prepared from control animals. It was suggested that the up-regulation was due to an increase in the number of transporters rather than a change in transporter affinity since there was an increase in V_{max} in the absence of change in K_m. In contrast, rats supplemented with pharmacological doses of biotin showed a reduced biotin uptake. Again, changes were mediated through alterations in V_{max} and not in K_m (Said *et al* 1989).

36. It has been shown that protein kinase C (PKC) and Ca^{2+}/calmodulin may be involved in the regulation of the biotin uptake process. Inhibitors of PKC (staurosporine and chelerythrin) have been shown to stimulate biotin uptake in Caco-2 cells whereas PKC

activators (phorbol 12-myristate 13-acetate [PMA], *sn*-1,2-dioctanoylglycerol) inhibited uptake (Said, 1999*a* and references cited therein). Inhibition of the Ca^{2+}/calmodulin pathway by calmidazolium and trifluoroperazin also inhibited biotin uptake (Said 1999*a* and references cited therein).

Absorption from the large intestine

37. The normal microflora of the large intestine synthesises substantial amounts of biotin. However, the extent to which this source of biotin may be absorbed is uncertain. *In vivo* studies in rats, minipigs and humans confirm that the colon is capable of absorbing considerable amounts of lumenal biotin (Barth *et al* 1986, Bowan and Rosenberg 1987, Innis and Allardyce 1983, Sorrell *et al* 1971, Oppel 1948). Studies in non-transformed colonic epithelial NCM460 cells (known to possess characteristics similar to normal colonic cells) suggest that the uptake process in the lower gut is similar to that present in the epithelium of the small intestine, and as such, is mediated by a Na^+-dependent carrier and inhibited by PKC activators (reviewed by Said 1999*a*). However, *in vivo* studies in pigs have indicated that the efficiency of biotin absorption in the colon is less than in the upper intestine and that biotin synthesised by the enteric bacteria is unlikely to be in the form or at a location that contributes significantly to total amount of biotin absorbed (Kopinski *et al* 1989 *a* & *b*).

Distribution

Transport in plasma

38. The mechanism by which biotin is transported from the site of absorption to the liver and peripheral tissues remains ill-defined. In contrast to the process of uptake, the exit of biotin from the absorptive epithelial cells occurs via a Na^+-independent carrier mechanism which is electrogenic and does not transport biotin against a concentration gradient (Said *et al* 1988*a*). Biotinidase has been reported to be the only protein present in plasma that specifically binds biotin. Consequently, it has been suggested that the enzyme may act as a specific biotin plasma-carrier protein or serve to transport biotin into cells (Chuahan and Dakshinamurti 1988). However, other workers do not concur. Although the concentration of biotinidase greatly exceeds that of biotin, less than 10% of the total biotin pool in humans is reversibly bound to plasma proteins and this may be accounted for as serum albumin-bound. Additional biotin is covalently bound to plasma protein. The percentages of free, reversibly bound and covalently bound biotin are ~ 81, 7 and 12%, respectively (reviewed by Mock 1998 and Mock 1996).

Hepatic uptake

39. Uptake of free biotin into the liver and peripheral tissues is mediated by both diffusion and a specific carrier-mediated process. As with the uptake in the gut, the active transport mechanism appears to be dependent upon a Na^+ gradient. The process is electron-neutral and specific for a free carboxyl group, although the structural specificity does not appear to be as rigid as that required by the small intestinal transporter. Once inside the cell, biotin is metabolically trapped, presumably through covalent binding to holocarboxylase

enzymes. Upon entering the hepatocyte, biotin then diffuses into the mitochondria via a pH-dependent process (reviewed by Mock 1998).

Transport into the CNS

40. Transport of biotin across the blood-brain barrier is via a saturable process that has a structural requirement for a terminal carboxylate group on the valerate side-chain (Spector and Mock 1987). Transport into the neurons is also thought to involve a specific transport mechanism followed by metabolic trapping through covalent binding to brain proteins likely to be carboxylases (Mock 1998).

Transplacental transport

41. *In vitro* studies have shown the presence of a specific transport system for the transfer of biotin from mother to fetus. Again, the process is Na^+-dependent and causes the active accumulation of biotin within the placenta, although biotin release into the fetal compartment is probably via a slow passive process (Grassl, 1992; Schenker *et al*, 1993; Prasad *et al*, 1997; Karl and Fisher, 1992). Limited studies in humans have shown biotin levels in the cord blood of human fetuses (at 18-24 weeks) and neonates to be 3-17-fold and ~2-fold higher than in maternal blood, respectively (Mantagos *et al* 1998, Baker *et al* 1975).

Biotin in breast milk

42. The concentration of biotin in human milk is quite variable but exceeds the concentration in the maternal plasma by one to two orders of magnitude. This suggests the involvement of an active transport process. (Mock 1998 and references cited therein). More than 95% of the total biotin in human milk is present in the skimmed fraction and most of this is found in the free form. The composition of human milk in terms of biotin and its metabolites changes with maturation. In early and transitional milk, metabolites bisnorbiotin and biotinsulphoxide account for ~50% and ~10%, respectively, of the total biotin plus metabolites pool. With postpartum maturation, the biotin concentration increases. At 5 weeks postpartum, the two metabolites account for only 25% and 8% of the biotin pool, respectively. There is currently no evidence to suggest the existence of a predominating trapping mechanism or a soluble biotin-binding protein in milk (reviewed by Mock 1998).

Re-adsorption in the kidney

43. There are specific mechanisms for the reabsorption of water-soluble vitamins from the glomerular filtrate which contribute to the recovery and conservation of these vitamins within the body. Biotin is reclaimed against a concentration gradient by a saturable Na^+-dependent, structurally specific system. Subsequent exit from the tubular cells occurs via a basolateral membrane transport system. Renal wasting of biotin and biocytin in biotinidase deficient individuals suggests that this enzyme may be important in the renal handling and salvage of biotin (Mock 1998 and references cited therein).

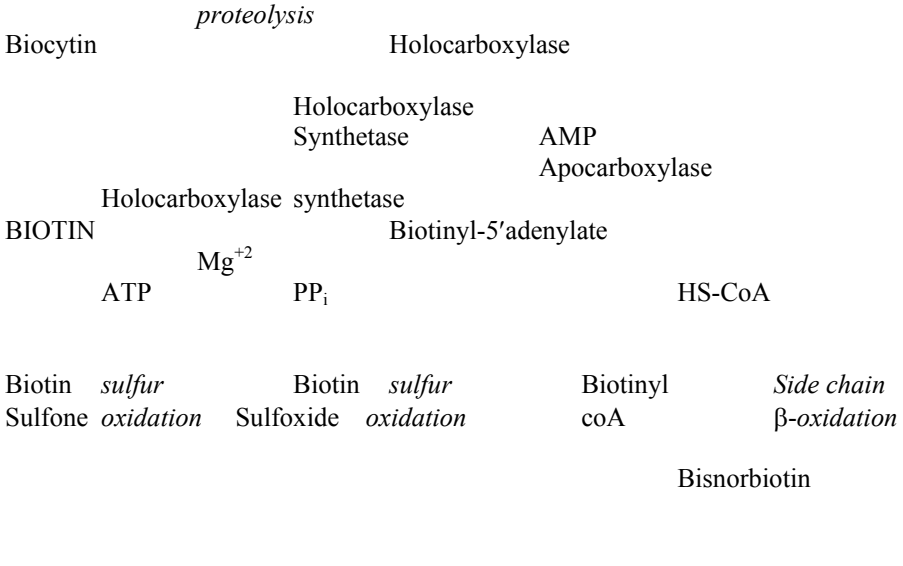
Metabolism and excretion

44. In the normal turnover of cellular proteins, biotin-containing holocarboxylases are degraded to biocytin or short oligopeptides containing biotin-linked lysyl residues. Biotinidase is required for the hydrolysis of biotin from lysyl amino acid residues and therefore may be important for the release and recycling of biotin (Mock 1998 and references therein).

45. Biotin that is not incorporated into carboxylase enzymes or their various intermediates is available for further metabolism. McCormick and co-workers elucidated two pathways of biotin catabolism in micro-organisms (Figure 2). In one pathway, biotin is catabolised by β-oxidation of its valeric acid side-chain. The repeated cleavage of two-carbon units leads to the formation of bisnorbiotin, tetranorbiotin and related intermediates such as α,β-dehydro, β-hydroxy and β-keto-intermediates. In a second pathway, biotin is catabolised by the oxidation of the sulphur present in its heterocyclic ring which leads to the formation of biotin L- and D -sulphoxides and biotin sulphone. Combinations of both pathways of catabolism can occur (reviewed by Wright and McCormick 1971 and by Mock 1998). Biotin metabolites originating from these two pathways of metabolism have been identified in the urine and plasma of mammals including rats, pigs and humans (Lee *et al* 1972, Wang *et al* 1996, Mock *et al* 1993, 1995 & 1997, Zempleni *et al* 1997).

46. Biotin metabolites in urine could theoretically result from catabolism of the vitamin within the tissues and/or from the absorption of biotin catabolites generated by micro-organisms present in the lower gut. Studies by Zempleni *et al* (1999 *a* & *b*) and Mock and Heird (1997) suggest that some if not all metabolites found in human urine are derived from the tissues. Biotin metabolites, such as bisnorbiotin and biotin sulphoxide, are inactive as vitamins.

Figure 2. Pathways of biotin metabolism (adapted from Mock, 1988)



HS-CoA = coenzyme A

47. At physiological doses, approximately 50% of biotin is converted to bisnorbiotin and biotin sulphoxide prior to excretion and the molar ratio of biotin, bisnorbiotin and biotin sulphoxide present in human urine and plasma is ~ 3:2:1. Other minor metabolites, bisnorbiotin methylketone and biotin sulphone, have also been identified in human urine (Zempleni *et al* 1997). Following administration of pharmacologic doses, the molar percentage excreted in human urine as unmetabolised biotin may be increased slightly (Mock and Heird 1997). It is speculated that this is unlikely due to saturation of the pathways of biotin metabolism but a reflection of a more rapid urinary excretion of biotin when serum biotin concentrations are high and the renal transporter for reabsorption of biotin is saturated (Zempleni and Mock 1999*b* and references therein).

48. Biliary excretion of absorbed biotin is thought to be quantitatively unimportant and, in the rat, accounts for ~2% of total excretion following intravenous administration (Zempleni and Mock 1999 *a & b*). However, faecal excretion of biotin is 3-6 times greater than normal intake, due to the substantial amount of biotin synthesised by the enteric bacteria.

Pharmacokinetics of biotin in humans

49. The pharmacokinetics of orally administered biotin have been described in two studies in humans. Clevidence *et al* (1988) measured plasma and urinary biotin in healthy men (n=12) and women (n=10), 0, 2, 4 and 24 hours after oral administration of 0, 75, 150 or 300 μg of biotin. For each dose, the highest plasma biotin concentrations were found in samples taken 2 hours after dosing. Levels fell between 2 and 4 hours after dosing and, after 24 hours, were not significantly different from pre-dose levels. Levels were increased ~ 4-fold in men and 6-fold in women taking the 300 μg dose. However, the mean percentage of the dose present in plasma was never > 2.4 %. Up to 33% of the dose was excreted in urine within 4 hours of dosing. A smaller percentage was recovered in urine following administration of the low (75 μg) dose. The authors suggested this reflected measurable amounts of biotin being retained by tissue stores. It should be noted, however, that this study did not take into account the presence of biotin metabolites. Bitsch *et al* (1989) demonstrated that single oral doses of biotin (600 μg [n=9; 4M, 5F] and 900 μg [n=7; 3M, 4F]) were rapidly eliminated from plasma in male and female volunteers and resulted in a marked increase in urinary excretion. Following a dose of 600 μg, T_{max} occurred between 1 and 2 hours after dosing and the plasma elimination half-life was found to be 1.8 hours. Pre-dose plasma levels were approached within 24 hours, whereas the rate of urinary excretion, which was increased 12-fold on the day of dosing, was still slightly (2-fold) but significantly elevated 2 days later. Prolonged enhancement of plasma levels occurred only after the administration of 300 μg/day for one week followed by 900 μg/day (n=28) for a further week. Again, this study did not take into account the presence of biotin metabolites.

Function

Carboxylase coenzyme

50. Biotin acts as an essential cofactor for the acetyl-CoA, propionyl-CoA, β-methylcrotonyl-CoA and pyruvate carboxylase (ACC, PCC, MCC and PC) enzymes. These four enzymes catalyse critical steps in pathways of intermediary metabolism; the

synthesis of fatty acids, the catabolism of branched-chain amino acids and the gluconeogenic pathway. The enzymes are mechanistically similar to each other and incorporate bicarbonate into a substrate in the form of a carboxyl group.

51. Biotin becomes covalently attached to the apocarboxylase enzymes via a condensation reaction catalysed by holocarboxylase synthetase (Figure 2). An amide bond forms between the carboxyl group of the valeric acid side-chain of biotin and the ε-amino group of a specific lysyl amino acid residue of the apoprotein. This particular region of the apocarboxylase protein is generally highly conserved among species and also within the individual carboxylase enzymes (Mock 1998 and references cited therein). Each carboxylation reaction involves the attachment of a carboxyl moiety to the biotin molecule at the ureido nitrogen opposite the valeric acid side-chain and its subsequent transfer to the substrate. The reaction is driven by the hydrolysis of ATP to ADP and inorganic phosphate. PCC, MCC and PC are all strictly mitochondrial enzymes whereas ACC is found in both the mitochondria and the cytosol. Inactive mitochondrial ACC may serve as a biotin storage pool (Mock 1998 and references cited therein).

52. ACC catalyses the incorporation of bicarbonate into acetyl-CoA to form malonyl-CoA. Malonyl-CoA, in turn, serves as the substrate for the fatty acid synthetase complex, donating two of its carbons to the fatty acid elongation process with the loss of the third as carbon dioxide. PC catalyses the incorporation of bicarbonate into pyruvate to form oxaloacetic acid (OAA), a Kreb's tricarboxylic acid cycle intermediate. In gluconeogenic tissues, such as liver and kidney, OAA can be converted into glucose. MCC catalyses a critical step in the degradation of the branch-chain amino acid, leucine. PCC catalyses the carboxylation of propionyl-CoA to form D-methylmalonyl-CoA. D-methylmalonyl-CoA is racemised to the L-isomer and subsequently undergoes isomerisation to form the tricarboxylic acid intermediate succinyl-CoA.

Interaction with histone proteins and the regulation of gene expression

53. In addition to its role in the hydrolysis of biotin (see paragraphs 23 & 44), biotinidase has been shown *in vitro* to catalyse the biotinylation of histone proteins. The hydrolase or transferase activity of biotinidase appears to be determined by pH and therefore may be organelle specific. The specific transfer of biotin to histones may explain the presence of the vitamin inside the nucleus and suggests a role in the regulation of protein transcription. Outside of the nucleus, it is interesting to note that biotin and histones perform similar functions. In cultured cells, both have insulin-like action, by increasing glucose uptake, and both stimulate cyclic GMP synthesis and nitric oxide formation. However, it remains to be seen whether biotinylated histones or biotinylated histone fragments are active components in these systems (Hymes and Wolf 1999 and references therein).

Deficiency

54. Dietary biotin deficiency is a rare occurrence within the developed world. However, biotin deficiency has been observed in patients receiving long-term total parenteral nutrition (TPN), or in people who have consumed large amounts of uncooked eggs. In the latter, the so-called "egg white injury", biotin deficiency is attributed to ingestion of

large amounts of avidin, a glycoprotein abundant in egg white. The avidin binds biotin with a very high affinity and prevents the absorption of both dietary biotin and that synthesised by the gut microflora. Signs of biotin deficiency have also been observed in people suffering from biotin malabsorption, including short-gut syndrome. Long-term anticonvulsant therapy in adults can also result in a depletion of biotin (see paragraph 28) that is severe enough to interfere with amino acid metabolism. Marginal states of deficiency may also develop during normal pregnancy, possibly due to an accelerated metabolism (Said and Mock 1998, see also paragraph 29). Benton *et al* (1996) reported that biotin status (assessed by plasma concentration) was marginal or deficient in a minority (~20-25%) of a sample of young British adults. However, all the subjects (113F, 130M) included in this study were students and it was noted that caution should be taken in generalising the data to other groups.

55. Biotin deficiency is characterised by development of a fine scaly dermatitis, hair loss, conjunctivitis, ataxia and delayed development. Histologically, the skin shows an absence of sebaceous glands and atrophy of hair follicles (Mock 1998 and references therein). Experimental studies of biotin depletion in humans have shown that diets providing up to 30% of energy intake from raw egg white can result in glossitis (inflammation of the toungue), anorexia, nausea, hallucinations, depression and somnolence, as well as fine scaly desquamating dermatitis and a characteristic skin rash frequently observed around the eyes, nose and mouth. In these studies, urinary excretion of biotin fell to about 10% of that of subjects maintained on a normal diet (DH, 1991 and references cited therein; FNB, 2000 and references therein).

56. Infants who receive biotin-free TPN develop signs attributed to biotin deficiency within 3-6 months of the commencement of TPN treatment. This is earlier than in adults, probably because of an increased requirement for biotin in infants related to growth. The characteristic rash, which first appears around the eyes, nose and mouth, along with an unusual distribution of facial fat commonly observed in these infants is known as *biotin deficiency facies.* The rash later extends to the ears and perineal orifices and is similar in appearance to that of cutaneous candidiasis. Hair loss can occur after 6-9 months of TPN. Biotin deficient infants show signs of hypotonia, lethargy, developmental delay and withdrawn behaviour, all of which are characteristic of biotin deficiency-related neurological disorder (FNB 2000 and references therein).

57. There are a number of inherited (autosomal recessive trait) disorders that result in functional biotin deficiency that are responsive to biotin supplementation. These include holocarboxylase synthetase (HCS) deficiency and biotinidase deficiency, both of which result in multiple carboxylase deficiency (MCD). MCD results in a block in carboxylase-related metabolic pathways and can lead to serious life-threatening illness. The MCD arising from HCS deficiency is seen in neonates whereas MCD resulting from biotinidase deficiency has a later on-set. The underlying mechanism for HCS deficiency is an increased K_m of HCS for biotin or a decreased V_{max} resulting in reduced holocarboxylase activity at physiological biotin concentrations. The MCD in biotinidase deficiency results from a progressive biotin deficiency due to the inability to liberate and recycle biotin from biocytin or to utilise protein-bound biotin from the diet (Baumgartner and Suormala 1999 and references therein; Hymes and Wolf 1999; Mock 1998 and references therein).

58. Biotinidase deficiency results in biochemical abnormalities and clinical findings that are similar, although not identical, to those of biotin deficiency. Seizures, irreversible neurosensory hearing loss and optic atrophy have been reported in cases of human biotinidase deficiency but not in biotin deficiency (Wolf *et al* 1985, Mock 1998 and references therein). It has been shown that doses of free biotin that are not in great excess of the estimated dietary intake (50-150 μg/day) are sufficient to prevent the symptoms of biotinidase deficiency (Diamantopoulos *et al* 1986).

59. The fatty liver and kidney syndrome, which can result in sudden death of chicken flocks, is associated with biotin deficiency (Bannister 1976). There are data to suggest that inadequate biotin nutrition may also be associated with sudden infant death syndrome (SIDS). A UK study (Johnson *et al*, 1980) reported that the livers of children dying from unknown causes (SIDS) contained 25% less biotin than infants dying of known causes (non-SIDS). Data from a study in Australia (Heard *et al* 1983) confirmed the UK findings in infants aged between 1-6 months. Whether this is anything other than a casual association remains to be ascertained. The Australian study found no significant differences in liver biotin in SIDS and non-SIDS infants aged < 1 month or 6-12 months.

60. Biotin deficiency in pregnancy has been shown to be teratogenic in several species including mice, hamsters, chickens and turkeys (Said 1999*b* and references therein). A high incidence of skeletal malformations (cleft palate, micrognathia, micromelia) was observed in mice born to dams maintained on a biotin deficient diet throughout pregnancy. However, there were no significant effects on reproductive performance, number of implantation sites, litter size or resorption frequency. Furthermore, the dams showed no physical evidence of biotin deficiency or significant difference in food intake or body weight gain (Watanabe and Endo 1990). Although reports are conflicting, some data indicate a marginal degree of biotin deficiency develops in a proportion of women during normal pregnancy (reviewed by Zempleni and Mock 2000). However, as yet, there is no direct evidence to associate marginal biotin deficiency in expectant mothers with an increased incidence in fetal malformations.

61. Studies in humans and rodents suggest that biotin is required for the normal functioning of the immune system; in the production of antibodies, immunological reactivity, protection against sepsis, macrophage function, T- and B- cell differentiation, afferent immune response and cytotoxic T-cell response (reviewed by Mock 1996).

62. Glucokinase is a high K_m isoform of the enzyme hexokinase. In the liver, the glucokinase form is responsible for increased glucose uptake and metabolism following a meal, when the glucose concentration in portal blood is high. It has been shown in rats that biotin acts, relatively specifically, to induce the synthesis of glucokinase in fasted rats implying that dietary biotin may be responsible for its regulation. It has been suggested, therefore, that biotin deficiency may be associated with decreased glucose tolerance, although impairment of gluconeogenesis, as a results of reduced pyruvate carboxylase activity, may lead to fasting hypoglycaemia (Bender *et al* 1999 and references therein). Koutsikos *et al* (1996) demonstrated an improved glucose tolerance following intravenous biotin therapy in haemodialysis patients and Maebashi *et al* (1993*a*) reported that long-term oral biotin therapy can improve glucose metabolism in non-insulin-dependent diabetes mellitus patients.

63. Biotin deficiency has also been reported or inferred in Leiner's disease, haemodialysis patients, gastrointestinal disease, alcoholism, inflammatory bowel disease and brittle nails (reviewed by Mock 1998).

64. Studies of the biochemical pathogenesis of biotin deficiency indicate that clinical manifestations are generally a direct or indirect result from deficient activities of the four biotin-dependent carboxylase enzymes. For example, CNS effects have been attributed to the effects of lactic acidosis (resulting from inhibition of pyruvate carboxylase activity). Skin rash and hair loss have been attributed to abnormal fatty acid metabolism and impaired synthesis or metabolism of long-chain polyunsaturated fatty acids (resulting from deficiency of acetyl-CoA carboxylase activity) (reviewed by Mock 1996).

65. In a study by Ho and Cordain, 2000, it was suggested that biotin insufficiency could occur, resulting in reduced fatty acid synthesis. This in turn may contribute to endothelial cell dysfunction and be a contributing factor in the development of cardiovascular disease.

Overview of reported non-nutritional beneficial effects

Treatment of biotin-responsive inborn errors and TNP

66. Large oral doses of biotin (1-10 mg/day) are administered to babies and older individuals with manifestations of biotin deficiency due to the presence of biotin-responsive inborn errors such as biotinidase deficiency, holocarboxylase synthetase and isolated deficiencies of PC, PCC and MCC. Individuals with holocarboxylase synthetase deficiency may require larger doses of biotin (40-100 mg/day), determined on a case by case basis. The dose level may be reduced following resolution of signs and symptoms of deficiency. In cases where biotin-responsive disorders are suspected within a fetus, biotin may be administered prenatally, via the mother. Patients receiving long-term TPN or undergoing renal dialysis should also receive biotin supplements (Marcus and Coulston, 1996; Baumgartner and Suormala 1999; Said and Mock (1999).

Treatment of brittle nails

67. Several studies have suggested that oral biotin supplements may be beneficial in the treatment of brittle fingernails. In a randomised, controlled study, Columbo *et al* (1990) reported a 25% increase in nail thickness and an improvement in nail morphology in women treated with 2.5 mg biotin per day. However, the number of individuals included in the study was small (22 tests; 10 controls). In an uncontrolled retrospective study, Hochman *et al* (1993) reported improvement in nail condition in 63% of 42 women given biotin (1-3 mg/day). Floersheim (1989) reported that biotin supplementation (2.5 mg/day) resulted in firmer and harder fingernails in 41 of 45 women who were available for evaluation. The original number of individuals taking part in the study was 75. Further details of the study were limited.

Treatment of sternocostoclavicular hyperstosis patients with low serum biotin levels

68. Maebashi *et al* (1993*b*) reported that oral administration of biotin (9 mg/day, along with 3 g of an antimicrobial drug, Miya- BM, included to prevent intestinal microflora degradation of biotin) to 30 patients suffering from sternocostoclavicular hyperstosis resulted in the correction of previous metabolic abnormalities (hyperglycaemia, serum amino acids and fatty acids) and clinical improvement.

Treatment of hyperinsulinaemia and impaired glucose tolerance.

69. Oral administration of biotin (9 mg/day, along with 3 g of an antimicrobial drug, Miya-BM, x 28 days) resulted in the correction of hyperglycaemia, with no change in serum insulin levels, in 28 patients with non-insulin dependent diabetes (Maebashi *et al*, 1999*a*).

Toxicity

Human

70. It is generally accepted that biotin is well-tolerated in humans, even when high doses are administered repeatedly over considerable time periods, either parenterally or enterally. For example, Koutsikos *et al* (1996) reported no adverse effects related to the intravenous administration of biotin (50 mg, 3 times per week post-dialysis, for up to 2 months) to 11 haemodialysis patients.

Case reports and other anecdotal information

71. There are numerous case reports in the literature that describe the outcomes of oral biotin administration to patients (infants, juveniles and adults) for the treatment of biotin–responsive inborn errors of metabolism and other forms of biotin deficiency. Furthermore, in cases where biotin-responsive disorders have been suspected within a fetus, biotin has been administered prenatally, via the mother. Typically, doses of 10 mg /day or more have been found to be therapeutic without reported adverse side effects. The NRC (1989) stated that there had been no reports of toxicity associated with intakes as high as 10 mg per day, citing the Life Science Research Office Evaluation of the Health Aspects of Biotin as a Food Ingredient (SCOGS 92, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1978). In a review of biotin nutrition and therapy, Bonjour (1977) noted the beneficial influence of biotin in the treatment of seborrhoeic dermatitis, egg white injury, propionicacidaemia and methylcrotonyl-glycinaciduria. He mentioned that there had been over 200 individual cases (infants and adults) where biotin had been administered in doses of up to 10 mg per day, either orally or intramuscularly, for periods exceeding 6 months in the absence of any reported toxic side effects. The origins of this data were not provided. A review by Mock (1996) stated: “toxicity has not been reported in individuals who have received daily doses of as much as 200 mg orally and 20 mg intravenously to treat biotin-responsive inborn errors of metabolism and acquired biotin deficiency”. No supporting data were provided.

Clinical trials and supplementation studies (see Table 2)

72. Several studies have been conducted to assess the beneficial effects of relatively high doses of orally administered biotin in the treatment of: biotin deficiency, particularly in renal dialysis patients and malnourished children, seborrhoeic dermatitis, brittle fingernails (or onychoschizia), sternocostoclavicular hyperstosis and hyperglycaemia in non-insulin-dependent diabetes. There have also been clinical studies in healthy individuals to assess the effects of high doses of oral biotin on plasma lipid profile, carboxylase activity, proliferation in blood mononuclear cells and urinary excretion of biotin metabolites.

73. The doses of biotin used in these studies were between 20 and 500 times lower than the highest doses (~200 mg/day) reported anecdotally in the treatment of biotin-responsive inborn errors or acquired biotin deficiency. Nonetheless, daily doses equivalent to ~250-fold the average UK daily intake have been taken for up to 4 years without any reported adverse effects. In the study of Hochman *et al* (1993), one patient out of 35 receiving biotin (2.4 mg/day) for the treatment of brittle nails reported a gastrointestinal upset. Whether the upset was related to the biotin treatment seems unlikely. The patient's gastrointestinal complaint did not subside on reduction of dose to 1.2 mg. The study was not controlled.

Effect of high concentrations of biotin present in special care infant formula

74. The feeding of special-care infant formula containing 300 μg of biotin per litre to new-born babies resulted in plasma concentrations that were ~20-fold greater than those of breast-fed infants. The consequences of such high levels of plasma biotin, if any, are not known (Livaniou *et al* 1991).

Vulnerable groups

75. There are no genetic traits that have been identified as likely to increase susceptibility to biotin toxicity. However, there are several inherited (autosomal recessive trait) disorders that result in functional deficiency of biotin (see paragraph 57).

Adverse drug reactions

76. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting" of reactions. Very few adverse reactions have been reported for products containing biotin. As all reactions relate to multiconstituent products, they may not be directly attributable to the vitamin.

Animal toxicity

Acute, subchronic and chronic toxicity

77. Animal toxicity data for biotin are limited. Available data from acute, subchronic and chronic studies are given in Tables 3 and 4. There are no long-term carcinogenicity data

available. The LD50 for repeated (10 day) oral administration in rat (strain and numbers of animals used not specified) was found to be >350 mg/day (see table 4, original data from Korner and Vollm 1976, as cited by Bonjour 1984).

Reproductive toxicity (summarised in Table 5)

78. Paul and co-workers reported oestrus cycle disturbances and atrophic changes in the ovaries of female (Holzman) rats administered 50 and 100 mg/kg biotin (n=6) by single subcutaneous injection during the time of vaginal dioestrus (Paul *et al* 1973*a*). These workers also reported adverse effects on reproductive performance and fetal development in female rats administered 50 and 100 mg/kg biotin by single subcutaneous injection 7, 14 or 21 days prior to mating (n=12). Biotin-related effects included the inhibition of fetal and placental growth and increased number of fetal resorptions (Paul *et al* 1973*b*). However, neither of these studies included adequate control groups. Mittelholzer (1975) essentially repeated the study of Paul *et al* (1973*b*) in Ibm:RORO$_f$ rats (n=7-8) with appropriate control groups. No significant adverse effects were observed. However, it should be noted that low male fertility in the control group could have masked a high dose effect. Paul and co-workers further reported that subcutaneous administration of biotin (100 mg/kg) to (Holzman) rats (n=7-11 per group) at the pre- (first and second day of gestation) or the post- (day of gestation 14 and 15) implantation stage of pregnancy resulted in adverse effects that included increased fetal resorptions, inhibition of fetal and placental growth and reduction of fetal and maternal body weight (Paul and Duttagupta 1975 & 1976). However, these studies were also flawed due to a lack of adequate controls. An appropriately controlled study by Watanabe (1996) reported no adverse effects on reproductive outcome in ICR mice following the administration of high levels of biotin during pregnancy (50 mg/kg by subcutaneous injection on days of gestation 0, 6 and 12, n=10-16). Furthermore these workers demonstrated that the administration of high levels of biotin in the diet (1000 ppm; intake in excess of 100 mg/kg/day [present author's estimate]) throughout pregnancy was also without adverse effects, despite a significant accumulation of biotin in both fetal and maternal tissues, including a 200-fold increase in maternal serum biotin and a 75-fold increase in fetal hepatic biotin over controls. In addition, 20-25% increases in biotinidase activity were observed in the placenta and the maternal serum.

Genotoxicity

79. D-Biotin, tested over a dose range tested of 0.033-10 mg/plate, was found not to be mutagenic in the bacterial Ames test employing *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 and *E.coli* WP2, either in the presence or absence of an Aroclor-induced rat liver S9 metabolic activation system. (Prival *et al* 1991).

Mechanism of toxicity

80. It has been suggested that reproductive changes following biotin treatment may reflect the inhibition of oestrogen production in the ovary (Paul *et al*, 1973*b*; Paul and Duttagupta 1976). However, this remains to be confirmed.

Regulatory considerations

81. The Recommended Daily Allowance in the Food Labelling Regulations for biotin is 0.15 mg. The Infant Formula and Follow-on Formula Regulations (1995) recommend a minimum biotin content of 1.5 μg/100 kcal. The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations (1999) recommend a maximum biotin content of 10 μg/100 kcal. The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations (1997) recommend that whole diet products should provide 15 μg and meal replacements 4.5 μg.

82. The US FDA have said that biotin used as a dietary supplement in food for human consumption is generally recognised as safe (GRAS) when used in accordance with good manufacturing practice (as cited by HSBD, 2000).

Existing recommendations on maximum intake levels

83. The European Federation of Health Product Manufacturers Associations (EHPM) recommend an Upper Safe Level for long-term consumption of 2,500 μg biotin (EHPM 1997). COMA (DH, 1991) agreed that intakes between 10-200 μg/day were both safe and adequate.

84. The FNB (2000) indicated that there were not sufficient data on which to base a Tolerable Upper Intake level (UL) for biotin.

Existing recommendations on maximum supplementation levels

85. Shrimpton (1995) recommended an upper safe level for daily supplementation of 500 μg biotin/day

Summary

86. D-Biotin (biotin, coenzyme R, vitamin H) is a water-soluble vitamin. It has a bicyclic ring structure. One ring contains a ureido group and the other contains a sulphur and a valeric acid side-group.

87. All biotin is derived from micro-organisms. It is widely distributed in natural foodstuffs but at very low levels. Foods relatively rich in biotin include egg yolk, liver, kidney, muscle and organ meats, and some vegetables. Food supplements are usually in the form of crystalline D-biotin or brewer's yeast.

88. The average daily intake of biotin in the UK is 39 μg and 29 μg in adult males and females, respectively. There are no Dietary Reference Values (DRVs) for biotin, although intakes between 10-200 μg/day are considered both safe and adequate.

89. Measurement of biotin in plasma is not a reliable indication of status in all cases. Changes in urinary excretion of biotin, bisnorbiotin, 3-hydroxyisovaleric acid and 3-methylcrotonylglycine are good indicators of biotin status.

90. Factors determining the bioavailabilty of biotin present in the diet are uncertain. The bioavailability of biotin that is covalently bound to protein is reduced in individuals suffering from biotinidase deficiency. There are few data concerning the bioavailability of crystalline biotin supplements, but a recent study has suggested that doses as high as 22 mg may be completely absorbed. The nutritional significance of biotin synthesised by bacteria present in the lower gut is subject to controversy.

91. Biotin uptake from the small intestine occurs by a carrier-mediated process that operates with a low K_m and also by slow passive diffusion. The carrier is driven by an electron-neutral Na+ gradient, has a high structural specificity and is regulated by the availability of biotin. The number of transporter molecules is up-regulated when biotin is deficient. The colon is also capable of absorbing biotin via a similar transport mechanism. Transfer of biotin from the site of absorption into plasma occurs via a Na+-independent carrier which is electrogenic and does not transport the vitamin against a concentration gradient. Approximately 80% of biotin found in plasma is in the free form. The remainder is either reversibly or covalently bound to plasma proteins. The existence of a specific plasma biotin carrier protein is debatable. Uptake into tissues occurs by specific transport mechanisms also dependent upon Na+ gradients. Transplacental transport is thought to involve the active accumulation of biotin within the placenta followed by its passive release into the fetal compartment. Biotin is metabolically trapped within the tissues, presumably by its incorporation into carboxylase enzymes. In normal protein turnover, carboxylase enzymes are broken down to biocytin or oligopeptides containing lysyl-linked biotin. Biotin may be released for recycling by the hydrolytic action of biotinidase. Liberated biotin may be reclaimed in the kidney by a process that again involves a Na+-dependent transporter working against a concentration gradient. Biotin may be metabolised oxidatively at the sulphur present in the heterocyclic ring and/or at the valeric acid side-chain. The metabolites formed are vitamin inactive and excreted in the urine. Very little biotin is thought to undergo biliary excretion. The substantial amounts of biotin that appear in the faeces are derived from the colonic bacteria.

92. Biotin acts as an essential cofactor for the acetyl-CoA, propionyl-CoA, β-methylcrotonyl-CoA and pyruvate carboxylase enzymes, important in the synthesis of fatty acids, the catabolism of branched-chain amino acids and the gluconeogenic pathway. Biotin may also have a role in the regulation of gene expression arising from its interaction with nuclear histone proteins.

93. Biotin deficiency has been observed in individuals maintained on total parenteral nutrition, people who consume large amounts of uncooked egg white, sufferers of inherent or acquired biotin malabsorption, haemodialysis patients, and individuals receiving some forms of long-term anticonvulsant therapy. Pregnancy may be associated with marginal biotin deficiency in some women. Signs of biotin deficiency include a fine scaly desquamating dermatitis and characteristic skin rash frequently observed around the eyes, nose and mouth, hair loss, conjunctivitis and ataxia. Biotin deficient infants show signs of hypotonia, lethargy, developmental delay and withdrawn behaviour, all of which are characteristic of biotin deficiency-related neurological disorder. “Egg white

injury" may be associated with glossitis, anorexia, nausea, hallucinations, depression and somnolence. Inherited deficiencies in biotinidase and holocarboxylase synthetase result in multiple carboxylases deficiency. These deficiencies and those of specific carboxylase enzymes may produce the same or similar disorders and manifestations of biotin deficiency. Clinical manifestations of biotin deficiency are generally thought to result, directly or indirectly, from deficient activities of the carboxylase enzymes. Biotin deficiency in animals results in terata.

94. Oral biotin supplementation is indicated in cases of biotin deficiency e.g. in individuals maintained on total parenteral nutrition, sufferers of inherent or acquired biotin malabsorption, haemodialysis patients, and individuals receiving some forms of long-term anticonvulsant therapy. Biotin supplements are also indicated in the management of inborn biotin-associated enzyme deficiencies such as biotinidase, holocarboxylase synthetase and the individual carboxylase enzymes. Biotin supplements may also be beneficial in the treatment of brittle nails, hyperinsulinaemia and impaired glucose tolerance and sternocostoclavicular hyperstosis.

95. The toxicity of biotin is generally accepted as low. Anecdotal reports suggest that typical daily doses of 10 mg are without adverse effects and toxicity has not been reported in individuals receiving as much as 200 mg per day. Clinical data are limited but studies have reported no adverse affects following the administration of 9 mg/day for up to 4 years, 10 mg/day for 15 days, 4 mg for 3 weeks or 2.5 mg for 6-15 months.

96. The database on the toxicity of biotin in laboratory animals is limited. The LD50 in mice, following a single intravenous administration, was found to be >1000 mg/kg. The LD50 following repeated oral dosing for 10 days of biotin in rats has been reported to be >350 mg/kg/day. There is controversy as to whether high doses of biotin cause reproductive toxicity in laboratory animals. Administration of biotin by sub-cutaneous injection to female Holzman rats, up to 3 weeks prior to mating, resulted in effects on reproductive performance, inhibition of fetal and placental growth and the increased resorption of fetuses. These effects observed in a similar study inconducted in Ibm:$RORO_f$ rats. However, both studies were compromised due to inadequate controls. Biotin-related inhibition of fetal and placental growth and the increased resorption of fetuses were also reported following the administration of biotin to Holzman rats during the pre- and post-implantation stages of pregnancy. However, these studies lacked adequate controls. High doses of biotin administered to female ICR mice throughout pregnancy, either by *s.c.* injection or via the diet, resulted in no adverse effects to either mother or fetus.

97. Biotin has been shown to be negative in the Ames test. Data for the effects of biotin in other types of mutagenicity tests are lacking. No carcinogenicity data are available for biotin.

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ANNEX 1 TO EVM/01/02.REVISEDAUG2002

TABLES REFERRED TO IN THE REVIEW

Table 1. Summary of Biotin AIs set by the FNB (2000)

Age	Adequate Intake of Biotin μg/day
0-6 months	5
7-12 months	6
1-3 years	8
4-8 years	12
9-13 years	20
14-18 years	25
>19 years	30
Lactation	35

AI – Adequate Intake (US)
No increment for pregnancy
Persons receiving haemodialysis or peritoneal dialysis may have an increased requirement for biotin as would persons with genetically determined biotin deficiency

Table 2. Biotin oral supplementation studies

study type	biotin dose	duration	adverse effects related to supplementation/comments	reference
Randomised double –blind placebo controlled cross-over study in infants (age not specified) with seborrhoeic dermatitis (n=19)	2 mg, 2x/day	3 weeks per treatment period (7 patients went through 3 courses of treatment)	there was no report of any adverse effects endpoint: grading of changes in skin in affected areas	Keipert 1976
double-blind placebo controlled study in 40 healthy individuals to assess effect of supplementation on plasma lipids.	0.9 mg/day	71 days	there was no report of any adverse effects endpoint: change in plasma lipid profile	Marshall *et al* 1980
biotin administered to healthy males (n=2) to investigate the effect on leucocyte carboxylase enzymes	5 mg, 2x/day	7 days	there was no report of any adverse effects endpoint: changes in leucocyte carboxylase activity	Bartlett *et al* 1980
Uncontrolled study of healthy males to investigate the effect on leucocyte carboxylase enzymes (n=5)	0.14 mg/kg/day (~8-10 mg/day)	5 days	there was no report of any adverse effects endpoint: changes in leucocyte carboxylase activity	Stirk *et al* 1982
Uncontrolled study in 71 women to investigate beneficial effect on finger nail condition	2.5 mg/d	not specified	there was no report of any adverse effects	Floersheim 1989 [Abstract in English]
an uncontrolled, retrospective study of 35 (out of 42, 44 or 46 approached initially – the paper was confused as to how many) patients with brittle nails, from a single nail consultation practice to investigate biotin supplementation as a treatment for brittle nails	1.0 -3.0 mg/day	1.5 -7 months	one patient reported a gastrointestinal upset which did not subside on reduction of dose from 2.4 to 1.2 mg/day; the patient discontinued the therapy after 6 weeks but the report does not indicate whether symptoms ceased at this point confusion as to the total number of individuals approached initially, therefore uncertain if all are accounted for endpoint: assessment of improvement in nail condition	Hochman *et al* 1993
study of 30 (16 M, 14 F) patients with sternocostoclavicular hyperstosis to investigate effect on metabolic	9 mg/day in combination with 3g of anti-	>2 months, up to 1 year	there was no report of any adverse effects the number of patients undergoing the maximum 1 years	Maebashi *et al* 1993*b*

study type	biotin dose	duration	adverse effects related to supplementation/comments	reference
abnormalities. The study included 20 (10M, 10F) healthy untreated controls	microbial drug Miya-BM (3 divided doses)		treatment duration was unclear; reasons for patient withdrawal were not made clear endpoint: analysis of serum amino acids, fatty acids; advancement of bone lesions as viewed by chest radiography	
Randomised placebo controlled study of 28 (18 treated; 10 control) patients with non-insulin- dependent diabetes as therapy for hyperglycaemia.	9 mg/day in (3 divided doses)	1 month	there was no report of any adverse effects endpoint: measurement of fasting blood glucose	Maebashi *et al* 1993*a*
Uncontrolled study of 20 patients with non-insulin- dependent diabetes as therapy for hyperglycaemia	9 mg/day in combination with 3g of anti-microbial drug Miya-BM (3 divided doses)	up to 4 years	it was reported that there were no undesirable side effects 15, 15, 10 and 5 patients were monitored for 24, 30, 36 and 48 months, respectively; reasons for patient withdrawal were not made clear end point: measurement of fasting blood glucose	Maebashi *et al* 1993*a*
Randomised controlled study of patients with brittle fingernails and onychoschizia (22 treated, 10 untreated controls)	2.5 mg/day	6-15 months	there was no report of any adverse effects endpoint: assessment of improvement in nail condition and thickness	Columbo *et al* 1990
placebo-controlled study in healthy males (test n=11, controls n=11) to determine effects of a ”high-potency” supplement on vitamin and mineral status	Multivitamin and mineral supplement containing ~ 0.4 mg/day	12 weeks	there was no report of any adverse effects endpoint: vitamin status assessed by measurement of plasma and urinary biotin	Singh *et al* (1992)
double-blind placebo controlled study in protein-energy deficient children (n=22)	10 mg/day	15 days	there was no report of any adverse effects endpoint: plasma biotin concentrations and lymphocyte carboxylase enzymes were measured	Velazquez *et al* 1995
Uncontrolled study of 14 (9F, 5M) healthy	1.2 mg/day	14 days	there was no report of any adverse effects	Mock and Heird

study type	biotin dose	duration	adverse effects related to supplementation/comments	reference
adult volunteers to investigate urinary biotin metabolites				1997
Uncontrolled study of five (3F, 2M) healthy adult volunteers (non-smokers)	1.2 mg/day	14 days	decreases in peripheral blood mononuclear cells and synthesis of interleukin-1β and interleukin-2	Zempleni et al 2001

Table 3. Acute Toxicity of biotin in animals: LD_{50} values mg/kg body weight

	route		
species*	oral	*i.v.*	*i.p.*
Mouse		> 1000	
Rat	>354		>29
Cat			>0.24

Data as cited by Bonjour, 1984; *strain and number of animals not specified

Table 4. Sub-chronic and chronic toxicity of biotin by oral administration in animals:

species*	LD_{50} µg/kg body weight/day	duration (days)
Rat	>350 x 10^3	10
Rabbit	>200	102
Piglet	>100	122

Data as cited by Bonjour, 1984; *strain and numbers of animal not specified

Table 5. Effects of biotin on reproductive function and/or fetal development following administration to experimental animals during pregnancy

Species	dose/route	biotin treatment-related effects	comments	reference
rat (Holzman) n=6	50 mg/kg, *s.c.* (in 0.2 ml 0.1 M NaOH; two divided doses) at the dioestrus stage of the oestrus cycle 7, 14 or 21 prior to termination or vehicle only 7 day before termination	disturbances in oestrus cycle as assessed by vaginal smears; a progressive increase in vaginal leucocyte numbers followed by a sharp decline after day 14; enhanced formation of corpora lutea; atrophic changes in corpora lutea and stroma; reduced liver glycogen;	rats selected for study showed 3 normal oestrus cycles prior to treatment ; vehicle alone did not effect oestrus cycle but these animals were monitored for only 7 days post-treatment	Paul *et al*, 1973*a*
rat (Holzman) n=12	50 mg/kg, *s.c.* (in 0.5 ml 0.1 M NaOH; two divided doses) on the day of vaginal oestrus, 7, 14 or 21 days prior to mating ± 1 μg/day E2 (olive oil as vehicle) from DG6	increased fetal resorptions; reduced fetal and placenta weights; failure to maintain pregnancy and effects on fetal and placenta weights rectified by E2 treatment	inadequate controls; an untreated control group included but no vehicle (0.5 ml of 0.1 M NaOH for biotin; 0.1 ml of olive oil for E2) control groups were included in the study .	Paul *et al*, 1973*b*
	100 mg/kg *s.c.* (in 1.0 ml 0.1 M NaOH; four divided doses) on the day of vaginal oestrus and the day after, 7 days prior to mating	failure to mate within 2 months	No vehicle control group included	
rat (Ibm:RORO$_f$) n=7-8	0, 5, 50 mg/kg, *s.c.* (in 1.0 ml 0.1 M NaOH; two divided doses) on the day of vaginal oestrus, 7, 14 or 21 prior to mating	no significant dose-related effect on reproductive performance, number of implantation sites fetal resorptions or ovary, placenta or fetal body weights; no histological changes in ovaries; vaginal cycle not changed	vehicle 0.1 M NaOH and untreated control groups were included; a satellite study demonstrated that low numbers of full-term fetuses in the untreated control and high dose groups mated 7 days after dosing observed in the main study were possibly due to the low fertility of males used. However, the satellite study was not taken to full-term, so a high dose effect cannot be discounted, although numbers of implantations and resorption sites were not significantly different from vehicle controls	Mittelholzer, 1975
rat (Holzman) n=7-9	100 mg/kg, *s.c.* (in 0.2 ml 0.1 M NaOH) on DG 1 and 2 (pre-implantation stage) ± 0.1	conceptus resorption, reduction in hepatic and uterine glycogen and protein, reduction in glucose-	inadequate controls; an untreated control but no NaOH or olive oil vehicle control groups were	Paul and Duttagupta,

	μg/day E2 (in 0.05 ml olive oil) or 4 mg progesterone (in 0.2 ml olive oil) from DG 15-21. Control rats were untreated	6- phosphate dehydrogenase activity in liver, uterus, ovary and adrenal	included in the study; no hormone control groups were included	1975
rat (Holzman) n=7-11	100 mg/kg, *s.c.* (in 0.2 ml 0.1 NaOH) on DG 14 and 15 (post-implantation stage) ± 0.1 μg/day E2 (in 0.05 ml olive oil) or 4 mg progesterone (in 0.2 ml olive oil) from DG 15-21	inhibition of fetal and placental growth, resorption of fetuses and placentas in 2/11 rats; reduction of maternal body and uterine weights; reductions in uterine and placental glycogen, RNA and protein levels and decreased glucose-6-phosphate dehydrogenase activity in ovary, liver and uterus; E2 therapy corrected adverse and biochemical effects while progesterone therapy only served to maintain maternal body weight and uterine weights with no effect on placental and fetal growth	inadequate controls; an untreated control but no NaOH or olive oil vehicle control groups were included in the study; no hormone control groups were included	Paul and Duttagupta, 1976
Mouse (ICR) n=10-16	0, 50 mg/kg, *s.c.* (in 0.5 ml 0.1 M NaOH) on DG 0, 6 & 12 0, 50 mg/kg, *s.c.* (in 0.5 ml olive oil) on DG 0, 6 & 12 0, 1000 mg/kg diet throughout gestation	none; no effect on successful pregnancy rate, number of dead or reabsorbed fetuses, litter size, placenta or fetal body weights; no significant increase in external malformations; no abnormal histology in liver, ovaries or placenta; biotinidase activity was increased in maternal serum and placenta	animals were killed on DG 17; appropriate control groups were included; maternal weight gain in *s.c.* control groups were 90% of dietary controls; accumulation of biotin in serum, maternal and embryonic tissues demonstrated in all biotin-treated treated groups with a 200-fold increase in maternal serum concentration in dietary treated group	Watanabe (1994) [abstract]; Watanabe, 1996

E2 – 17β estradiol; DG - day of gestation

ANNEX 2 TO EVM/01/02.REVISEDSEPT2001

INTAKES OF BIOTIN FROM FOOD AND SUPPLEMENTS

The data presented on biotin intakes are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years[4,5,6,7,8]. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

Total intakes of biotin

Table 1 provides information on the absolute intakes of biotin by the British population, from food sources and from all sources (including dietary supplements) classified by age and sex. Mean and median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively), are given.

Average intakes of biotin were lowest for young children aged 1½ to 4½ years, and highest for males aged 16 to 64 years. Mean biotin intakes increased significantly with age for boys aged 4 to 18 years and adults aged 16 to 34 years and decreased significantly with age for older people free-living in the community. The contribution of supplements was small and the inclusion of supplements had little effect on mean intakes.

There are no Dietary Reference Values set for biotin, however mean biotin intakes for all groups were within the range considered to be safe and adequate (between 10 and 200 μg).

Intakes at the 97.5%ile were about twice the median in all groups and were well within the upper level for safe and adequate intakes (200 μg).

Table 2 provides information on biotin intakes from food and supplements adjusted for body weight and classified by age and sex. Body weight adjusted biotin intakes are highest in infants and show a trend to decrease with age for children, young people and older males free-living in the community.

Sources of biotin in the diet

Table 3 indicates the contribution made by different types of food to average intakes of biotin by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices.

[4] Food and nutrient intakes of British infants. 1986
[5] National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3
[6] National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8
[7] Dietary and nutritional survey of British adults. 1986/7
[8] National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

The main food source of biotin in this age group is cereals and cereal products (24%), of which 6% came from breakfast cereals (fortification of breakfast cereals with biotin is uncommon). Milk and milk products provided 22% of the average daily intake, the majority of which came from milk. Meat and meat products provided 15%. Beer and coffee made a significant contribution to intake in some age groups; beer contributed 12% of total intake for males aged 16-64 years and coffee contributed 12% for females in the same age group.

Infants obtained about a third of their biotin intake from infant formulas, approximately a quarter from cow's milk and a further eighth from commercial infant foods.

Biotin intakes from supplements

The contribution of dietary supplements to biotin intakes in children aged 1½ to 4½ years was negligible. For other groups (except infants, where data is unavailable), dietary supplements containing biotin provided between about 1% and 4% of mean intakes. However, for older females aged 85 and over free-living in the community, supplements containing biotin provided 8% of mean intake from all sources.

Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately. Table 4 shows the number of consumers of dietary supplements containing biotin in each age group, together with the mean, median and range of intakes of biotin from supplements for those who consumed them. No more than 2% of any group studied used supplements containing biotin.

The range of intakes from supplements was wide with the maximum intake from this source at 243μg per day in females aged 65 years and over free-living in the community (above the upper range for safe and adequate intake). The highest intake from supplements in children was 171 μg/day for one female aged 11-14 years. Some of the supplements taken by young people contained 150 μg biotin per tablet.

Diet and Nutrition Surveys Branch
Food Standards Agency
January 2001

Table 1: Total intakes of Biotin

	Absolute Biotin intake (µg/day)							
Age/sex	*Food Only*				*Food and Supplements*			
	2.5% ile	Mean	Median	97.5%ile	2.5% ile	Mean	Median	97.5%ile
Infants (1986)								
6-12mths/M&F	10.9	25.0	22.0	70.6	*	*	*	*
Pre-school children								
1½-2½ yrs/M/F	6.3	17.1	16.0	33.3				
2½-3½ yrs/M/F	7.3	17.0	15.9	32.5	**	**	**	**
3½-4½ yrs/M	7.5	17.7	17.2	33.2				
3½-4½ yrs/F	7.0	16.8	15.9	32.7				
Young people (1997/8)								
4-6 yrs/M	8	22	20	43	8	22	20	43
4-6 yrs/F	8	19	18	32	8	19	18	32
7-10 yrs/M	11	24	22	41	11	24	22	50
7-10 yrs/F	7	20	19	39	7	21	20	40
11-14 yrs/M	9	25	24	51	9	25	24	51
11-14 yrs/F	9	20	20	42	9	21	20	44
15-18 yrs/M	12	29	27	57	12	29	27	57
15-18 yrs/F	9	21	21	40	9	21	21	40
Adults (1986/7)								
16-24 yrs/M	12.8	34.6	33.5	64.9	12.8	35.1	33.5	66.6
16-24 yrs/F	8.1	23.7	23.6	42.6	8.1	23.7	23.6	42.6
25-34 yrs/M	15.7	40.2	39.1	70.6	15.7	40.5	39.1	70.8
25-34 yrs/F	8.3	26.6	24.9	56.7	8.3	26.6	24.9	56.7
35-49 yrs/M	16.2	40.8	39.3	72.1	16.2	40.8	39.5	72.1
35-49 yrs/F	11.4	31.4	28.6	69.5	11.8	32.0	28.8	72.8
50-64 yrs/M	14.1	38.6	37.4	67.4	14.1	38.8	37.4	71.1
50-64 yrs/F	11.0	28.8	27.1	51.1	11.0	29.4	27.3	55.1
Older people free-living in the community (1994/5)								
65-74yrs/M	12	34	33	63	12	35	33	63
65-74yrs/F	10	26	24	47	10	27	25	49
75-84 yrs/M	12	30	30	55	12	31	30	57
75-84 yrs/F	10	24	22	44	10	25	22	46
85 and over/M	11	28	27	45	11	28	27	45
85 and over/F	8	23	20	45	8	25	21	47
Older people living in institutions (1994/5)								
65-84 yrs/M	13	29	27	57	13	29	28	57
65-84 yrs/F	15	27	25	43	15	27	25	44
85 and over/M	12	30	27	54	12	31	27	66
85 and over/F	12	24	23	43	12	25	23	43

* Data unavailable

** The contribution of dietary supplements to biotin intakes in pre-school children was negligible

Table 2: Bodyweight adjusted Biotin intake

	Bodyweight adjusted Biotin intake (µg/kg bwt /day)[9]		
Age/sex	*intakes from food and supplements*		
	Mean	Median	97.5%ile
Infants (1986)[10]			
6-12mths/M&F	2.66	2.22	5.98
Pre-school children (1992/3)			
1½-2½ yrs/M&F	1.40	1.31	2.79
2½-3½ yrs/M&F	1.17	1.11	2.24
3½-4½ yrs/M	1.08	1.01	2.10
3½-4½ yrs/F	1.03	0.97	1.82
Young people (1997/8)			
4-6 yrs/M	1.01	0.95	2.00
4-6 yrs/F	0.95	0.91	1.55
7-10 yrs/M	0.81	0.73	1.53
7-10 yrs/F	0.69	0.63	1.39
11-14 yrs/M	0.56	0.52	1.04
11-14 yrs/F	0.44	0.40	0.93
15-18 yrs/M	0.44	0.41	0.89
15-18 yrs/F	0.37	0.34	0.68
Adults (1986/7)			
16-24 yrs/M	0.50	0.49	0.99
16-24 yrs/F	0.40	0.39	0.78
25-34 yrs/M	0.54	0.52	0.99
25-34 yrs/F	0.45	0.41	0.93
35-49 yrs/M	0.54	0.53	0.95
35-49 yrs/F	0.51	0.45	1.30
50-64 yrs/M	0.50	0.48	0.87
50-64 yrs/F	0.47	0.43	0.93
Older people free-living in the community (1994/5)			
65-74 yrs/M	0.45	0.43	0.80
65-74 yrs/F	0.42	0.38	0.86
75-84 yrs/M	0.42	0.40	0.77
75-84 yrs/F	0.39	0.35	0.75
85 and over/M	0.41	0.39	0.69
85 and over/F	0.42	0.35	0.90
Older people living in institutions (1994/5)			
65-84 yrs/M	0.43	0.39	0.86
65-84 yrs/F	0.46	0.43	0.84
85 and over/M	0.46	0.43	1.03
85 and over/F	0.43	0.39	0.86

[9] Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

[10] Intakes for infants aged 6-12 months are from food only.

Table 3[11]: Sources of Biotin in the diet

Food Type	Contribution of food types to average daily intake of Biotin µg/day	% of total
Cereal and cereal products	6.1	24
- of which breakfast cereals	*1.4*	*6*
Milk and milk products	5.6	22
- of which milk	*4.5*	*18*
Egg and egg dishes	1.8	7
Fat spreads	0.0	0
Meat and meat products	3.6	15
Fish and fish dishes	0.5	2
Vegetables, potatoes and savoury snacks	2.2	9
Fruits and nuts	1.4	5
Sugar, confectionery and preserves	0.9	3
Beverages	2.5	10
Miscellaneous	0.5	2
Total intake from food	**25.1**	**100***
Intake from dietary supplements	*0*	*0*
Total intake from food and supplements	**25.1**	**100**

*Total allows for rounding

[11] NDNS: young people aged 4-18 years. 1997/8. 15-18 year group

Table 4: Biotin intake from supplements

	Consumers of Biotin supplements		**Biotin intake from supplements (consumers only) (µg/day)**		
Age/sex	*Number*	*%*	*Mean*	*Median*	*Range*
Infants (1986)					
6-12 mths/M&F	*	*	*	*	*
Pre-school children (1992/3)					
1½-4½ yrs/M&F	3	<1	2.7	1.5	1.0-5.0
Young people (1997/8)					
4-6 yrs/M&F	1	<1	1.4	1.4	1.4
7-10 yrs/M&F	4	<1	74.5	55.4	0.4-150.0
11-14 yrs/M	0	0	0.0	0.0	0.0
11-14 yrs/F	1	<1	171.4	171.4	171.4
15-18 yrs/M	2	1	9.7	4.0	4.0-17.9
15-18 yrs/F	1	<1	5.0	5.0	5.0
Adults (1986/7)					
16-64 yrs/M	12	1	18.2	3.3	0.1-71.4
16-64 yrs/F	27	2	16.0	1.1	0.1-128.6
Older people free-living in the community (1994/5)					
65 and over/M	10	2	16.8	6.5	0.7-150.0
65 and over/F	15	2	33.6	10.0	0.3-242.7
Older people living in institutions (1994/5)					
65 and over/M	2	<1	35.4	7.5	7.5-66.3
65 and over/F	5	2	11.7	7.1	4.7-23.3

* Data unavailable

ANNEX 3 TO EVM/01/02.REVISEDSEPT2001

Biotin: Summary table of selected nutrition related information and existing guidance on regulations

Unit of usage	**µg/day**		**µg/100 kcal**
UK Safe Intake	10-200		
Mean adult UK dietary intake from food (all sources)	Male	Female	
Adults (16-64)[12]	38.9 (39.1)	28.3 (28.7)	
65 years and over[13]			
free living	33 (33)	28 (29)	
institutionalised	29 (30)	26 (27)	
EU labelling RDA[14]	0.15 mg		
Supplemental doses			
Regulations			
Infant formula[15]			minimum 1.5
Infant foods[16]			10
Weight reduction[17]			
whole daily diet replacement	15		
meal replacement	4.5		
Maximum total safe daily intake[1]			
EHPM 1997[18]	2500		

[12] Dietary and nutritional survey of British adults. 1986/7
[13] National Diet and Nutrition Survey of people aged 65 years and over. 1994/5
[14] The Food Labelling Regulations 1996
[15] The Infant Formula and Follow-on Formula Regulations 1995
[16] The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1999 (amended)
[17] The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.
[18] Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.