Regulation of intestinal vitamin B₂ absorption
Focus on “Riboflavin uptake by human-derived colonic epithelial NCM460 cells”

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RIBOFLAVIN OR VITAMIN B₂ (7,8-dimethyl-10-ribitylisoalloxazine) is an essential water-soluble vitamin. Riboflavin is important for normal cell functioning and for cell growth and development. Riboflavin is a major component of coenzymes FAD and flavin mononucleotide (FMN) that play a key role in the metabolism of carbohydrates, amino acids, and lipids. Riboflavin is also intimately involved in the conversion of pyridoxine (vitamin B₆) and folic acid (vitamin B₉) into their coenzymes (2, 5).

Humans and other mammals cannot synthesize riboflavin. However, this vitamin is found abundantly in milk, leafy green vegetables, and eggs. The dietary riboflavin is found primarily in the forms FAD and FMN. These coenzymes are hydrolyzed by intestinal luminal phosphatases before they can be absorbed (1, 3, 8). Transport of riboflavin has been extensively studied in human, rabbit, and rat small intestine. Riboflavin absorption in the mammalian small intestine occurs on the brush-border membrane (BBM) of the absorptive villus cells and is carrier mediated (9, 11–15). The role of Na⁺ dependency for riboflavin uptake in the small intestine is not completely clear, because BBM vesicle studies suggest no Na⁺ dependency, whereas intact tissue studies suggest a role for Na⁺. Taken together, these findings may suggest a secondary role for Na⁺ dependency in riboflavin small intestinal absorption compared with a more direct dependency necessary for Na⁺-nutrient cotransport processes such as Na⁺-glucose cotransport (9, 13–15).

An equally important source of riboflavin is bacterially synthesized riboflavin in the colon. Colonic flora synthesize a considerable amount of this vitamin and which in the colon is available for absorption in the free form (6, 7). Although the small intestinal assimilation of riboflavin appears to be regulated by the amount of the vitamin ingested (18), the colonic absorption is more dependent on the type of diet ingested. The colonic bacteria appear to produce more riboflavin when a more fiber-based diet (e.g., green leafy vegetables) is ingested compared with a meat-based diet. Thus with the former type of diet there is more of the vitamin for the colonic epithelium to absorb (6).

The wide availability of riboflavin in food sources and the redundancy of intestinal absorption of this vitamin may point to its importance for the overall well-being of the organism. Indeed, deficiency of riboflavin results in a variety of pathophysiological states. Vitamin B₂ deficiency is characterized by glossitis, cheilosis, angular stomatitis, seborrhea-like dermatitis, pruritus, photophobia, visual impairment, growth retardation, alopædia, and degenerative changes of the nervous system (2, 4, 5, 10). Thus better understanding of the intestinal assimilation of riboflavin is essential to prevent the wide range of disease entities associated with its deficiency.

However, until recently very little was known about the cellular regulation of riboflavin transport. This was chiefly owing to the lack of suitable in vitro cell culture systems to study the transport of this vitamin. Recently, Said’s group in a series of studies has elegantly detailed the cellular mechanism of regulation of riboflavin transport in the colon. In one study using Caco-2 cells they demonstrated that riboflavin uptake is carrier mediated, Na⁺ independent, and inhibitable by cation exchange (e.g., amiloride) but not anion exchange inhibitors (e.g., stilbene derivatives), furosemide, or probenecid. Further, extracellular substrate concentration appeared to regulate the transporter by increasing the BBM transporter numbers (17). Whether this increase in transporter numbers is secondary to altered membrane trafficking and/or transcriptional changes of the riboflavin transporter has yet to be deciphered. Said et al. then demonstrated that protein kinase A (PKA), but not protein kinase C regulates the riboflavin transporter in Caco-2 cells. Increasing intracellular cAMP levels inhibited the uptake of riboflavin in these cells. The mechanism of PKA-mediated inhibition was not secondary to a decrease in the synthesis or membrane trafficking of the riboflavin transporter, but most likely secondary to a decrease in the activity of the transporter (16).
The current article in focus by Said et al. (Ref. 19, see page C270 in this issue) describes the regulation of riboflavin transport in a human-derived nontransformed colonic epithelial cell line, NCM 460. Riboflavin uptake in these colonocytes was carrier mediated, Na⁺ independent, and inhibitable by structural analogs. The transporter is amiloride sensitive. This last observation in both NCM 460 and Caco-2 cells raises the intriguing possibility that this may be a riboflavin/proton exchanger. Further studies will need to be done to establish this. In NCM 460 colonocytes the riboflavin transporter number is regulated up or down by the availability of extracellular vitamin levels. Intracellular Ca²⁺/calmodulin, but not protein kinase C seems to regulate this transporter. Specifically, inhibition of the Ca²⁺/calmodulin pathway results in the inhibition of riboflavin transport. The mechanism of inhibition is both at the level of the transporter numbers as well as secondary to altered affinity of the transporter for the vitamin. Future molecular studies will be necessary to determine whether the Ca²⁺/calmodulin pathway regulation of riboflavin transport is primarily at the transcriptional or posttranslational level.

It is evident from the study in focus as well as all of the previous work by Said’s group that their effort has greatly enhanced our knowledge of riboflavin assimilation in the intestine. Further, their work to date has set the stage to examine the regulation of riboflavin transport at the molecular level. Better understanding of the regulation of this transporter in health will lead to future investigations aimed at the deregulation of this vitamin’s transport in pathophysiological states. This will undoubtedly result in more efficacious treatment modalities for disease states that result when there is an imbalance in the homeostasis of this very important vitamin.

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REFERENCES