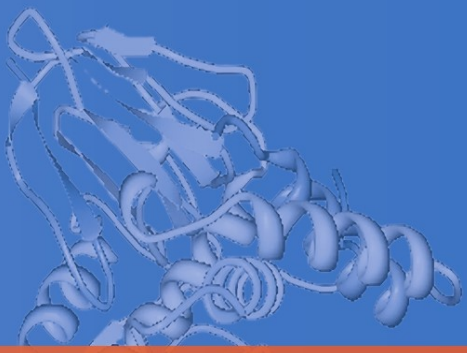
Subcellular Biochemistry 56



Olaf Stanger
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Editor
Olaf Stanger, MD, PhD, MBA
Royal Brompton and Harefield NHS Foundation
Imperial College of Science, Technology
and Medicine
Heart Division
Cardiothoracic Surgery
Sydney Street
London SW3 6NP
United Kingdom
o.stanger@rbht.nhs.uk

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Preface

For many years, there were vague ideas that some diseases were due to dietary deficiencies. The discovery of vitamins in the early 1900s, their chemical characterization and the clarification of their metabolic functions are sequential aspects of a brilliant chapter in the history of modern nutritional sciences and medicine. The research in vitamins and deficiency-related pathologies has indeed seen a remarkable past, and the path to still better understanding is likely to continue far into the future.

Numerous observations led to the discovery of vitamins and their function, including some truly pioneering investigations that made history.

For example, perhaps one of the first randomized trials was done by James Lind (a British naval surgeon, 1716–1794) in 1747 on board the HMS Salisbury: he randomized 6 groups (2 sailors each) to either "a quart of cyder", or "two oranges and one lemon", or "half a pint sea water", or "twenty five gutts of elixir vitriol", or "two spoonfuls of vinegar", or "the bigness of a nutmeg of an electuray made of garlic, mustard seed, raddish root, Peruvian balsam and gum myrrh", -in addition to the regular diet. The idea was to find out which of the accepted antiscorbutic therapies really worked and the outcome was clear pointing towards the oranges and lemon-treatment (and cyder to some extent).

Another thrilling story refers to the clinical work of William Castle leading to the conclusion that there must be a reaction that takes place between an unknown intrinsic factor (IF) in the gastric juice and an unknown extrinsic factor in beef muscle. That extrinsic antianaemic factor turned out to be cobalamin (vitamin B12) and IF is needed for its absorption. Presumably, the gastric juice in pernicous anemia lacks IF.

Most of the mentioned work was done in the early 1900s. The period from 1912 to 1948 saw the isolation and identification of the variety of compounds and structures collectively termed vitamins. The name, derived from "vital-amines", indicates their elementary metabolic key functions in human metabolism. Indeed, severe deficiency is often associated with malfunction, symptoms and potentially fatal disease. The population-wide impact of overt deficiency-associated disease was so large that key discoveries in the field allowing prevention and leading to therapies were rewarded with several Nobel prizes.

The next logical step was synthetic mass production. Today, the general availability of vitamins to practically everyone and numerous national health programs have saved many lives and prevented complications. True (congenital) metabolic disorders and serious deficiency-associated diseases are rare and in general limited to particular geographic areas and high risk groups. Their identification is of utmost importance to make the best use of the appropriate vitamin's therapeutic potential. When used properly to treat or cure of diseases, vitamins are, indeed, "magical" substances. Due to their efficacy, they should therefore be regarded as drugs with effects and side effects to be weighted against each other. Sometimes a toxic dose is difficult to define and a longstanding misapprehension has led many people to believe that vitamins are harmless in any dose and, particularly in generous dose, can cure almost all of mankind's ills.

Today, it is not the previously fatal deficiency-associated diseases such as pernicious anemia or beri-beri that are in the focus of interest, but rather the relation of suboptimal vitamin bioavailability to chronic disease, which is much more difficult to observe and document. This is complicated by genetic susceptibility, lifestyle, and the presence or absence of health-compromising habits, such as smoking.

Vitamins are truly families of compounds, which include precursors and various bound forms, all with individual roles in metabolism and function. A more recent approach therefore searches for the components and optimal intake of a physiologically complete diet aiming at preventing disease and assuring optimal health. Any state of nutrition can be defined in theory, as deficient, marginal, satisfactory, excessive, or toxic. But in practice, this may prove to be extremely difficult requiring the development of appropriate tests and various status indicators.

In turn, the development and application of new and more sensitive and specific assays further enable us to look more closely into the many functions of vitamins.

At a national level, recommended daily allowances for vitamins become policy statements. Nutrition policy has far-reaching implications in the food industry, in agriculture, and in food provision programs. In fact, the broad availability of vitamins means that a great deal of work concentrates on aspects of overdosage and toxicology, interactions and also on regulations of direct and indirect intake. Water soluble vitamins are complex molecular structures and even today, many areas in vitamin biochemistry are not yet fully understood. Novel effects and functions of vitamins remain and continue to be discovered.

This book in your hands adds fantastic new insights into the biochemistry, metabolism, function, and therapeutic and diagnostic use of water soluble vitamins.

Serious clinical abnormalities including growth retardation, neurological disorders, and dermatological abnormalities occur in conditions of biotin (vitamin H) deficiency. Hamid M. Said (Irvine, California) presents the latest information on the biochemical, physiological, and clinical aspects of biotin nutrition.

Niacin is covered in two very interesting chapters. James B. Kirkland (Guelph, Ontario) explains the association between niacin status and genomic instability in bone marrow cells. The results are important for cancer patients, who tend to be niacin deficient, are exposed to large doses of genotoxic drugs, and suffer short-term bone marrow suppression and long-term development of secondary leukemias.

The recent identification of the nicotinic acid receptor has allowed distinction of the drug-like roles of nicotinic acid from its vitamin functions. The group of Elaine L. Jacobson (Tucson, Arizona) reviews niacin as an antidyslipidemic and cardioprotective drug.

The chapter on thiamin by Derrick Lonsdale (Westlake, Ohio) emphasizes beri-beri as the model for high calorie malnutrition and reviews the biochemistry of the three phosphorylated esters of thiamin and of their transporters. The pathophysiology of thiamin deficiency and the role of synthetic thiamin derivatives as therapeutic agents are discussed.

Riboflavin (vitamin B2) is essential for energy generation in the aerobic cell, through oxidative phosphorylation. Hilary J. Powers and co-workers (Sheffield, U.K.) provide insight into specific functions associated with cell fate determination, and review mechanisms and consequences of riboflavin depletion through effects on the expression of regulatory genes, exerted at both the transcriptional and proteomic level.

Sang Woon Choi (Boston, Mass.) and Simonetta Friso (Verona, Italy) and their co-workers review the evidence indicating a relationship between pyridoxin (vitamin B6) status and cancer as well as cardiovascular disease. Their discussion of the potential mechanisms of action is complemented by the chapter by Georg T. Wondrak (Tucson, Arizona), also from the Jacobson group, presenting insight to the structural basis of pyridoxin activity as a potent antioxidant, metal chelator, carbonyl scavenger and photosensitizer.

Novel aspects of the very complex biochemistry and metabolism of cobalamin (vitamin B12) are elaborated in three chapters. Bernhard Kräutler (Innsbruck, Austria) provides an in-depth survey of the physiological chemistry of cobalamin in the context of the metabolic transformation of cobalamin-derivatives and their use in cobalamin-dependent enzymes, whereas Sergey N. Fedosov (Aarhus, Denmark) reviews the molecular mechanisms of cobalamin transport with emphasis on interaction of corrinoids with the specific proteins and protein-receptor recognition. Practical novel aspects concerning early detection of cobalamin-related disorders are described including medical application of Cbl-conjugates, and purification of corrinoids from biological samples. To round out the subject, Wolfgang Herrmann (Homburg, Germany) suggests a revised definition of cobalamin deficiency.

Ascorbic acid (vitamin C) is covered in three further outstanding chapters. Mario C. De Tullio (Bari, Italy) focuses on largely unknown facets of the role of ascorbic acid in cell metabolism and its involvement in cell signalling and gene expression that are, at least in part, unrelated to antioxidant functions. John X. Wilson and F. Wu (Buffalo, New York) discuss in depth the role of ascorbic acid in correcting microvascular dysfunction and protecting capillary blood flow and arteriolar responsiveness in septic syndromes. Finally, neurons in the central nervous system (CNS) contain some of the highest ascorbic acid concentrations of mammalian tissues. James M. May (Nashville, Tenn.) reviews the role of the specific ascorbate transporter SVCT2 (Slc23a2) in regulating neuronal ascorbate homeostasis and the extent to which ascorbate affects brain function, including antioxidant protection,

peptide amidation, myelin formation, synaptic potentiation, and protection against glutamate toxicity in the CNS.

There is increasing research into the importance of folate-derived one-carbon units for DNA and histone methylation reactions, which exert crucial epigenetic control over cellular protein synthesis. Numerous polymorphisms have now been identified in folate related genes and it is thus becoming clear that genetic aspects of folate metabolism are wide-ranging and may touch on events as disparate as prenatal imprinting and cancer susceptibility. This topic is covered by Anne M. Molloy (Dublin, Ireland), who provides a detailed account of genetic aspects of folate metabolism. Willi Wonisch (Graz, Austria) and myself have elaborated on novel enzymatic and non-enzymatic antioxidative effects of folic acid and its reduced derivates that help explain the beneficial effects of folic acid supplementation in various clinical conditions. As an important clinical application J. D. Williams (Tucson, Arizona) reports on recent advances in research on folates in skin cancer and their potential in its prevention. And finally, J. Yang and Philipp Low (W. Lafayette, Indiana), expand upon themes introduced earlier and give a brilliant overview of the biology of the folate receptor (FR) and FR expression in immune cells with relevance for cancer and inflammatory and autoimmune disease. Their description of folate conjugates acting as drug carriers and diagnostic imaging tools holds strong promise for future successful efforts to cure certain types of cancer and chronic inflammatory disease.

This work in sum total so adds a new leaf to the exciting book on vitamin research that today's and tomorrows's scientists will continue to write.

I am deeply thankful to each of the contributing authors. All of them are highly esteemed and leading scientists very much dedicated to the demanding biochemistry of vitamins. They all have willingly agreed to share the latest research results from their respective working groups, laboratories and institutes. I truly thank them for all of their efforts and patience in the preparation of this book.

London, UK Olaf Stanger

Contents

Contributors

Sang Woon Choi Tufts University, Medford/Somerville, MA, USA; Department of Clinical and Experimental Medicine, School of Medicine, University of Verona, Verona, Italy; Vitamins and Carcinogenesis Laboratory, Jean Mayer USDA HNRCA, Tufts University, Boston, MA 02111-1524, USA, sang.choi@tufts.edu

B.M. Corfe Human Nutrition Unit, The School of Medicine, The University of Sheffield, Sheffield S10 2RX, UK, b.m.corfe@sheff.ac.uk

R. Corrocher Tufts University, Medford/Somerville, MA, USA; Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy, roberto.corrocher@univr.it

Mario C. De Tullio Dipartimento di Biologia e Patologia Vegetale, Universita' di Bari, 70125 Bari, Italia, detullio@botanica.uniba.it

Sergey N. Fedosov Protein Chemistry Laboratory, Department of Molecular Biology, University of Aarhus, Aarhus, Denmark, snf@mb.au.dk

Simonetta Friso Tufts University, Medford/Somerville, MA, USA; University of Verona School of Medicine, Department of Medicine, 37134 Verona, Italy, simonetta.friso@univr.it

Wolfgang Herrmann Department of Clinical Chemistry and Laboratory Medicine, University of Saarland, 66421 Homburg, Germany, kchwher@uniklinik-saarland.de

Elaine L. Jacobson Division of Medicinal Chemistry, Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Niadyne Development, Inc., Tucson, AZ, USA, jacobse@pharmacy.arizona.edu; elaine.Jacobson@pharmacy.arizona.edu

M.K. Jacobson Division of Medicinal Chemistry, Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Niadyne Development, Inc., Tucson, AZ, USA, mjacobson@pharmacy.arizona.edu

H. Kim Division of Medicinal Chemistry, Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Niadyne Development, Inc., Tucson, AZ, USA, hkim@pharmacy.arizona.edu

M. Kim Division of Medicinal Chemistry, Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Niadyne Development, Inc., Tucson, AZ, USA, mkim@pharmacy.arizona.edu

James B. Kirkland Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1, jkirklan@uoguelph.ca

Bernhard Kräutler Institute of Organic Chemistry and Centre of Molecular Biosciences (CMBI), University of Innsbruck, Innsbruck, Austria, Bernhard.kraeutler@uibk.ac.at

Derrick Lonsdale Preventive Medicine Group, Westlake, OH 44145, USA, dlonsdale@pol.net

V. Lotto Tufts University, Medford/Somerville, MA, USA; Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy, valentine.lotto@univr.it

Philip Low Purdue University, West Lafayette, IN, USA, plow@purdue.edu

James M. May Departments of Medicine and Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA, james.may@vanderbilt.edu

Anne M. Molloy School of Medicine, Trinity College Dublin, Dublin 2, Ireland, amolloy@tcd.ie

E. Nakano Human Nutrition Unit, The School of Medicine, The University of Sheffield, Sheffield S10 2RX, UK, e.nakano@sheff.ac.uk

Rima Obeid Department of Clinical Chemistry and Laboratory Medicine, University of Saarland, 66421 Homburg, Germany, rima.obeid@uniklinikum-saarland.de

Hilary J. Powers Human Nutrition Unit, The School of Medicine, The University of Sheffield, Sheffield S10 2RX, UK, h.j.powers@sheff.ac.uk

Hamid M. Said University of California-School of Medicine, Irvine, CA, USA; VA Medical Center, Long Beach, CA 90822, USA, hmsaid@uci.edu

Olaf Stanger Royal Brompton and Harefield NHS Foundation, Imperial College of Science, Technology and Medicine, Heart Division, Cardiothoracic Surgery, Sydney Street, London SW3 6NP, United Kingdom, o.stanger@rbht.nhs.uk

Erina Vlashi University of California, Los Angeles, CA, USA; Purdue University, West Lafayette, IN, USA, evlashi@purdue.edu

J.D. Williams Division of Medicinal Chemistry, Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Niadyne Development, Inc., Tucson, AZ, USA, williams@pharmacy.arizona.edu

John X. Wilson Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY 14214-8028, USA, jxwilson@buffalo.edu

Georg T. Wondrak Department of Pharmacology and Toxicology, College of Pharmacy and Arizona Cancer Center, University of Arizona, Tucson, AZ, USA, wondrak@pharmacy.arizona.edu

Willibald Wonisch Clinical Institute of Medical and Chemical Laboratory Diagnostics, Institute of Physiological Chemistry, Center for Physiological Medicine, Medical University of Graz, A-8010 Graz, Austria, willi.wonisch@aon.at

F. Wu Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY 14214-8028, USA, fengwu@buffalo.edu

Jun Yang St. Jude Children's Research Hospital, Memphis, TN, USA; Purdue University, West Lafayette, IN, USA, jun.yang@stjude.org

Chapter 1
Biotin: Biochemical, Physiological and Clinical Aspects

Hamid M. Said

Abstract Significant progress has been made in our understanding of the biochemical, physiological and nutritional aspects of the water-soluble vitamin biotin (vitamin H). It is well know now that biotin plays important roles in a variety of critical metabolic reactions in the cell, and thus, is essential for normal human health, growth and development. This is underscored by the serious clinical abnormalities that occur in conditions of biotin deficiency, which include, among other things, growth retardation, neurological disorders, and dermatological abnormalities (reviewed in 1). Studies in animals have also shown that biotin deficiency during pregnancy leads to embryonic growth retardation, congenital malformation and death (Watanabe 1983; Cooper and Brown 1958; Mock et al. 2003; Zempleni and Mock 2000). The aim of this chapter is to provide coverage of current knowledge of the biochemical, physiological, and clinical aspects of biotin nutrition. Many sections of this chapter have been the subject of excellent recent reviews by others (Wolf 2001; McMahon 2002; Mock 2004; Rodriguez-Melendez and Zempleni 2003; Said 2004; Said et al. 2000; Said and Seetheram 2006), and thus, for more information the reader is advised to consider these additional sources.

Keywords Biotin · Vitamin H · Carboxylases · Gluconeogenesis · Fatty acids

1.1 Chemical Structure, Sources and Availability of Biotin

The chemical structure of the biotin molecule (see Fig. 1.1 for the structure of biotin and related/relevant compounds) is composed of two rings to which a valeric acid moiety is attached as a side chain. One of the rings contains a ureido group (N-CO-N) which is involved in the binding of the vitamin to avidin, a glycoprotein found in egg-white that has an extremely high binding affinity toward biotin (Green 1990). The other ring contains a tetrahydrothiophene group to which a valeric acid moiety is attached. While the biotin molecule can exist in eight stereoisomers, the

H.M. Said (✉)
University of California-School of Medicine, Irvine, CA, USA

VA Medical Center, Long Beach, CA 90822, USA
e-mail: hmsaid@uci.edu

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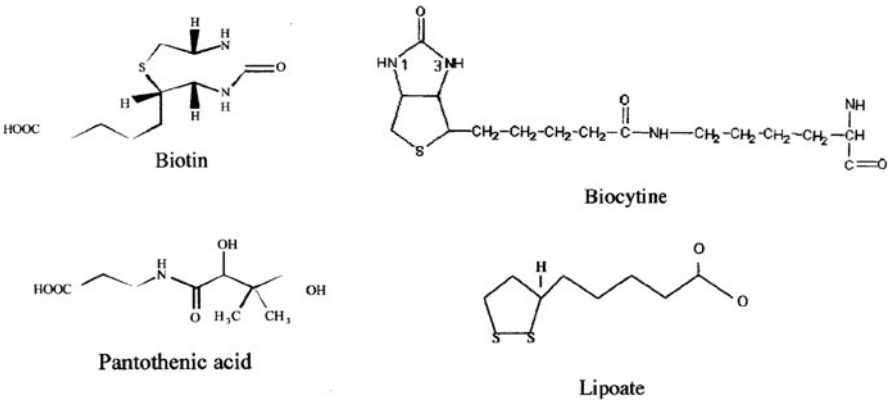

Fig. 1.1 Chemical structure of biotin and related compounds

D-biotin isomer is the only stereoisomer that is biologically active. Biotin has a pKa of 4.5, thus, it exists at physiological pHs mainly in the anionic de-protonated form.

Biotin is essential for all organisms. Animal cells cannot synthesize this micronutrient, but microorganisms (like certain bacteria and yeast) and plant cells can synthesize biotin endogenously (McMahon 2002). Biotin is widely distributed in foodstuff but at a level that is lower than that of other water-soluble vitamins (Combs 1992). Good sources for biotin include organ meat (like liver and kidney), egg yolk, some vegetables and cow's milk; poor sources include lean meat, cereal and fruits (Combs 1992; Harding and Crooks 1961). Bioavailability of dietary biotin varies from one food source to another; it is close to 100% in the case of corn, and as low as 5% in case of wheat (Combs 1992). In addition to the dietary source of biotin, humans have an additional source for this essential micronutrient, which is the bacterial source in the large intestine where the vitamin is produced and can be absorbed (see below). The relative contribution of this latter source of biotin toward overall body requirement of the vitamin is not well defined (Wolf 2001); however, it is becoming increasingly evident that this source of biotin does contribute to the host nutrition, and especially to the cellular nutrition of the localized colonocytes (see below).

1.2 Biotin Requirements and the Incidence of Biotin Deficiency

The recommended daily allowance of biotin has not been established due to the uncertainty about the contribution of the bacterially synthesized biotin in the human large intestine. However, a safe daily intake of the vitamin for both infants and adults has been estimated to be at 35 ug and 150–300 ug, respectively (National Research Council 1980). Biotin appears to be relatively nontoxic even at doses of greater than 60 mg/day for several months (Wolf 2001; Paul 1978; Watanabe 1996).

Biotin-deficiency and sub-optimal levels have been reported with increased frequency in recent years. Deficiency of biotin occurs in patients with inborn errors

of biotin metabolism (Wolf 2001; Sweetman and Nyhan 1986), in patients on long-term therapy with anticonvulsant agents (Krause et al. 1982a), and in patients on long-term parenteral nutrition (Krause et al. 1985; Forbes and Forbes 1997). Sub-optimal levels of biotin have been reported during pregnancy (Mock et al. 1997), in substantial numbers of alcoholics (Fennelly et al. 1969; Bonjour 1980), in patients with inflammatory bowel disease (Urabe et al. 1986; Banares et al. 1989), and in patients with seboric dermatitis and Leiner's disease (Nisenson 1957; Messaritakis et al. 1975).

1.3 Metabolic Role of Biotin

In mammals, biotin acts a cofactor for four carboxylases catalyzing the transfer of a carboxyl group to targeted substrates (reviewed in McMahon 2002; Mock 2004; Sweetman and Nyhan 1986; Dakshinamurti and Chauhan 1988). These carboxylases play a critical role in the intermediate metabolism of gluconeogenesis, fatty acid synthesis, and amino acid catabolism (McMahon 2002; Mock 2004; Sweetman and Nyhan 1986; Dakshinamurti and Chauhan 1988). The four biotin-dependent carboxylases are: pyruvate carboxylase (PC; EC 6.4.1.1), propionly-CoA carboxylase (PCC; EC 6.4.1.3), β-methylcrotonyl-CoA caboxylase (MCC; EC 6.4.1.4), and acetyl-CoA carboxylase (ACC; EC 6.4.1.2), with the latter enzyme existing in two genetically distinct forms one of which is in the cytosol (ACC1) and the other is in the mitochondria (ACC2). These carboxylases exist in the inactive apo-forms, which are converted to the active hollo-forms by the action of the enzyme holocarboxylase synthetase (HCLS; EC 6.3.4.10). The conversion occurs via a two-step, ATP-dependent reaction that involves the covalent attachment of a biotin molecule to a lysine residue in the carboxylases. This lysine moiety is located in a highly conserved domain common to all the biotin-dependent carboxylases. The HCLS exists in different cellular compartments including the nucleus, cytoplasm and mitochondria (Gravel and Narang 2005).

PC plays a role in the conversion of pyruvate to oxalate in the tricarboxylic acid cycle. Deficiency of this enzyme leads to lactic acidemia. PCC plays a role in the conversion of methylmalony-CoA to propionyl-CoA, which then becomes succinyl-CoA and enters into the tricarboxylic acid cycle. Deficiency of the latter enzyme leads to an increase in the excretion of the organic acids 3-hydroxypropionic acid and 2-methylcitric acid. MCC plays a role in the metabolism of leucine and deficiency of this enzyme leads to an increase in the urinary excretion of organic acids like 3-hydroxyisovaleric acid and 3-methylcrotonylglycine. ACC1 and ACC2 are involved in the generation of malonyl CoA.

In addition to the classical function of biotin described above, recent findings have indicated a role for the vitamin in histone modification (via biotinylation) (Gravel and Narang 2005; Ballard et al. 2002; Stanley et al. 2004). This function may be important for cell proliferation (Stanley et al. 2002) and DNA repair (Peteres et al. 2002) and may explain a number of findings on the effect of biotin on cell function. Both the HCLS and biotinidase (see latter) were suggested to play a role

in histones biotinylation (Gravel and Narang 2005; Ballard et al. 2002; Stanley et al. 2004). Patients with HCLS deficiency have very low histone biotinylation level in their lymphocytes (Narang et al. 2004).

Evidence for a role for biotin in the regulation of expression of a variety of genes has also been reported. An excellent review of this subject by Rodriguez-Melendez and Zempleni has recently been published (Rodriguez-Melendez and Zempleni 2003). Briefly, biotin appears to stimulate the level of expression of the insulin receptor (De La Vega and Stockert 2000), glucokinase (a key enzyme in glycolysis) (Chauhan and Dakshinamurti 1991, Dakshinamurti and Cheah-Tan 1968), and that of the human thiamin transporter-2 (Vlasova et al. 2005), while it suppresses the level of expression of the hepatic phosphoenolpyruvate carboxykinase (a key enzyme in gluconeogenesis; Dashinamurti and Li 1994). In addition, expression of the genes of the holocarboxylase synthetase and of the biotin-dependent carboxylases is affected by the prevailing level of biotin (Rodriguez-Melendez et al. 2001; Solorzano-Vargas et al. 2002; Wiedmann et al. 2003). Furthermore, the level of expression of the human sodium-multivitamin transporter (hSMVT, a major biotin transporter in different tissues, see latter) has also been shown to be modulated (in a tissue specific manner) by the prevailing level of biotin (Rodriguez-Melendez and Zempleni 2003; Crisp et al. 2004; Reidling and Said 2006; Balamurugan et al. 2005). Evidence has also been forthcoming demonstrating an effect for biotin on expression of oncogenes like N-myc, c-myb, N-ras and raf (Scheerger and Zempleni 2003). A post-transcriptional regulation of the asialoglycoprotein expression in hepatocytes by biotin has also been reported and is believed to be due to improper cell surface targeting of the glycoprotein (Collins et al. 1988). The mechanisms through which biotin exerts its above effects are not fully understood but could involve activation of soluble guanylate cyclase (by biotinyl-AMP), translocation of NF-kB to the nucleolus, and histone modifications via biotinylation (Rodriguez-Melendez and Zempleni 2003; Gravel and Narang 2005; Ballard et al. 2002; Stanley et al. 2004; Solorzano-Vargas et al. 2002).

Biotin also appears to play a role in normal immune functions including the production of antibodies, normal macrophage function, and the differentiation of T and B lymphocytes (Rabin 1983; Pruzansky and Axelrod 1955; Petrelli et al. 1981; Kung et al. 1979; Kumar and Axelrod 1978; Baéz-Saldaña et al. 1998). The vitamin may also be important for the normal function of natural killer cells as biotin supplementation alleviates the level of suppression in the activity of these cells in patients with Crohn's disease (Okabe et al. 1988). A role for biotin in cell proliferation has also been reported (Dakshinamurti et al. 1985; Mathey et al. 2002).

1.4 Biotin Catabolism

Biotin appears in the intact form in the urine and feces with the output being always higher than the daily intake of the vitamin. Some biotin, however, undergoes a limited-degree of catabolism to biotin sulfoxide (a process that takes place mainly in the liver; McCormick 1975) and to bisnorbiotin (a process that takes place

mainly in the mitochondria; McCormick and Wright 1971). These catabolic events appear to increase in pregnancy, with cigarette smoking, and following the use of anticonvulsant medications (Mock et al. 1997, 2002).

1.5 The Physiology of Biotin

The intestine and the kidney play important roles in regulating biotin body homeostasis via their involvement in the entry and the exit processes of the vitamin. Thus, significant attention has been paid toward the understanding of the mechanisms involved in biotin uptake by absorptive epithelial cells of these organs and how these processes are regulated at the cellular and molecular levels. In this section, we will describe in details the current knowledge of the mechanisms and regulation of the intestinal and renal transport process of biotin; more extensive reviews in the area also exist (Said 2004; Said et al. 2000; Said and Seetheram 2006). We will also provide a description of biotin transport into other tissues that are important in biotin metabolism/function.

1.5.1 Intestinal Absorption of Biotin

1.5.1.1 Digestion of Dietary Biotin

As mentioned earlier humans are exposed to two sources of biotin, the first being the dietary source, and the second being the bacterial source in the large intestine (Wrong et al. 1981). Dietary biotin exists in the free and protein-bound forms with the ratio of the two in a given dietary source being dependent on the type of that source (Lampen et al. 1942). Ingested protein-bound forms of biotin are first broken down by gastrointestinal proteases and peptidases to biocytin (biotinyl-L-lysine; Fig. 1.2) and biotin-oligopeptides (Wolf et al. 1984). These products are then further processed in the intestinal lumen (i.e., prior to absorption) to release the free biotin (Fig. 1.2). The latter process is performed enzymetically by the action of biotinidase (EC 3.5.1.12). The source of the involved intestinal biotinidase is believed to be the pancreas (Wolf et al. 1984). Clinical and experimental evidence have demonstrated the importance of the hydrolysis step of biocytin and biotin-oligopeptides to free biotin for the efficient absorption and optimal bioavailability of dietary biotin (Wolf et al. 1984; Said et al. 1993). The liberated biotin is then absorbed in the small intestine.

1.5.1.2 Mechanism of Intestinal Biotin Uptake

The mechanism of biotin uptake by the small intestine has been the subject of intense investigations and has been thoroughly reviewed in recent years (Said 2004; Said et al. 2000; Said and Seetheram 2006). Using a variety of intestinal in vitro and in vivo preparations, it has been well established that the intestinal biotin uptake

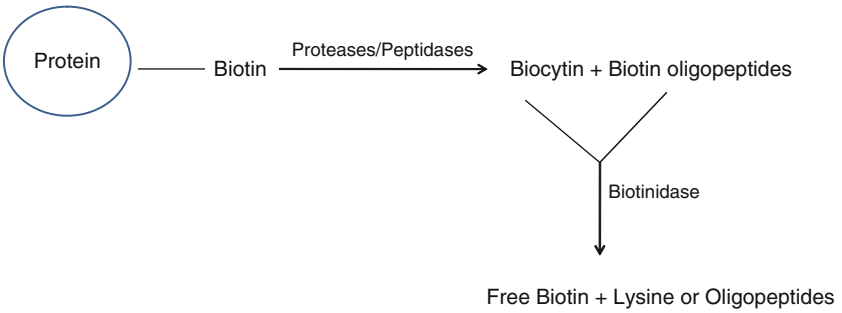

Fig. 1.2 Uptake of dietary biotin

process occurs via a Na+-dependent, carrier-mediated mechanism. This mechanism is inhibited by biotin structural analogues with a free carboxyl group at the valeric acid moiety of the biotin molecule (as in the case with desthiobiotin), but not by analogues with a blocked carboxyl group (as in the case of biocytin). Uptake of biotin in both human and animals is higher in the proximal compared to the distal part of the small intestine (Said et al. 1988; Said and Redha 1987). Functional and immunological studies have shown that the biotin Na+-dependent, carrier-mediated mechanism is expressed only at the apical membrane domain of the polarized enterocytes (Said et al. 1987, 1998; Said and Redha 1988b; Said and Derweesh 1991; Said 1991; Nabokina et al. 2003). This has been further confirmed by means of confocal imaging of living intestinal epithelial cells transfected with fluorescently tagged biotin transporter, SMVT (Fig. 1.4; Subramanian et al. 2006). The apical Na+-dependent biotin uptake system appears to be the rate-limiting step in the overall movement of biotin across the intestinal epithelial cells and is capable of transporting the substrate against a concentration gradient (Said et al. 1987; Said and Redha 1988a; Said and Derweesh 1991). The role of Na+ in biotin transport across the intestinal apical membrane domain is mediated via the inwardly directed Na+ gradient (which provides the needed energy for the transport of biotin against the concentration gradient) and not through the mere existence of Na+ in the incubation medium (Said et al. 1987) (Fig. 1.3). Internalized biotin then leaves the intestinal epithelial cells via the basolateral membrane by means of a Na+-independent, electrogenic, carrier-mediated mechanism (Said and Redha 1988c; Said 1991) (Fig. 1.3).

One of the interesting features of the Na+-dependent biotin uptake system is its ability to also transport two other functionally unrelated nutrients, namely pantothenic acid and lipoate (Fig. 1.1) (Said et al. 1998; Nabokina et al. 2003). Pantothenic acid is a member of the B family of water-soluble vitamins which is required for the biosynthesis of coenzyme A and acyl carrier proteins, and thus, it assumes important roles in carbohydrate, fat and protein metabolism. Lipoate is a potent intracellular and extracellular antioxidant in mammalian cells and is involved in the redox cycling of other antioxidants like vitamins C and E; it is also involved in regulating the intracellular level of glutathione. The ability of the intestinal biotin

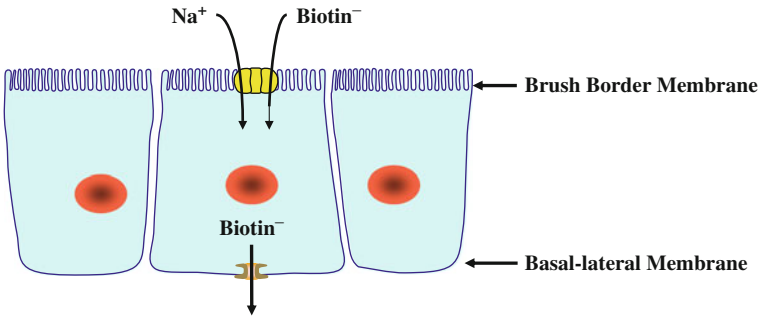

Fig. 1.3 Processing of dietary protein-bond biotin in the intestinal lumen

uptake system to also transport pantothenic acid and lipoate is not unique to the intestine but has also been observed in other cellular systems such as the brain, heart, placenta and kidney (Said 1991; Spector and Mock 1987; Beinlich et al. 1990; Grassl 1992). This ability increases the physiological and nutritional significance of the involved uptake system and was the basis for its naming as the sodium-dependent multivitamin transporter (SMVT).

With regards to the bacterially synthesized biotin, a substantial amount of this biotin exists in large intestinal lumen in the absorbable/unbound form (Wrong et al. 1981; Streit and Entcheva 2003). Also, in vivo studies in humans and animal models have shown that the large intestine is capable of absorbing luminal biotin (Barth et al. 1986; Brown and Rosenberg 1987; Sorrell et al. 1971). The mechanism involved in biotin uptake in the large intestine has also been the subject of investigation in recent years (Said et al. 1998). Using the human-derived colonic epithelial NCM460 cells as a model system for human colonocytes, studies have shown the existence of an efficient, Na+ -dependent, carrier-mediated mechanism for biotin uptake in these cells, which is again shared by pantothenic acid and lipoate (Said et al. 1998). These findings provided further evidence for the accessibility of the bacterially synthesized biotin into the human and its contribution toward the overall biotin pool, and especially that of the localized colonocytes.

1.5.1.3 Molecular Identity of the Intestinal Biotin Transport System

Cloning studies have determined the molecular identity of the intestinal SMVT of humans and of a number of animal models (Prasad et al. 1998; Wang et al. 1999; Chatterjee et al. 1999; and GenBank accession # AY572835). The human SMVT gene is located on chromosome 2p23 and consists of 17 exons (Wang et al. 1999). Significant sequence homology, at both the nucleotide and the amino acid levels, was found in the SMVT of the different mammalian species, and the polypeptide was predicted to have 12 trans-membrane domains with both of its ends (i.e., the N- and the C-terminal tails) oriented inwardly. The hSMVT polypeptide also appears to have a number of potential post-translational modification sites

including sites for phosphorylation by protein kinases and glycosylation sites. When expressed in heterologus systems, the cloned SMVT displayed Na+-dependency transport specificity for biotin, pantothenic acid and lipoate, and showed similar kinetic parameters to those observed for biotin uptake in the native intestine. The SMVT transcripts were shown to be expressed at a markedly higher level in the differentiated epithelial cells of the intestinal villi compared to those of the immature and undifferentiated cells of the crypt, a finding that corresponds with the higher level of biotin uptake in the former compared to the latter cells (Chatterjee et al. 1999). Distribution of the SMVT transcripts along the longitudinal axis of the intestine has also been delineated and shown to be similar in the different regions of the gut (Prasad et al. 1998; Wang et al. 1999; Chatterjee et al. 1999). This is in contrast to the observations of a higher biotin uptake in the proximal compared to the distal part of the small intestine, and the colon (Said et al. 1988; Said and Redha 1987). These findings suggest the possible involvement of specific post-translational modification(s) that may regulate biotin transport activity in the different regions of the intestinal tract. Cellular localization of the SMVT protein in human intestinal epithelial cells have also been examined using confocal imaging approach with results showing exclusive expression of the protein the apical membrane of polarized cells (Fig. 1.4; Unpublished observation from one laboratory). Other studies, have reported significant heterogeneity in the 5′ un-translated region of the rat SMVT, with four distinct variants (I, II, III, IV) being identified (Chatterjee et al.

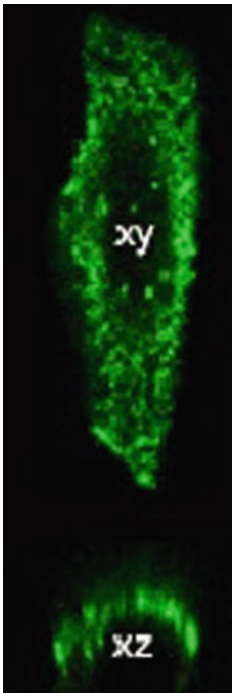

Fig. 1.4 Confocal imaging of live intestinal epithelial Caco-2 cells grown on filter showing exclusive expression of the human SMVT at the apical membrane domain. The XZ image shows exclusive expression of the human SMVT at the apical membrane domain of the polarized Caco-2 cells

1999); variant II was shown to be the predominant variant expressed in the intestinal tract (Chatterjee et al. 1999). A major role for the human SMVT system in intestinal carrier-mediated biotin uptake has been recently established with the use of a SMVT gene-specific siRNA approach (Balamurugan et al. 2003).

1.5.1.4 Regulation of the Intestinal Biotin Absorption Process

The intestinal biotin uptake process is regulated by a number of intracellular and extracellular factors. A role for intracellular protein kinase-C (PKC)- and Ca^{2+}/calmodulin-mediated pathways in the regulation of the intestinal biotin uptake process has been reported (Said 1999; Said et al. 1998). While each of these intracellular regulatory pathways was found to act via altering the activity (but not the affinity) of the SMVT system, they appeared to do so via different mechanisms (Said 1999; Said et al. 1998).

Extracellular biotin levels exert adaptive regulatory effects on the intestinal biotin uptake process in both humans and animal models (Reidling and Said 2006; Said et al. 1989a). Biotin deficiency leads to a specific and significant up-regulation in intestinal carrier-mediated biotin uptake, while biotin over-supplementation appears to have the opposite effect (ref). The up-regulation in intestinal biotin uptake observed in biotin deficiency occurs in association with a parallel increase in the level of SMVT protein and mRNA in intestinal epithelial cells with no changes in mRNA stability (Reidling and Said 2006). These findings suggest the involvement of transcriptional regulatory mechanism(s) in the observed adaptive regulatory effects in the intestinal biotin uptake process in biotin deficiency.

The intestinal biotin uptake process also undergoes developmental regulation via changes in the preferential site of biotin absorption and via changes in the kinetic parameters of the uptake process (Nabokina et al. 2003; Said and Redha 1988b). The latter changes involve the entry step of biotin across the apical membrane domain of the polarized intestinal epithelial cells and occurs in association with parallel changes in the level of SMVT protein and mRNA, as well as the transcription rate of the SMVT gene (Nabokina et al. 2003).

Recent studies using the human-derived intestinal epithelial Caco-2 cells as a model have also shown that the intestinal biotin uptake process is under differentiation-dependent regulation. Biotin uptake and the level of expression of hSMVT mRNA and protein as well as activity of the hSMVT promoter were found to be higher in post-confluent (differentiated) Caco-2 cells compared to pre-confluent (undifferentiated) cells (Reidling J and Said HM; unpublished observations). These findings clearly point to the possible involvement of transcriptional regulatory mechanism(s) in the differentiation-dependent regulation of the biotin uptake process.

Insight into the transcriptional regulation of the SMVT gene under basal and regulated conditions has also been forthcoming following the cloning and the characterization of the 5′-regulatory region of the human and rat SMVT genes (Chatterjee et al. 2001; Dey et al. 2002). In the case of the rat SMVT gene, three distinct promoters were identified (Chatterjee et al. 2001), while two promoters were

identified in the case of the human SMVT gene (Dey et al. 2002). In both cases activities of the cloned promoters were demonstrated by fusing the promoter fragments with the Firefly luciferase reporter gene followed by expression of the constructs in the appropriate cellular systems. Human promoter I was found to be more active than promoter II and required functional GKLF and AP-2 cis-regulatory elements for its activity in intestinal epithelial cells (Reidling and Said 2006). Activity of the cloned human SMVT promoter has also been confirmed in vivo in transgenic mice (Reidling and Said 2006).

1.5.1.5 Effect of Anti-Epileptic Drugs and Alcohol on Intestinal Biotin Uptake

As mentioned earlier, long-term use of anticonvulsant drugs leads to impairment in normal biotin status (Krause et al. 1982a, b). While the mechanism(s) involved in causing this abnormality is not fully clear, competitive inhibition of intestinal biotin uptake by these agents (Said et al. 1989b; Prasad and Ganapathy 2000) as well as accelerated biotin catabolism (Mock et al. 1997, 1998) and impairment renal reclamation of the vitamin (Chauhan and Dakshinamurti 1988) have all been reported. Similarly, the reduced blood biotin levels observed in alcoholics (Fennelly et al. 1969; Bonjour 1980) is believed to be, at least in part mediated via impairment in intestinal uptake of biotin (Said et al. 1990b).

1.5.2 Renal Uptake of Biotin

Circulating biotin undergoes filtration in the renal glomeruli. The vitamin is then salvaged via reabsorption by renal proximal tubular epithelial cells. Studies on the mechanism of renal biotin uptake have shown the involvement of a concentrative Na-dependent, carrier-mediated mechanism localized at the apical membrane domain of the polarized renal epithelial cells (Balamurugan et al. 2005; Baur and Baumgartner 1993; Baur et al. 1990; Podevin and Barbarat 1986). The system involved in renal biotin uptake is also shared by pantothenic acid and lipoate (Balamurugan et al. 2005) and is inhibited by biotin structural analogues with a free carboxyl group in the valeric acid moiety (like desthiobiotin), but not by analogues with a blocked carboxyl group (e.g., biocyin and biotin sulfoxide) (Balamurugan et al. 2005; Baur and Baumgartner 1993; Baur et al. 1990; Podevin and Barbarat 1986). Exit of biotin from the renal epithelial cells occurs via a carrier-mediated mechanism that is Na-independent and electrogenic in nature (Podevin and Barbarat 1986).

Regulation of the renal biotin uptake process has also been examined using the human-derived renal proximal epithelial HK-2 cells as in vitro model to human renal epithelial cells (Balamurugan et al. 2005). The results showed the process to be under the regulation of intracellular PKC- and Ca^{2+}/calmodulin (CaM)-mediated pathways. The renal biotin uptake process is also adaptively regulated by extracellular biotin levels via a mechanism that appears to be transcriptionally mediated (Balamurugan et al. 2005). Other studies have shown that native human renal

epithelial cells and the culture renal epithelial HK-2 cells to both express hSMVT at the protein and the mRNA levels (Balamurugan et al. 2005; Chatterjee et al. 1999). This hSMVT appears to be the main (if not the only) carrier-mediated mechanism for biotin uptake by renal epithelial cells as shown in studies utilizing gene silencing approach with gene-specific siRNA (Balamurugan et al. 2005).

An interesting finding with the renal biotin uptake process when compared to the intestinal uptake process is the finding that the former process does not appear to undergo developmental regulation (Nabokina et al. 2003). This conclusion is based on the observations that biotin uptake as well as the levels of the SMVT protein and mRNA is similar in suckling and adult rat kidney epithelial cells (Nabokina et al. 2003).

1.5.3 Biotin Uptake by the Liver

The liver plays an important role in normal biotin physiology and represents the major organ for biotin metabolism and utilization. While the liver contains the highest amount of biotin compared to other tissues, its capacity to store the vitamin is limited compared to other water-soluble vitamins like cobalamin, folate and riboflavin (Danford and Munro 1982). The liver extracts (from the portal circulation) a major portion of the newly absorbed biotin (Brown and Rosenberg 1987), and evidence exists that recycled biotin (i.e., the biotin that is generated as a result of degradation of holocarboxylase) is generated not in cells but in the extracellular compartment (Dakshinamurti and Chauhan 1988; Freytag and Utter 1983; Heard et al. 1985). Thus, the liver relies heavily on circulating and extracellular biotin for its needs, and on its transport across the hepatocyte basolateral membrane. Using isolated and cultured hepatocytes as well as purified liver basolateral membrane vesicle preparations of human and rat origin (Komro and McCormick 1985; Said et al. 1990a, 1992b, 1994), studies have shown the involvement of a concentrative, Na+-dependent carrier-mediated mechanism. This mechanism is sensitive to the effect of sulfhydryl group inhibitors and is inhibited by biotin structural analogues like desthiobiotin but not by biocytin. Human liver and the human-derived liver HepG2 cells both express the hSMVT at the protein and mRNA level (Balamurugan et al. 2003), and gene silencing studies with siRNA have shown that this system is the main biotin uptake system that operates in human hepatocytes (Balamurugan et al. 2003).

1.5.4 Biotin Transport Across the Blood Brain Barrier and the Placenta

Biotin transport across the blood brain barrier is carrier-mediated and is inhibited by probenecid and pantothenic acid but it is not affected by biocytin. This has been shown in vivo in the rat (Spector and Mock 1987) and in vitro using cultured

calf brain microvessel endothelial cells (Baur and Baumgartner 2000). The recent description of patients with a novel biotin-responsive basal ganglia disease (Ozand et al. 1998; Zeng et al. 2005), and a patient with sudden onset biotin-responsive coma (Mardach et al. 2002), underscore the importance of biotin for brain function.

The concentration of biotin in the plasma of human fetuses is many folds higher than that in the plasma of the mother, clearly suggesting the involvement of an efficient transport system that transports biotin in the direction of the fetus. The existence of such a system has indeed been demonstrated using different placental preparations (Karl and Fisher 1992; Schenker et al. 1993; Hu et al. 1994). This system transports biotin by a Na-dependent, carrier-mediated mechanism and the system is again shared with pantothenic acid and lipoate. These are characteristics of SMVT, which is expressed at a high level in the human placenta (Balamurugan et al. 2003).

1.5.5 Intracellular Transport of Biotin: Transport into the Mitochondria

Studies have shown that free biotin can be transported into the mitochondria for utilization in biotinylation reactions of apo carboxylases (Ahmad and Ahmad 1991). The mechanism involved in this transport has been studied using isolated liver mitochondria and shown to occur by an acid pH-dependent, non-mediated simple diffusion process (Said et al. 1992). It is believed that biotin enters the intramitochondrial space in the protonated (neutral) form, dissociates into its anionic form (pKa = 4.5) at the alkaline pH of the mitochondria, and becomes trapped within (Said et al. 1992).

1.6 Disorders of Biotin Metabolism and Physiology

As mentioned earlier, biotin in humans acts as a coenzyme to four carboxylases, and that these enzymes exist in the inactive apo forms which are converted to the holoactive forms by the action of HCLS by means of biotinylation (McMahon 2002; Mock 2004; Sweetman and Nyhan 1986; Dakshinamurti and Chauhan 1988). At the end of their functional life, the holocarboxylases undergo proteolytic degradation (in the lysosomes) that lead to the generation of biocytin and biotin-short peptides. Free biotin is then released from biocytin and biotin-oligopeptides via the action of biotinidase. The freed biotin is then reutilized by cells, i.e., recycled, in what is sometimes referred to as the "biotin cycle."

There are two major genetic disorders in biotin metabolism in humans, both of which lead to multiple carboxylase deficiency (reviewed in McMahon 2002; Mock 2004; Sweetman and Nyhan 1986). The first metabolic disorder leads to a defect in the process of biotinylation due to deficiency in HCLS. The second metabolic disorder leads to a defect in biotin recycling (release of free biotin from its

conjugated forms) due to deficiency in biotinidase. These two genetic disorders have also been classified according to the age at which their symptoms appear and are called early-onset (neonatal) multicarboxylase deficiency, and late-onset (juvenile) multiplecarboxylase deficiency syndromes, respectively.

HCLS deficiency is an autosomal recessive disorder. Patients with this disorder exhibit hypotonia, seizures, difficulties in breathing and in feeding, skin rash, and alopecia; in extreme conditions, patients may also exhibit developmental delay and coma. Metabolically, children affected by this disorder exhibit metabolic acidosis, organics aciduria and hyperammonemia. Multiple mutations in the HLCS gene have been identified and shown to lead to the generation of an enzyme with an impaired activity mainly due to decreased affinity. This condition can be diagnosed prenatally by determining the level of the relevant organic acids in the amniotic fluid, activity levels of the mitochondrial caboxylases in amniocytes, and by means of mutational analysis. This disorder can be treated with oral administration of pharmacological doses of biotin (10 mg/day), with most of the affected children demonstrating significant improvement.

Biotinidase deficiency is also an autosomal recessive disorder. The clinical symptoms associated with this disorder vary and include hypotonia, seizures, difficulty in breathing, ataxia, visual and hearing disturbances, skin rash, alopecia, immunological disturbances, and developmental delay. Metabolically, most of the affected children exhibit metabolic acidosis and organic aciduria. Multiple mutations in the biotinidase gene have been identified, which leads to the generation of an enzyme that is severely or partially incapable of recycling/re-utilization endogenous conjugated biotin; it is also incapable of releasing free biotin from its conjugated forms in the ingested food. Biotinidase deficiency can be diagnosed by measuring the biotinidase activity in the serum. Prenatally, the condition can also be diagnosed by means of mutational analysis. When diagnosed early all affected children can be successfully treated with oral administration of pharmacological doses of free biotin (5–20 mg).

Another inherited disorder related to biotin is the recently described biotin-responsive basal ganglia disease (BRGD). BBGD is a recessive disorder with childhood onset that exhibit subacute encephalopathy, confusion, dysarthria, disphagia with occasional external opthalmopelgia or supranuclear facial nerve palsy (Ozand et al. 1998; Zeng et al. 2005). The condition could progress to severe cogwheel rigidity, dystonia and quadriparesis, and even death if untreated. Patients with this disorder respond well to pharmacological doses of biotin (5–10 mg/kg/day), but the symptoms re-appear within a month if biotin supplementation is discontinued (Ozand et al. 1998; Zeng et al. 2005). Recent studies have shown that the cause of BRDG is mutations in the SLC19A3 gene, a member of the solute transporter family of genes (Zeng et al. 2005). More recent studies, however, have shown that the product of the SLC19A3 is not a biotin transporter but rather a specific membrane transporter for the water-soluble vitamin thiamin (Subramanian et al. 2006). Thus, it is still unclear at this stage how a disease like BRGD with mutations in a thiamin transporter responds to biotin over-supplementation, and further studies are needed to understand the connection(s).

A biotin-dependent sudden onset coma in an 18 months old child has also been recently described and is believed to be due to a defect in a biotin transport other than SMVT (Mardach et al. 2002). The patient in this case did not have holocarboxylase synthetase or biotinidase deficiencies, nor did the child appear to have nutritional biotin deficiency or accelerated biotin catabolism. The actual cause behind this case is under further investigation.

1.7 Concluding Remarks

Significant progress has occurred in recent years in our understanding of the physiology, biochemistry and nutritional roles of biotin. Despite that, there is a clear need for better understanding of the effects of suboptimal (marginal) biotin status on cellular metabolism and on human health and well-being. Additional effort is also needed for better appreciation of the degree of occurrence of suboptimal biotin status in the general population. There is also a need for better understanding of the nature of the molecular and biochemical defects in children who respond to biotin over-supplementation yet they are neither biotinidase nor holocarboxylase deficient. Finally a better understanding of the biotin physiology at the integrated whole animal level in vivo is required.

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