Introduction to “Advances in Understanding of the Biological Role of Biotin at the Clinical, Biochemical, and Molecular Level”

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This symposium highlighted the significance of biotin status for the health of the whole organism and explored the cell biological and biochemical mechanisms of biotin’s action. In view of recent studies of a wide range of organisms, including humans, indicating that biotin status influences fetal development, the symposium focus on rapidly evolving areas of general scientific interest was particularly timely. These areas included integration processes occurring at the cell membrane, in cytoplasm and in the nucleus, epigenetics, and communication between nutritional status and transcriptional events.

Communication between the cytoplasmic and nuclear events is critical for an organism’s response to changing environmental and metabolic states. Elucidation of the mechanisms of this communication has revealed important interactions between cellular events that were heretofore assumed to function independently. With respect to the water-soluble vitamin biotin, recent studies on biotin sensing in organisms across the evolutionary spectrum illustrate the importance of biotin for physiological processes beyond the well-established function in biotin-dependent carboxylases. Potential communication mechanisms include transcriptional events affected by an epigenetic mechanism. The novel phenomenology includes translocation of the protein biotin ligase (holocarboxylase synthetase) to the nucleus in response to biotin availability; mechanisms under active investigation include chromatin modification by biotinylations histones and effects on the transcriptional activity of specific genes.

Mock (1) reviewed evidence that biotin deficiency develops during normal human gestation and that biotin deficiency is teratogenic in some species. Mock presented a post-hoc analysis of data from the classic study by Czeizel and Dudás (2) suggesting that biotin deficiency might be teratogenic in humans. Studies of marginal biotin deficiency experimentally induced in adults were reviewed; these indicated that decreased urinary excretion of biotin, increased urinary excretion of 3-hydroxyisovaleric acid (which likely reflects decreased activity of the biotin-dependent enzyme β-methylcrotonyl-CoA carboxylase), and activity of the biotin-dependent enzyme propionyl-CoA carboxylase in peripheral blood lymphocytes were valid as indices of biotin status. Studies in pregnant women consistently find that more than half of pregnant women excrete increased amounts of urinary 3-hydroxyisovaleric acid. However, urinary biotin excretion is not generally decreased in pregnancy, and interpretation of urinary excretion rates is problematic because renal function is altered by pregnancy per se. Propionyl-CoA carboxylase data were presented from Mock’s recent study (D. Mock, unpublished data) indicating that a substantial proportion of pregnant women are marginally biotin deficient. Given that marginal biotin deficiency in the mouse dam caused high rates of fetal malformations and that these were likely mediated by a much more severe degree of deficiency in the fetus, increased concern about the potential for biotin deficiency to cause human birth defects is justified. Potential mechanisms for the teratogenicity were discussed, including deficiency of fatty acids and prostaglandin synthesis, and the recent groundbreaking work of Zempleni and coworkers (3) demonstrating that biotin effects on gene expression mediated by histone biotinylation changes at very specific loci in human and animal retrotransposons could be acting synergistically or in place of effects on carboxylase activity to mediate the teratogenic effects of biotin deficiency. Decreased abundance of biotinylated histones at these loci increases the transcriptional activity of retrotransposons, the production of viral particles, and the frequency of retrotranspositions and chromosomal abnormalities.

Said (4) reviewed molecular aspects of the human intestinal biotin absorption process. Because humans cannot synthesize biotin, they must obtain this vitamin from exogenous sources. The intestine is exposed to 2 sources of biotin: a dietary source and a bacterial source (normal microflora of the large intestine). Digestion of protein-bound biotin and absorption of free biotin in the small and large intestine was reviewed. Work conducted largely by Said et al. (4) clearly indicates that biotin is absorbed by a saturable and Na+-dependent carrier that can also transport pantothenic acid and lipoate; hence, the Sodium-dependent Multi-Vitamin Transporter (SMVT). The human SMVT (hSMVT) system has been cloned, demonstrated to be exclusively

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6 Abbreviations used: bioO, biotin operator; HCS, holocarboxylase synthetase; hSMVT, human sodium-dependent multi-vitamin transporter; SMVT, sodium-dependent multi-vitamin transporter.
expressed at the apical membrane of enterocytes, and shown to be the main biotin uptake system that operates in human intestinal epithelial cells. Said reviewed studies that indicate that the human intestinal biotin uptake process is adaptively upregulated in biotin deficiency via a transcriptionally mediated mechanism(s) that involves KLF4 sites. The 5′- regulatory region of the hSMVT gene has also been cloned and characterized both in vitro and in vivo, and 2 distinct and functional promoters (P1 and P2) were identified. Studies identified a region in the cytoplasmic C-terminal domain of the polypeptide that targets SMVT to the apical membrane domain of epithelial cells. Evidence was reviewed indicating that intracellular trafficking of the hSMVT protein involves distinct trafficking vesicles that require an intact microtubule network and the motor protein dynein.

Zempleni (3) reviewed the evidence that supports a novel role for holocarboxylase synthetase (HCS) in sensing and regulating levels of biotin in eukaryotic cells. He presented his hypothesis that biotin regulates its own cellular uptake by participating in HCS-dependent chromatin remodeling events at the SMVT promoter 1 locus. Specifically, he proposed that nuclear translocation of HCS increases in response to biotin supplementation; HCS then biotinylates histone H4 at SMVT promoters, silencing this biotin transporter. He reviewed supporting studies in human lymphoid (Jurkat) cells showing that nuclear translocation of HCS is a biotin-dependent process that likely involves protein kinases, histone deacetylases, and histone methyltransferases. These studies indicate that nuclear translocation of HCS correlated with biotin concentrations in cell culture media; the relative enrichment of both HCS and K12BioH4 at SMVT promoter 1 (but not promoter 2) almost doubles in cells cultured in medium containing 10 nmol/L biotin compared with 0.25 nmol/L biotin. This increase of K12BioH4 at the SMVT promoter was inversely linked to SMVT expression. Biotin homeostasis by HCS-dependent chromatin remodeling at the SMVT promoter 1 locus was disrupted in HCS knockdown cells.

Beckett (5) described molecular details of biotin sensing. She reviewed the substantial evidence, including her own pioneering studies, indicating that sensing of biotin availability occurs at the level of gene transcription in a broad range of organisms. The biotin sensing is mediated through biotin ligase, the enzyme that catalyzes covalent linkage of biotin to biotin-dependent carboxylases. In higher organisms, the ligase is designated holocarboxylase synthetase. This biotinylation of histones correlates with changes in transcription of several genes. In *Saccharomyces cerevisiae*, the ligase is an integral component of the response to biotin starvation that results in increased transcription of a number of genes. In many bacteria, the ligase is bifunctional and, in addition to catalyzing biotin addition, represses transcription initiation of biotin-related genes. The *Escherichia coli* biotin protein ligase, BirA, belongs to this class of bifunctional ligases. BirA binds to the biotin operator (bioO) in a biotin-dependent manner. However, the activated derivative of biotin, bio-5′-AMP, rather than biotin, is the physiological corepressor of BirA. Bio-5′-AMP enhances bioO binding to BirA by enhancing BirA homodimerization. Structural analysis suggests that the allosteric effect of bio-5′-AMP is linked to folding of portions of the BirA structure. This mechanism has been further characterized by combined site-directed mutagenesis, followed by thermodynamic and structural analysis of the resulting mutants. The results indicate that folding of a single 20-amino-acid loop is critical for initiating the allosteric response to bio-5′-AMP binding. Further analysis by hydrogen-deuterium exchange linked to mass spectrometric detection indicates that this initial folding event percolates to distal regions of the protein structure to enhance repressor dimerization, and subsequent bioO binding and transcription repression at the biotin biosynthetic operon.

Taken together, these molecular, cellular, microbial, mouse, and human studies from the laboratories of the presenters at this symposium have further elucidated the biological mechanisms and biotin homeostasis and cellular action and supported concern that biotin deficiency may be a significant human teratogen. These studies and those from other laboratories around the world mandate intensive investigation into the normal homeostasis and pathogenic mechanisms of biotin deficiency at the molecular, cellular, and organism level.

Literature Cited